Cardiac Magnetic Resonance Quantitative Perfusion Imaging in Health and Diabetic Heart Disease

Louise Anne Elizabeth Brown

Submitted in accordance with the requirements for the degree of Doctor of Philosophy (PhD)

The University of Leeds
School of Medicine
Leeds Institute of Cardiovascular and Metabolic Medicine

July, 2021

Intellectual Property and Publication Statements

The candidate confirms that the work submitted is his/her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

Publications arising from this work:

Chapter 3

Publication:

Brown, L.A.E., Onciul, S.C., Broadbent, D.A. et al. Fully automated, inline quantification of myocardial blood flow with cardiovascular magnetic resonance: repeatability of measurements in healthy subjects. J Cardiovasc Magn Reson 20, 48 (2018). https://doi.org/10.1186/s12968-018-0462-y

Authorship:

LB: Conception and design, recruitment, supervision of scans, analysis and interpretation of data, drafting of manuscript. **SO**: Supervision of scans, analysis and interpretation of data, critical & intellectual revision of manuscript. **DB**: analysis and interpretation of data, critical & intellectual revision of manuscript. **KJ**: Recruitment, supervision of scans, critical & intellectual revision of manuscript. **GF**: Critical & intellectual revision of manuscript. **JF**: Critical & intellectual revision of manuscript. **PG**: Critical & intellectual revision of manuscript. **PC**: Critical & intellectual revision of manuscript. **KK**: Critical & intellectual revision of manuscript. **ED**: Critical & intellectual revision of manuscript. **PS**: Critical & intellectual revision of manuscript. **HX**: Conception and design, processing and interpretation of CMR data, critical & intellectual revision of manuscript. **JG**: Critical & intellectual revision of manuscript. **JM**: Critical & intellectual revision of manuscript. **PK**: Conception and design, processing and interpretation of CMR data, critical & intellectual revision of manuscript. **SP**: Conception and design, interpretation of data and drafting of manuscript. All authors read and approved the final manuscript.

Chapter 4

Authorship:

LB: Conception and design, recruitment, supervision of scans, analysis and interpretation of data, drafting of manuscript, **SO**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **DB**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **JY**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **AW**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **GG**: Recruitment,

supervision of scans, critical & intellectual revision of manuscript, **CS**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **AD**: critical & intellectual revision of manuscript, **NJ**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **AC**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **ST**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **NS**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **KK**: critical & intellectual revision of manuscript, **EL**: critical & intellectual revision of manuscript, **PS**: Recruitment, supervision of scans, critical & intellectual revision of manuscript, **HX**: Conception and design, processing and interpretation of CMR data , critical & intellectual revision of manuscript, **JG**: critical & intellectual revision of manuscript, **JM**: critical & intellectual revision of manuscript, **DA**: critical & intellectual revision of manuscript, **GM**: critical & intellectual revision of manuscript, **PK**: Conception and design, processing and interpretation of CMR data, critical & intellectual revision of manuscript, **SP**: critical & intellectual revision of manuscript.

Chapter 5

Publication:

Brown, L.A.E., Saunderson, C.E.D., Das, A. et al. A comparison of standard and high dose adenosine protocols in routine vasodilator stress cardiovascular magnetic resonance: dosage affects hyperaemic myocardial blood flow in patients with severe left ventricular systolic impairment. J Cardiovasc Magn Reson 23, 37 (2021). https://doi.org/10.1186/s12968-021-00714-7)

Authorship:

LB: Conception and design, recruitment, scanning and supervision of scans, analysis and interpretation of data, drafting of manuscript. **CS**: recruitment, supervision of scans, critical and intellectual revision of manuscript. **AD**: critical and intellectual revision of manuscript. **TC**: critical and intellectual revision of manuscript **EL**: critical and intellectual revision of manuscript **KK**: critical and intellectual revision of manuscript **ED**: critical and intellectual revision of manuscript **HX**: processing and interpretation of CMR data, critical and intellectual revision of manuscript **JM**: critical and intellectual revision of manuscript **JG**: critical and intellectual revision of manuscript **PK**: processing and interpretation of CMR data, critical and intellectual revision of manuscript **PS**: recruitment,

supervision of scans, critical and intellectual revision of manuscript, **SP**: concept and design and drafting of manuscript. All authors read and approved the final manuscript.

Chapter 6

Authorship:

LB: conception and design, recruitment, data acquisition, scanning and supervision of scans, analysis and interpretation of data, drafting of manuscript, **AW**: recruitment, data acquisition, critical and intellectual revision of manuscript, **EI**: recruitment, data acquisition, critical and intellectual revision of manuscript, **CS**: supervision of scanning, critical and intellectual revision of manuscript, **AD**: critical and intellectual revision of manuscript, **TC**: critical and intellectual revision of manuscript, **AC**: critical and intellectual revision of manuscript, **EL**: critical and intellectual revision of manuscript, **ED**: critical and intellectual revision of manuscript, **KK**: critical and intellectual revision of manuscript, **JG**: critical and intellectual revision of manuscript, **JM**: critical and intellectual revision of manuscript, **HX**: critical and intellectual revision of manuscript, **PK**: critical and intellectual revision of manuscript, **SP**: supervision, drafting of manuscript, **PS**: concept and design, drafting of manuscript, recruitment, data acquisition.

Chapter 7

Authorship:

LB: conception and design, recruitment, data acquisition, scanning and supervision of scans, analysis and interpretation of data, drafting of manuscript, **AW**: recruitment, data acquisition, critical and intellectual revision of manuscript, **EI**: recruitment, data acquisition, critical and intellectual revision of manuscript, **CS**: supervision of scanning, critical and intellectual revision of manuscript, **AD**: critical and intellectual revision of manuscript, **TC**: critical and intellectual revision of manuscript, **AC**: critical and intellectual revision of manuscript, **EL**: critical and intellectual revision of manuscript, **ED**: critical and intellectual revision of manuscript, **KK**: critical and intellectual revision of manuscript, **JG**: critical and intellectual revision of manuscript, **JM**: critical and intellectual revision of manuscript, **HX**: critical and intellectual revision of manuscript, **PK**: critical and

intellectual revision of manuscript, **SP**: supervision, drafting of manuscript, **PS**: concept and design, drafting of manuscript, recruitment, data acquisition.

Contributors' Initials/Names

AC	Amrit Chowdhary
AD	Arka Das
AW	Alice Wood
AW	Ali Wahab
CS	Chris Saunderson
DA	David Adlam
DB	David Broadbent
ED	Erica Dall'Armellina
EI	Eunice Ikongo
EL	Eylem Levelt
GF	Graham Fent
GG	Gaurav Gulsin
GM	Gerry McCann
HX	Hui Xue
JF	James Foley
JG	John Greenwood
JM	James Moon
JY	Jian Yeo
KK	Kris Knott
LB	Louise Brown
NJ	Nick Jex
NS	Noor Sharrack
PC	Pei Gee Chew
PG	Pankaj Garg
PK	Peter Kellman
PS	Peter Swoboda
SO	Sebastian Onciul
SP	Sven Plein
ST	Sharmaine Thirunavukirasu
TC	Thomas Craven

Abstracts

1. Brown LAE, Saunderson CED, Onciul SC, Broadbent DA, Fent GJ, Foley JRJ, Garg P, Chew PG, Knot KD, Dall'Armellina E, Xue H, Greenwood JP, Swoboda, PP Moon JC, Kellman P, Plein S. Reduced myocardial perfusion reserve in systolic heart failure – a therapeutic target? Heart Jun 2018, 104 (Suppl 6) A44-A45. Poster Presentation at BCS, Manchester UK, June 2018.

2. Brown LAE, Onciul SC, Broadbent DA, Fent GJ, Foley JRJ, Garg P, Chew PG, Knot KD, Dall'Armellina E, Xue H, Greenwood JP, Swoboda, PP Moon JC, Kellman P, Plein S. Reduced Myocardial Perfusion Reserve in Systolic Heart Failure is related to NYHA class but not Degree of LV Dysfunction. Abstract poster - CMR, Barcelona, Spain, February 2018.

3. Brown LAE, Onciul SC, Broadbent DA, Fent GJ, Foley JRJ, Garg P, Chew PG, Knot KD, Swoboda, PP, Dall'Armellina E, Xue H, Greenwood JP, Moon JC, Kellman P, Plein S. Gender Difference in Response to Adenosine Stress Perfusion CMR. Oral Abstract Presentation at CMR 2018 Barcelona, Spain, February 2018.

4. Brown LAE, Saunderson CED, Chew PG, Das A, Xue H, Knott KD, Dall'Armellina E, Levelt E, Moon JC, Kellman P, Greenwood JP, Plein S. Swoboda. Underlying mechanisms in heart failure and dysglycaemia. Oral Abstract Presentation at SCMR, Seattle, USA January 2019

5. L Brown, CED Saunderson, A Das, T Craven, H Xue, K Knott, E Levelt, E Dall'Armellina, PP Swoboda, J Moon, JP Greenwood, P Kellman, S Plein. Assessing myocardial perfusion in heart failure - are we achieving adequate stress? Abstract at EuroCMR, Venice Italy, May 2019.

6. L Brown, CED Saunderson, A Das, T Craven, H Xue, K Knott, E Levelt, J Moon, E Dall'Armellina, JP Greenwood, P Kellman, S Plein, PP Swoboda. Defining the phenotype of heart failure with mid-range ejection fraction by cardiovascular magnetic resonance. Abstract at EuroCMR, Venice Italy,

May 2019.

7. L Brown, A Wahab, C Saunderson, A Das, T Craven, A Chowdhary, N Jex, K Knott, E Levelt, E Dall'Armellina, H Xue, J Moon, J Greenwood, P Kellman, S Plein, P Swoboda. Underlying Mechanisms in Non-ischaemic Heart Failure. Abstract presented at SCMR Orlando, USA, February 2020.

Acknowledgements

This work would not have been possible without the contribution and support of others. I would like to express my gratitude to my supervisors Professor Sven Plein and Professor John Greenwood. Their guidance, leadership and support has been invaluable, and it is a real privilege to have been part of the University of Leeds CMR team.

My thanks go to Dr Peter Kellman, without whom this research would not have been possible. His knowledge, enthusiasm and patience will stay with me throughout my career. And to Dr Peter Swoboda, for all his help, which remains ongoing, his enthusiasm, advice and guidance have been invaluable.

To the AIC team, from beginnings where we were all learning as we went along, special thanks to David Shelley, for all his hard work in scanning, organising and administration, and for everything else that was asked of him, without him this would not have been possible. Thanks to Dr David Broadbent, for his expertise and the patience in his explanations, his help in scanning and setting up our protocols and the many cups of coffee we consumed along the way. And to Dr Sebastian Onciul, for all his help in getting things up and running in the early days. Special thanks to my ‘buddy’ Dr Chris Saunderson for his support with recruitment, scanning and anything else, and for making work a more enjoyable place throughout this period.

I am grateful to the research nurses, Petra, Hannah, Fiona and Eunice for all their invaluable help in setting up these studies and ensuring that they ran smoothly. Particular thanks must go to Petra for her patience and help in my initial attempts at producing study paperwork and ethics, and to Hannah, who has gone above and beyond what could be expected in helping to recruit and scan patients, as well as providing friendship and support through some of the more challenging moments in research.

And to the rest of the team; fellows, radiographers and assistants; Drs Fent, Foley, Garg, Chew, Das, Craven, Jex and Chowdhary, Gavin, Margaret, Lisa,

Dave, Julian and Debbie, whose organisational skills are unmatched. For all the support, discussion, knowledge and friendship over the three years, I have thoroughly enjoyed my time in Leeds and gained not only colleagues but friends.

I am most grateful to all the patients and volunteers who participated in this research project. Their time and altruism have hopefully furthered our knowledge of this field and will lead to progress in patient care.

Lastly, I would like to acknowledge the support of my family and friends outside work, for all their help and encouragement throughout this time. Most importantly, my parents whose support, love and guidance are with me in whatever I pursue.

Abstract

Introduction

CMR is the gold standard imaging technique for assessment of cardiac function and volumes and can assess myocardial perfusion and tissue characteristics. Quantitative myocardial perfusion can detect microvascular disease in addition to obstructive coronary artery disease but has not been widely available due to time-consuming manual processing. Newly developed inline quantitative perfusion mapping allows assessment of myocardial perfusion in non-ischaemic myocardial disease in clinical practice.

Aims

To assess 1) repeatability of inline quantitative perfusion mapping by CMR, 2) establish normal values and variation with age and sex, 3) methodology for achieving adequate stress in patients, particularly in those with impaired ventricular function, 4) characteristics of patients with heart failure, including associated microvascular dysfunction and 5) characteristics of diabetic heart failure and markers of prognosis in this condition.

Methods

Participants were recruited between January 2017 and February 2020. All underwent comprehensive CMR scans with inline quantitative perfusion. Healthy volunteers were scanned to establish repeatability and normal values, patients with coronary artery disease and heart failure to assess adenosine protocols for stress imaging, and tissue characteristics of heart failure and diabetic heart disease.

Results

1) Quantitative perfusion CMR was shown to have repeatability similar to widely used methods of perfusion imaging including PET. 2) Normal values were established with a decline in stress MBF and MPR with age, and significantly higher MBF in females. 3) Patients with impaired ventricular function had an increase in stress MBF with higher dose adenosine, indicating they may require higher doses to achieve hyperaemia. 4) Native T1, ECV and MPR correlated with ejection fraction in patients with heart failure, worsening with impaired ejection fraction. 5) Patients with dysglycaemia and heart failure had higher T1 and lower

MPR than those with normoglycaemia. MPR was an independent prognostic marker for cardiovascular events in dysglycaemia and heart failure.

Table of contents

List of Figures

List of Tables

List of Abbreviations

AF	atrial fibrillation
AHA	American Heart Association
AIF	arterial input function
ANOVA	analysis of variance
BP	blood pressure
BTEX	blood tissue exchange
CAD	coronary artery disease
CFR	coronary flow reserve
CMR	cardiovascular magnetic resonance
CTCA	computed tomography coronary angiogram
CV	coefficient of variation
Cx	circumflex
DCM	dilated cardiomyopathy
ECG	electrocardiogram
ECV	extracellular volume
EDV	end diastolic volume
EF	ejection fraction
ESC	European Society of Cardiology
ESV	end systolic volume
FFR	fractional flow reserve
FLASH	fast low angle shot
GBCA	gadolinium based contrast agent
Hct	haematocrit
HF	heart failure
HFmrEF	heart failure with mid-range ejection fraction

HFpEF	heart failure with preserved ejection fraction
HFrEF	heart failure with reduced ejection fraction
HR	heart rate
ICC	intraclass correlation coefficient
IHD	ischaemic heart disease
IQR	interquartile range
LAD	left anterior descending
LGE	late gadolinium enhancement
LV	left ventricle
LVH	left ventricular hypertrophy
MACE	major adverse cardiovascular event
MBF	myocardial blood flow
MI	myocardial infarction
MOCO	motion-corrected
MOLLI	MOdified Look Locker Inversion recovery
MPR	myocardial perfusion reserve
NYHA	New York Heart Association
PD	proton density
PET	positron emission tomography
RC	reproducibility coefficient
RCA	right coronary artery
RPP	rate pressure product
SD	standard deviation
TE	echo time
TR	repetition time
TS	saturation recovery time

Chapter 1
Background

Coronary artery disease (CAD) is a leading cause of morbidity and mortality globally(1,2). Management is guided by the presence of significant stenoses and the degree of flow limitation and can be medical or interventional, either with percutaneous intervention or surgical revascularisation. It is now recognised that knowledge of the functional significance of disease is preferable to guide management(3,4).

The current gold standard for assessment of CAD is invasive coronary angiography, with the use of fractional flow measurements to assess pressure drop over a lesion and determine its functional significance(4,5).

Coronary angiography is however an invasive procedure, exposing patients to ionising radiation and procedural risk. Without suitable screening prior to referral for this test, a high proportion of patients may have no significant stenosis on angiography(6).

A number of imaging techniques exist that are able to assess the presence of coronary disease and provide additional information including myocardial viability and burden of ischaemia, as well as the ability to quantify left ventricular (LV) function. Computed tomography coronary angiography (CTCA), positron emission tomography (PET) and cardiovascular magnetic resonance (CMR) all feature in guidelines for the assessment of coronary syndromes(4) showing high diagnostic accuracy.

1.1 Non-invasive assessment of ischaemia and blood flow

1.1.1 Computed tomography

CTCA is recommended by NICE as the first line investigation for patients with typical or atypical chest pain(7), and by the European Society of Cardiology (ESC) in patients with low clinical likelihood and no history of CAD(4).

Without direct visualisation of the coronary artery lumen, a CT scan be used to provide a calcium score, an estimation of the calcium burden within the coronary tree. This is a simple procedure, acquired during one breath-hold with no requirement for intravenous contrast or additional medication. Scores above 1 are

associated with increasing risk(8). In asymptomatic patients, calcium scores can predict future risk of cardiovascular events(9), while in symptomatic patients, the score has been shown to correlate to the degree of artery stenosis(10). A calcium score of zero is associated with low cardiovascular risk, although this cannot fully rule out the presence of significant coronary disease(11) therefore CT angiography is also required.

CTCA allows non-invasive anatomical evaluation of the coronary arteries. Using an intravenous iodinated contrast agent, the coronary artery lumen can be visualised in a scan which only takes minutes. This method provides a high degree of accuracy in detecting obstructive coronary disease defined by invasive angiography.

CTCA has been shown to have excellent sensitivity (99%) and negative predictive value (97%) for CAD(12) but has poor diagnostic accuracy in detecting haemodynamically significant lesions(13), overestimating occlusive plaque, with a sensitivity of 63%. This results in a grey area, with stenoses estimated between 50-90% on visual assessment not always resulting in functionally significant obstruction. In the absence of flow measurement, it is generally recommended that functional testing is required if this level of stenosis was detected.

The addition of CT-FFR (fractional flow reserve) adds a quantitative aspect to CT assessment. With the use of computational flow dynamics, algorithms allow the calculation of coronary flow and pressure from a CTCA scan enabling production of a CT FFR value(14). These results have been shown to be comparable to invasive FFR measurements(15) and values <0.8 are prognostically associated with higher risks of revascularisation, myocardial infraction and death(16).

Decision making in complex coronary disease has been shown to have high agreement when based solely on CTCA when compared to using conventional angiography(17).

CT examination has benefits to the patient with speed of the test and is generally well tolerated but has negatives in the use of ionising radiation and iodinated contrast agents, contraindicated in low GFR. Its accuracy relies on lower heart rates (ideally <60bpm) which may require administration of beta blockers at the time of scan.

CT provides some additional prognostic information; the presence of non-obstructive plaque can be used to guide therapy and medical prevention. The

addition of CTCA to standard care has been shown to increase use of antianginal and preventative therapies, and result in lower rate of death from coronary disease and non-fatal myocardial infarction(18).

1.1.2 Positron Emission tomography

PET imaging relies on the detection of photons emitted during the decay of a radiolabelled tracer specifically used for these scans. PET can produce three dimensional images of the distribution and uptake of these tracers, in the case of myocardial perfusion imaging, absolute concentration of the tracer can be mapped, allowing images of the distribution throughout the myocardium in first pass perfusion. Early PET studies relied on qualitative assessment of myocardial perfusion, or semiquantitative analysis, normalising all myocardial data to the area of highest perfusion using static uptake images of radioactive tracers such as 82Rb and 13NH3. These methods were widely used and shown to be a sensitive and specific method for diagnosis of coronary disease. However, the use of relative comparison meant that the presence of multivessel disease was more challenging to diagnose, where all segments would have reduced perfusion, with little distinction between regions.

Quantitative PET allowed an estimate of absolute perfusion without relying on this comparison and early studies showed that it was able to better assess the extent of coronary disease(19). As the first non-invasive modality to quantify myocardial perfusion, the majority of studies showing the additional value of quantification are from PET data.

In a study of 104 patients with moderate pre-test probability of CAD, it was shown that absolute quantification of MBF improved specificity and positive predictive value compared to relative perfusion analysis(20). This improvement was particularly seen in multivessel disease.

In addition to providing improved diagnostic accuracy, quantitative assessment was able to provide prognostic information. Abnormal perfusion and coronary flow reserve (CFR – the ratio of stress and rest blood flow) have been shown to predict poorer outcomes with increased risk of MACE (major adverse cardiac event) and cardiac death. CFR has also been shown to provide additional prognostic data above perfusion; in those with abnormal myocardial perfusion, normal CFR conveys lower risk than both abnormal perfusion and abnormal CFR(21).

In comparison to other imaging modalities, PET involves ionising radiation and produces limited anatomical information, although this may change as hybrid techniques involving CT or MRI are brought into practice.

1.1.3 Cardiac Magnetic Resonance

CMR provides the gold standard for assessment of cardiac function, structure and tissue characterisation. One of the main uses of CMR is for detection of myocardial ischaemia(22).

1.1.3.1 Qualitative assessment

In clinical practice, CMR is currently a qualitative technique. Hyperaemia is achieved using pharmacological stress, most commonly with adenosine, although regadenoson and dobutamine are also used. Adenosine produces vasodilation in the coronary circulation. In normal myocardium this leads to dilatation of the coronary vascular bed, but where coronary stenosis exists, the microvasculature distal to the stenosis is already dilated at rest, and therefore cannot increase perfusion. Adenosine therefore causes a 'steal effect' seen in MRI imaging with brighter areas of increased contrast in normally perfused myocardium, and darker areas of hypoperfusion where stenoses are present (Figure 1).

Short axis slices are obtained in the basal, mid and apical portions of the left ventricle to ensure coverage of the whole myocardium, and images are acquired during first pass of gadolinium-based contrast. Visual assessment by an experienced operator identifies areas of relative hypoperfusion, corresponding to reduced myocardial perfusion.

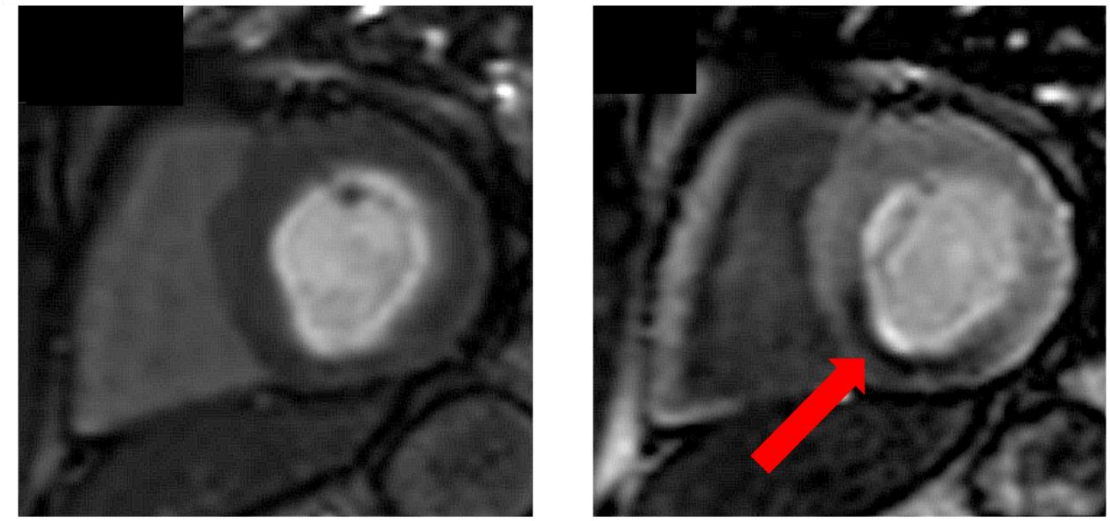
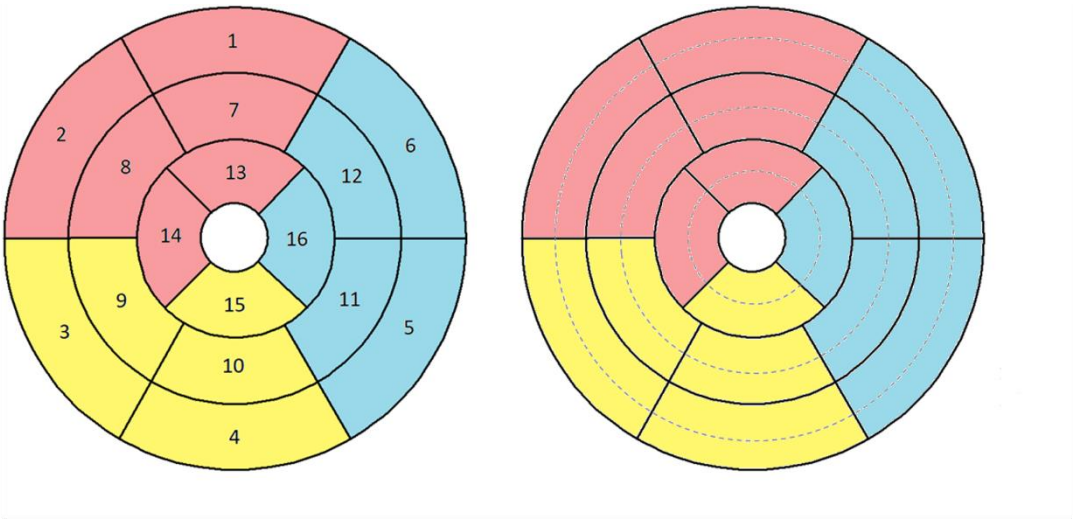


Figure 1-1 Perfusion defect on standard CMR imaging

The figure on the left shows uniform contrast uptake throughout the myocardium during rest perfusion. On the right, during adenosine induced stress, areas of hypoperfusion (indicated with arrow) are seen.

Resting perfusion images may also be obtained and can help identify artefacts but are not universally used. This method has been shown to be an effective diagnostic approach(23) and a negative scan carries a good prognosis with a 3 year event free survival of over 99%(24).

The extent of ischaemia can be evaluated using the AHA (American Heart Association) 16 segment model(25), or the adapted 32 segment model, where segments are subdivided into epicardial and endocardial components taking advantage of the spatial resolution of CMR(26). The ischaemia extent is then used to predict patient risk and guide management with involvement of 4 of 32 segments of the myocardium or ≥1.5 of 16 segments indicating moderate to severe ischaemia and recommending intervention (27,28).



1 – Basal anterior
2 – Basal anteroseptal
3 – Basal inferoseptal
4 – Basal inferior
5 – Basal inferolateral
6 – Basal anterolateral
7 – Mid anterior
8 – Mid anteroseptal
9 – Mid inferoseptal
10 – Mid inferior
11 – Mid inferolateral
12 – Mid anterolateral
13 – Apical anterior
14 – Apical septal
15 – Apical inferior
16 – Apical lateral

Left anterior descending
Right coronary artery
Circumflex

Figure 1-2 American Heart Association 16 segment model

The myocardium is divided into 16 segments, which can be further divided (as shown on the right image) into 32, with endocardial and epicardial segments. The burden of ischaemia can be estimated using these segments, and the presumed culprit coronary artery identified.

This method of assessment can be less accurate in 'balanced ischaemia' – where all three coronary territories have reduced perfusion, or in microvascular dysfunction, again resulting in global reduction in myocardial perfusion, a feature that can account for chest pain, and is common in cardiomyopathies.

In order to improve accuracy, and reproducibility, particularly in these challenging cases, quantitative perfusion can be used, providing absolute values of myocardial blood flow at stress and rest, and a myocardial perfusion ratio (stress MBF: rest MBF) equivalent of CFR in PET.

Early studies demonstrated that quantitative analysis of CMR data was accurate in detecting significant coronary stenoses and improved the ability to detect multivessel disease(29).

1.1.3.2 Semi quantitative perfusion

Initially semi-quantitative assessment of perfusion utilising time-signal intensity curve of contrast in myocardial segments was used. Time signal intensity curves plot the intensity of contrast signal within the myocardium against time, various methods of estimation are used (Figure 3):

i. initial area assessment - using area under the myocardial signal intensity curve, up until the peak of arterial input (signal from the blood pool). This method of assessment has been shown to correlate with microsphere measurement of MBF(30).
ii. upslope measurement – uses the maximal slope of the ascending signal intensity curve, and uses the ratio of this parameter in stress to rest to produce a relative perfusion index which has been shown to provide high diagnostic accuracy for the detection of significant coronary stenosis and to correspond with findings in PET and invasive angiography(31,32).
iii. contrast enhancement ratio - calculated as the increase in signal intensity of a region of interest/baseline signal intensity.

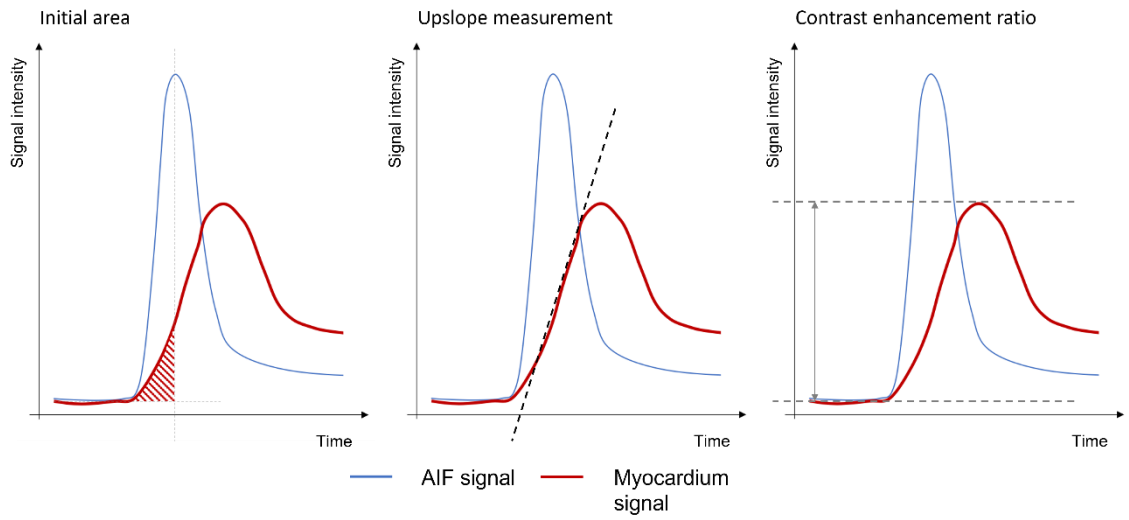


Figure 1-3 Semi-quantitative assessment in CMR

Initial area measures the area under the curve of myocardial signal, up to the peak of AIF (arterial input function) signal. AIF signal is taken from the blood pool. Upslope measurement utilises the gradient of myocardial signal intensity increase. Contrast enhancement uses a ratio of increase in myocardial signal intensity compared to baseline.

While calculation of these estimates may be automated, it is usual to require manual delineation of the myocardium or area of interest in each acquired phase. Semi-quantitative perfusion measurements show a linear relationship to absolute myocardial blood flow at lower rates, but as blood flow increases (in hyperaemia) this relationship is lost and there is a tendency to underestimate flow(33), potentially limiting their diagnostic ability and encouraging the use of fully quantitative CMR.

1.1.3.3 Fully quantitative perfusion

Absolute quantification of myocardial blood flow has been considered in CMR for some time, with the goals of providing a more objective assessment of perfusion and providing the ability to detect disease with global reduction in flow.

In order to achieve absolute quantification, this technique requires measurement of myocardial enhancement and the arterial input function (AIF). This information is then converted into Gd concentration and a model of contrast behaviour in myocardial tissue is required to go on to derive absolute myocardial blood flow.

The AIF models the arterial transit and delivery of contrast to the myocardium. This can be derived from signal intensity curves measured from the blood pool (usually left ventricular cavity).

Accurate signal intensity curves must be obtained for myocardium and AIF. Due to the high concentration of contrast in blood pool during first pass, resulting in T1, T2 and T2* effects not present when more diluted, the two curves cannot be obtained from a single measurement.

Contrast enhancement in the blood pool initially has a linear relationship to contrast concentration, but this is lost at higher concentrations as signal saturation occurs. (34). This can lead to underestimation of arterial contrast enhancement which would in turn lead to overestimation of MBF. In order to avoid this, a variety of methods have been developed to produce accurate measurements such as

i. dual bolus technique – a lower, dose of contrast is administered first to obtain the AIF curve, before a higher dose is given to obtain myocardial measurements. The 2 doses are in proportion to each other, and AIF results can then be scaled to use with myocardial results. The involvement of 2 separate boluses for both stress and rest perfusion means that this technique adds time and complexity to the CMR study.

ii. use of lower concentration contrast dosages – this avoids saturation of the blood pool signal but results in reduced contrast to noise in the myocardium.

iii. retrospective correction for signal saturation - using calibration curves to all conversion of signal intensity into a linear, Gd concentration

iv. dual sequence - providing full coverage of the myocardium, with one slice repeated and optimised for high Gd concentrations in the blood pool(35)

Signal intensity curves for the myocardium are influenced by the dynamics of contrast transit within the myocardium, and by AIF. The combination of tissue and AIF curves are deconvoluted to produce a residual function, reflecting only the transit through myocardium, and absolute value of MBF are extracted from this. Again, a variety of models have been developed for this step, based on

i. distributed parameters – using longitudinal variation in contrast concentration though the intravascular space and/or the extracellular, extravascular space(36)

ii. compartments - similar to distributed parameters but using the assumption that contrast agent concentration varies only with time and not axially.

iii. indicator dilution theory – such as the Fermi model where deconvoluted signal intensity is fitted to the Fermi function (modelling the probability that a contrast molecule has left the myocardium) and amplitude represents myocardial blood flow(37).

From early studies validating these techniques against microspheres in animal studies(38,39), they have gone on to be validated against coronary angiography and invasive FFR showing correlation between MBF and MPR with intracoronary doppler, visual assessment of stenosis at angiography and FFR, and a high sensitivity in detection of significant flow limiting disease (37,40,41). Studies validated against PET showed good correlation in values between PET and CMR, but weaker correlations in absolute values, suggesting absolute ranges or cut-offs from one modality may not apply to the other(42,43).

In some studies fully quantitative perfusion has been shown to be superior to semiquantitative and qualitative methods of diagnosis(44), however other studies, particularly using high resolution imaging with 3T scanners, or experienced operators have not observed a significant difference(45–47).

Although these techniques were shown to provide benefit in diagnosis in some studies, others were less positive showing no significant different in diagnosis of significant coronary disease. In addition, the manual segmentation and processing of the data adds greatly to analysis time, taking up to 1 hour per patient in some studies(45).

1.1.3.4 Automated quantitative perfusion

In order to make quantitative perfusion CMR viable for clinical usage, the time taken in processing and analysis needs to be reduced. Automating this process has been the focus of ongoing research and recently sequences have been produced that provide inline quantitative perfusion analysis, so that this data can be analysed at the same time as the clinical study(48,49).

This fully automated, inline approach was first developed by Kellman et al, using sequences and processing within the Gadgetron framework(50) to allow production of pixelwise perfusion maps within minutes of perfusion acquisition. This method uses a dual sequence approach, AIF is calculated from the LV blood pool using low resolution images, optimised for high gadolinium concentration. Higher spatial resolution images are acquired of the myocardium and used to estimate myocardial perfusion. The sequence used to obtain AIF is a low-resolution FLASH protocol with two echoes, resulting in short echo times to minimise T2* losses at high concentration and allow remaining T2* losses to be estimated and corrected.

Both AIF and myocardial imaging sequences include 3 measurements of proton density (PD) weighted images. Images are motion corrected and then corrected for surface coil intensity variation based on the proton density weighted images.

AIF data are extracted from the low-resolution Gd concentration images using automated segmentation of the LV cavity. Signal intensity data are converted to Gd concentration (mmol/L) based on automatically generated look-up tables for the magnetization Bloch simulation.

MBF is calculated on a pixel-wise basis in the high-resolution images by blood tissue exchange (BTEX) model(51) constrained deconvolution incorporating estimation of the delay time between bolus arrival in the LV cavity and the tissue of interest.

The output includes source signal intensity first-pass images with and without motion correction, and pixelwise MBF maps (Figure 4). Additional output to aid quality control include RR intervals throughout acquisition, blood pool segmentation and AIF curves.

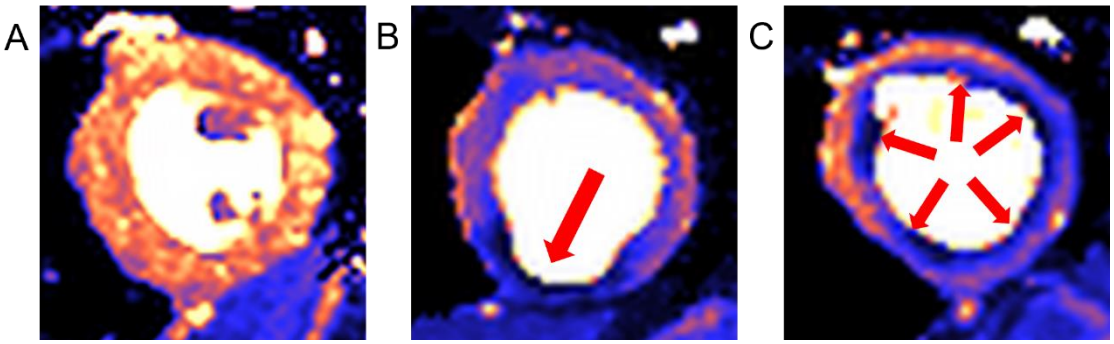


Figure 1-4 Stress perfusion maps

Pixelwise stress perfusion maps produced inline during clinical CMR scanning. A – normal myocardial perfusion with high, uniform MBF throughout the myocardium. B – Previous myocardial infarction shows in an area of low MBF (arrowed) and uniform perfusion throughout the rest of the myocardium. C – Multivessel coronary artery disease – areas of low MBF are seen throughout the myocardium (arrowed) with an epi-endocardial gradient.

This method has been validated against PET showing strong correlation both in global and regional absolute values of MBF at stress and rest, and a similarly strong correlation in MPR(52). It provides the ability to perform rapid, quantitative assessment of myocardial blood flow during a scan, so that sequences can be repeated or adjusted as required ensuring a comprehensive dataset is obtained.

1.2 Myocardial perfusion in the absence of epicardial coronary disease

Quantitative perfusion may also be useful outside epicardial coronary disease. Studies have shown decreased myocardial perfusion associated with impaired LV systolic function, and in one study of patients with idiopathic LV impairment, quantitative PET analysis demonstrated severely reduced stress MBF (≤1.36ml/g/min) to be a predictor of poor prognosis, independent of the degree of dysfunction(53).

Microvascular dysfunction is known to be a feature of cardiomyopathies such as HCM, Fabry's and amyloidosis. In HCM patients have been shown to have impaired stress perfusion associated with increased wall thickness and fibrosis, although also present in non-hypertrophied segments(54). In one study this has been shown to be an independent predictor of cardiovascular events and death(55).

PET studies have shown the presence of microvascular dysfunction in Fabry's disease, independent of the degree of hypertrophy or sex of the patient, and may be the first manifestation of the disease(56). Studies have used this dysfunction as a measurement of response to treatment(57) and it has been postulated that this dysfunction may play a part in the progression of fibrosis in this condition(58).

Microvascular dysfunction has been shown to be prevalent in amyloidosis, with lower levels of both stress and rest perfusion compared to patients with hypertensive LVH(59).

In a PET study of 58 patients with typical angina, but normal coronary arteries, a large proportion (72%) had reduced CFR with abnormal resting and stress MBF suggesting microvascular dysfunction and a potential treatment target in these patients(60).

1.3 Conclusions

Across modalities the benefits in quantitative perfusion have been demonstrated. Within cardiac imaging, CMR has the benefits of lack of ionising radiation, and ability to provide comprehensive data including LV volumes and function, and tissue characteristics alongside perfusion assessment. Quantitative perfusion has applications outside epicardial coronary disease, having been identified as abnormal across a spectrum of myocardial disease, and may be a prognostic marker in these conditions.

1.4 Cardiac Magnetic Resonance and Diabetic Heart Disease

1.4.1 Prevalence and burden of diabetes

The prevalence of type 2 diabetes mellitus and its global health burden has increased over the last 3 decades and is projected to continue to rise. It is currently estimated that there are over 450 million people with a diagnosis of

diabetes worldwide, projected to rise to near 693 million by 2045 (61–63). Nearly 10% of global health expenditure is spent on diabetes(64).

Diabetes is associated with significant increases in all-cause mortality, and in cardiovascular mortality(65,66). Diabetes greatly increases the risk of cardiovascular disease including coronary artery disease and heart failure and is one of the leading causes of death and of years lost due to disability. In those with type 2 diabetes, nearly 51% of all-cause mortality is contributed by cardiovascular disease(67). Cardiovascular complications are associated with the majority of diabetes related morbidity and mortality, emphasising the importance of early diagnosis, intervention and understanding of the pathology in order to guide treatment.

The category of 'pre-diabetes', defined by HbA1c 42-47mmol/mol(68), comprises patients who have evidence of dysglycaemia and are at risk of developing diabetes. This group may benefit from early assessment and targeted treatment to prevent progression to diabetes and associated complications.

1.4.2 CMR in diabetic heart disease

Cardiovascular magnetic resonance is increasingly used as a research tool to investigate subclinical changes in diabetes and can provide a wide range of information about the diabetic cardiovascular phenotype. In a single examination, it is possible to make accurate and reproducible assessment of structure and function, the presence of ischaemic scar using late gadolinium enhancement (LGE), and diffuse fibrosis using T1 mapping. In addition, the presence of ischaemia can be assessed with both quantitative and qualitative methodology.

While other imaging modalities can be used, CMR has multiple advantages over PET and echocardiography in the assessment of diabetic heart disease. The associated tendency to obesity in type 2 diabetes means imaging windows are often poor, and increased doses of ionising radiation are required to achieve adequate information in PET. CMR offers the advantages of better spatial resolution, lack of ionising radiation, and multiparametric assessment within a single test.

1.4.3 Ischaemic Diabetic Heart Disease

Coronary artery disease and its complications remain the most common cause of mortality and morbidity in patients with diabetes(69). Myocardial ischaemia and

obstructive coronary artery disease carry significantly increased rates of myocardial infarction (MI) and death in patients with diabetes(70). Early studies classed diabetes as an equivalent to coronary artery disease, with patients with a diagnosis of diabetes having as high a risk of myocardial infarction as patients without diabetes, but with known previous infarction(71).

While there is an increased risk of CAD, subsequent studies have suggested that this is an overestimation, with meta-analysis demonstrating a 43% lower risk of cardiac events in patients with diabetes without myocardial infarction compared to patients without diabetes, but previous MI(72). Registry data has shown that patients with diabetes are more likely to be on preventative medications including statins and aspirin, but then have the equivalent risk of cardiovascular events to those without diabetes following normal coronaries on angiography(73), potentially demonstrating the importance of preventative medication in this group and the importance of early identification of ischaemia or silent coronary disease.

Current techniques to assess risk in patients with suspected ischaemic heart disease involve assessment of global function (left ventricular ejection fraction - LVEF) and of the degree and extent of myocardial ischaemia and scar.

1.4.3.1 Diagnosis of ischaemia

The presence of ischaemia has been shown to be prognostic in diabetes, whether symptomatic or asymptomatic.

In a study including 328 patients with diabetes and suspected ischaemic heart disease, undergoing dobutamine stress CMR, 27% were found to have dobutamine induced wall motion abnormalities. The presences of these abnormalities was a predictor of cardiac death (Hazard ratio (HR) 8.6, CI 3.5-21)(74).

In another study of 1737 patients with diabetes, 44% of those with angina were shown to have ischaemia, with annual critical event rates of 2% in those with normal scans, 5.2% in those with ischaemia and 7.6% in those with both ischaemia and scar(75). Annual critical event rates for the same scan results were 1.6%, 2.9% and 4.0% in asymptomatic patients, demonstrating the effect of even asymptomatic ischaemia. In further analysis, extent of ischaemia was shown to be an independent predictor of events.

Results from the Swedish Heart Failure registry demonstrated a decreased mortality risk in patients with diabetes who had CAD and had received revascularisation (HR 0.82, CI 0.75-0.91)(76) underlining the importance of diagnosis in order to direct management.

Diagnostic sensitivity of stress perfusion CMR has been validated against coronary angiography for assessment of significant coronary disease(23,77,78) and allows the assessment of perfusion defects together with function and scar together with perfusion.

1.4.3.2 Detection of unrecognised MI

Although the majority of patients with diabetes and CAD present with angina, 20-30% may have silent ischaemia, occurring more commonly in those with diabetes than those without and associated with a worse prognosis(79,80).

CMR is able to detect unrecognised MI in patients with diabetes, due to the presence of late gadolinium enhancement in the characteristic pattern of infarction. In a study of 107 patients with diabetes, with no clinical history of MI, 28% were found to have LGE in keeping with a previous MI(81). Several other cardiovascular imaging studies have shown that type 2 diabetes is associated with increased rates of CAD(82), silent myocardial ischaemia(83) and silent MI (84,85). In a study of 350 patients with impaired fasting glucose or diabetes, the prevalence of prior MI was 15.9% in patients with type 2 diabetes undergoing clinical CMR scans(86) and 8.3% in patients with impaired fasting glucose, suggesting the risk of unrecognised MI occurs in prediabetes as well as those with confirmed diabetes.

Patients with prior MI have increased hazard for a major adverse cardiovascular event (HR 3.71, P<0.001) and all-cause mortality (HR 3.61, P=0.007)(81). In a study including 266 patients with diabetes, clinically unrecognised MI detected by LGE was associated with similar long-term risk or mortality when compared to patients with clinical MI (28% vs 33%, P=0.40)(87).

The mechanism for increased rates of unrecognised myocardial infarction in type 2 diabetes is thought to relate to neuropathy and decreased awareness of cardiac chest pain in this population although the increased incidence in the pre-diabetic population, where neuropathy would not be expected to be present, points to a more complex explanation.

The detection of both ischaemia and previous myocardial infarction allows the targeting of medical therapies and coronary intervention to attempt to reduce the associated morbidity and mortality. The benefits of quantitative perfusion and the ability to detect global decreases in myocardial perfusion are particularly important in this population, where three-vessel disease is more commonly found, and the presence of 'balanced ischaemia' can make non-invasive diagnosis of ischaemia more challenging.

1.4.4 Diabetic cardiomyopathy

The presence of heart failure in diabetes has been reported since early studies(88). Type 2 diabetes mellitus has been shown to be a risk factor for the development of heart failure, independent from conventional risk factors such as age, sex, existing coronary artery disease and hypertension(89–91).

Type 2 diabetes is an independent predictor of cardiovascular morbidity and mortality in heart failure in both impaired and preserved ejection fraction. Outcomes in the CHARM study showed higher relative risk of cardiovascular death or heart failure hospitalisation in both groups (HR 1.60, CI 1.44-1.77 in impaired EF and HR 2.0, CI 1.70-2.36 in preserved EF)(92). Heart failure (HF) with reduced ejection fraction is a predictor of poor outcomes in type 2 diabetes(93–95).

In a UK population study of nearly 2 million patients without known cardiovascular disease, heart failure was shown to be one of the earliest manifestations of cardiovascular disease in those with diabetes. Over a median period of follow up of 5.5 years, presentation with heart failure was more common than myocardial infarction, angina or death due to CAD.(96)

Another study of recently diagnosed patients with diabetes demonstrated an increased incidence of HF diagnosis with increasing severity of dysglycaemia. An incidence of 2.3/1000 person years was seen in those with HbA1c <6%(42.1mmol/mol), increasing to 11.9/1000 person years in those with HbA1c≥10% (85.8mmol/mol) (97). Similar results have been seen in other studies (98), suggesting HbA1c as an independent risk factor for heart failure.

In patients without diabetes, those with raised HbA1c (pre-diabetes) have also shown an increased incidence of heart failure(99,100) however in randomised controlled trials intensive control of glucose has been shown not to reduce the risk of hospitalisation from heart failure(101). More recently, newer hypoglycaemic

agents appear to reduce the risk of heart failure admissions in patients with diabetes, independently of glucose lowering properties(102–104) although the mechanism for this is unknown.

The precise mechanisms behind diabetic cardiomyopathy are unclear. Early changes are thought to be a product of non-ischaemic organ and tissue level remodelling(90) involving altered substrate metabolism, impaired calcium handling, increased reactive oxygen species and microvascular advanced glycation end product deposition(105).

These changes lead to extracellular cardiac fibrosis, which ultimately leads to myocyte dysfunction and adverse clinical outcomes(106–108). Patients with diabetes have shown a change in myocardial structure and function to produce diabetic cardiomyopathy, originating in the myocardial tissue with expansion of extracellular matrix, myocardial steatosis and hypertrophy(109–111). These mechanisms present parameters than can be assessed using CMR (Figure 5).

1.4.4.1 Detection of fibrosis and scar

CMR studies have shown the presence of abnormal diabetic pathophysiology occurring in the absence of ischaemic heart disease. Multiparametric cardiovascular imaging has been used to detect subclinical changes in cardiac structure and function in type 2 diabetes mellitus including increased left ventricular mass, concentric remodelling of the left ventricle (112–114) and diastolic dysfunction(115,116). In a study of over 4000 asymptomatic participants without known cardiovascular disease, patients with diabetes were found to have increased LV mass, decreased stroke volume and LVEF(113).

T1 mapping can be used to detect focal and diffuse fibrosis that may not be easily detected on LGE imaging. Changes in extracellular volume (ECV) affect T1 relaxation time of the myocardium and in both interstitial oedema and fibrosis, ECV is increased, leading to an increase in native T1 enabling detection of asymptomatic, potentially minor, cardiac remodelling.

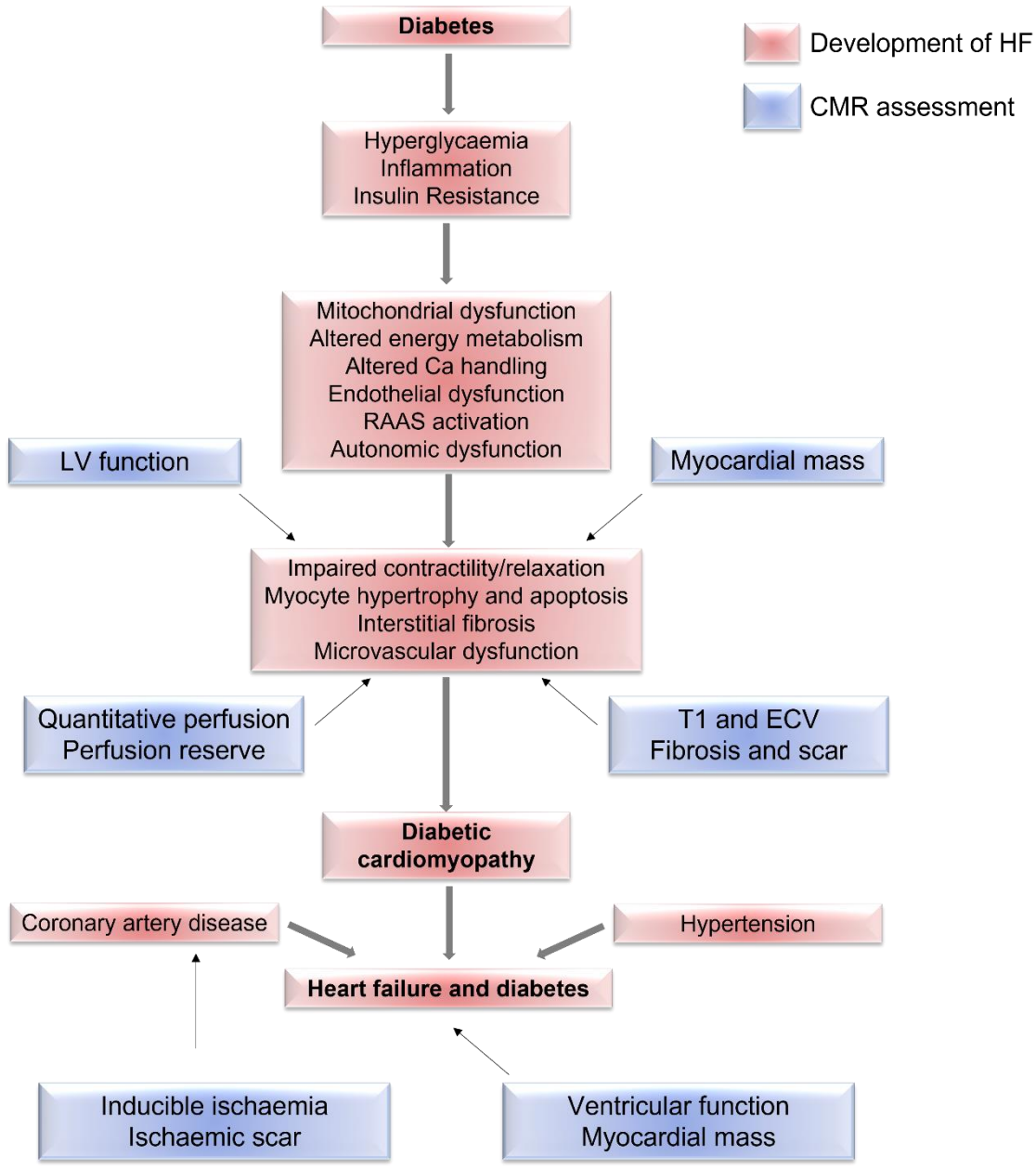


Figure 1-5 CMR assessment targets in diabetes and heart failure

CMR is able to accurately assess the phenotype in heart failure, but also has the ability to assess early, subclinical changes including microvascular impairment and interstitial fibrosis.

CMR T1 mapping studies have suggested that type 2 diabetes mellitus is associated with diffuse fibrosis and increased cardiac ECV (106,117–119). This increased ECV is associated with long term prognosis(120). T1 mapping in diabetes allows the detection of some of the earliest changes in diabetic heart disease. In a study of 50 patients with diabetes and normal EF, they were found to have reduced T1 and therefore increased EVC compared to healthy volunteers. Shortening of T1 was also found to be associated with reduction in global longitudinal strain(117). In a further study of patients with diabetes and no known CAD, decrease in T1 was shown to be associated with diastolic function, impaired exercise capacity and increased insulin resistance(118).

ECV, and the presence of diffuse fibrosis, has been suggested as a potential maker in disease. In order to use it as a marker, it would need to be independently associated with adverse risk, ideally with the risk being modifiable. This has not yet been shown in diabetic cardiomyopathy. In a study of 81 patients with type 2 diabetes and evidence of diastolic dysfunction on echocardiography randomised to either spironolactone 25mg od or placebo, spironolactone was associated with an improvement in diastolic function but there was no change in post contrast T1 time(121).

ECV has been shown to correlate with HbA1c (122) although no relationship was seen with either duration of the disease or diabetic complications(123).

1.4.4.2 Myocardial perfusion

Clinical use of CMR to look for the presence of ischaemia in patients with diabetes has largely relied on qualitative assessment of visual perfusion defects seen in adenosine stress perfusion and regional wall motion abnormalities in dobutamine stress perfusion.

Quantitative perfusion offers further benefits in the area of diabetes and cardiomyopathy; in addition to diagnosis of obstructive coronary artery disease, MPR has previously been found to be lower in patients with diabetes. With the ability to assess this accurately, it offers another insight into the pathophysiology of diabetic heart disease and potential for risk stratification potential therapeutic intervention.

Reduced MPR has been shown in other non-ischaemic heart disease, such as severe aortic stenosis and dilated cardiomyopathy (DCM)(124,125), representing

microvascular dysfunction. It has also been shown to associated with worsened contractility in idiopathic cardiomyopathy(126).

Impaired microvascular function has been demonstrated in diabetes and is likely to be multifactorial due to factors including interstitial fibrosis, myocardial hypertrophy and decreased capillary density (127–129).

In patients without obstructive coronary disease, microvascular dysfunction (reduced CFR) is known to be associated with increased of cardiac events(130). Therefore identifying the presence of dysfunction may be useful in stratification for risk and therapy.(131).

Limited quantitative perfusion assessment with CMR exists for diabetic cardiomyopathy. Impaired myocardial perfusion reserve appears to correlate with extracardiac complications in diabetes with lower values in those with uncomplicated diabetes compared to controls, and further decreased values in those with diabetes and complications such as retinopathy or albuminuria (132).

Semiquantitative CMR perfusion studies have found reduced time to maximal intensity and reduced maximal signal intensity in those with a longer diagnosis of diabetes compared to normal controls or those recently diagnosed(133), and have shown to be associated with an increased risk of microvascular dysfunction(134). A recent small study of 32 asymptomatic patients with diabetes showed reduced coronary flow reserve, but no association with either impaired systolic function or poor glycaemic control(135).

1.5 Conclusions

CMR has shown benefits in diabetes in the assessment of ischaemia, scar and fibrosis. With the addition of quantitative perfusion there may be further improvements in the knowledge of mechanisms in diabetic cardiomyopathy, diagnosis of microvascular disease and management or targeting of therapies.

1.6 Thesis Aims

With the potential increased availability of quantitative myocardial perfusion CMR, and inline processing giving the ability to include this technique in clinical scanning, the prospect of accurately quantified myocardial perfusion in day-to-day practice comes closer. This could enable improvements both in assessment of ischaemia and complex epicardial coronary disease, and evaluation of diffuse, microvascular

abnormalities that have to date been difficult to assess using CMR. These more subtle changes are often seen in non-ischaemic heart disease and may offer insights into the aetiology and progression of disease as well as the potential for treatment targets or research endpoints.

In order to allow confident clinical use of this technique in patients, it must first be validated and established in a normal population. This thesis aims to provide early information on inline quantitative myocardial perfusion in a healthy population and examine the broad spectrum of impaired myocardial function and diabetic heart disease.

The subsequent chapters each have a specific aim with individual introduction, methods, results and discussion:

Study 1: To establish repeatability of CMR quantitative perfusion in assessment of stress and rest myocardial blood flow in healthy volunteers.

Study 2: To establish normal ranges for quantitative perfusion parameters in the healthy population and investigate factors affecting these values including age and sex.

Study 3: Using quantitative perfusion, assess the ability of adenosine stress CMR to achieve hyperaemia, examining patient groups that have been proposed to have a decreased response to adenosine, such as heart failure.

Study 4: To investigate tissue characteristics and perfusion in heart failure when categorised using LV ejection fraction.

Study 5: To investigate heart failure in diabetes, assessing differences in heart failure in patients with dysglycaemia and normoglycaemia, and prognostic factors within these groups.

Chapter 2
Methods

Methods common to all the following results chapters are detailed in this section. Individual aims, methods and analysis are included in each results chapter.

2.1 Study populations

Details of study specific patient populations are described in individual chapters. Exclusion criteria common to all studies included:

- Contraindication to CMR (e.g.non-CMR conditional permanent pacemaker or defibrillator, intra-orbital metal, intracranial clips, claustrophobia etc.)
- Pregnant or breastfeeding patients
- Obesity where girth exceeds scanner diameter
- Known adverse reaction to gadolinium-based contrast agents
- Glomerular filtration rate < 30mL/min/1.73m2
- Inability to lie flat for the duration of the CMR scan
- Inability to give written, informed consent

2.1.1 Patient population for chapters 3, 4 and 5

Normal healthy volunteers with no background of cardiovascular disease or known cardiovascular risk factors of hypertension, hypercholesterolaemia, smoking or diabetes were recruited from two tertiary cardiology centres.

2.1.2 Patient population for chapters 5, 6 and 7

Patients who had been seen in cardiology outpatients with a new, clinical diagnosis of heart failure and referred for cardiac MRI were prospectively recruited. All patients were recruited from a single, tertiary cardiology centre. Inclusion criteria included ≥18 years, no known coronary artery disease or cardiomyopathy such as HCM, amyloidosis, sarcoidosis.

2.1.3 Patient population for chapter 5

In addition to the healthy volunteers and patients with heart failure described above, patients with known or suspected coronary artery disease, referred for coronary angiography were prospectively recruited.

2.2 Ethics and approvals

All scans were performed in accordance with the Declaration of Helsinki and approved by the National Research Ethics Service: 12/YH/0551 (Chapters 3, 4 and 5), 18/YH/0125, 14/EM/0056 and 17/WM/0192 (Chapter 4), 18/YH/0168 (Chapters 4 and 5) and 17/YH/0300 (Chapters 6 and 7). All patients providing informed written consent.

2.3 MRI protocols

All scans for Chapters 3, 5, 6 and 7 were performed on a 3T Siemens Prisma scanner (Siemens Healthcare, Erlangen, Germany). The components of the basic CMR protocol performed in results chapters 3-7 are outlined in the sections below. Any additions to this basic protocol specific to the individual results chapters are detailed within each relevant chapter.

Each study was typically performed within 60 minutes in the order illustrated in Figure 1.

At the start of any CMR protocol, free breathing low-resolution survey scans of the chest were done to mark anatomical landmarks. For each pulse sequence, images with artefact were removed or minimised. The highest quality images were used for analysis.

2.3.1 Cine imaging

Long axis cines: 4 chamber (4Ch), 2 chamber (2Ch) and 3 chamber (3Ch) single slice images were acquired using Trufi (true fast imaging with steady state free precession) sequence obtained breath-held at end expiration. Typical parameters were: echo time (TE) 1.4ms, repetition time (TR) 37ms, flip angle 43°, field of view 320 to 420mm depending on patient size, slice thickness 8mm, 25 phases

Short axis cines: (for right and left ventricular volumes and function) were assessed with a contiguous stack of multiphase ventricular short axis Trufi cines. 10-15 slices were acquired to ensure full ventricular coverage, over 25 phases, 8mm slice thickness, 2mm gap, with pulse sequence parameters the same as long axis cines.

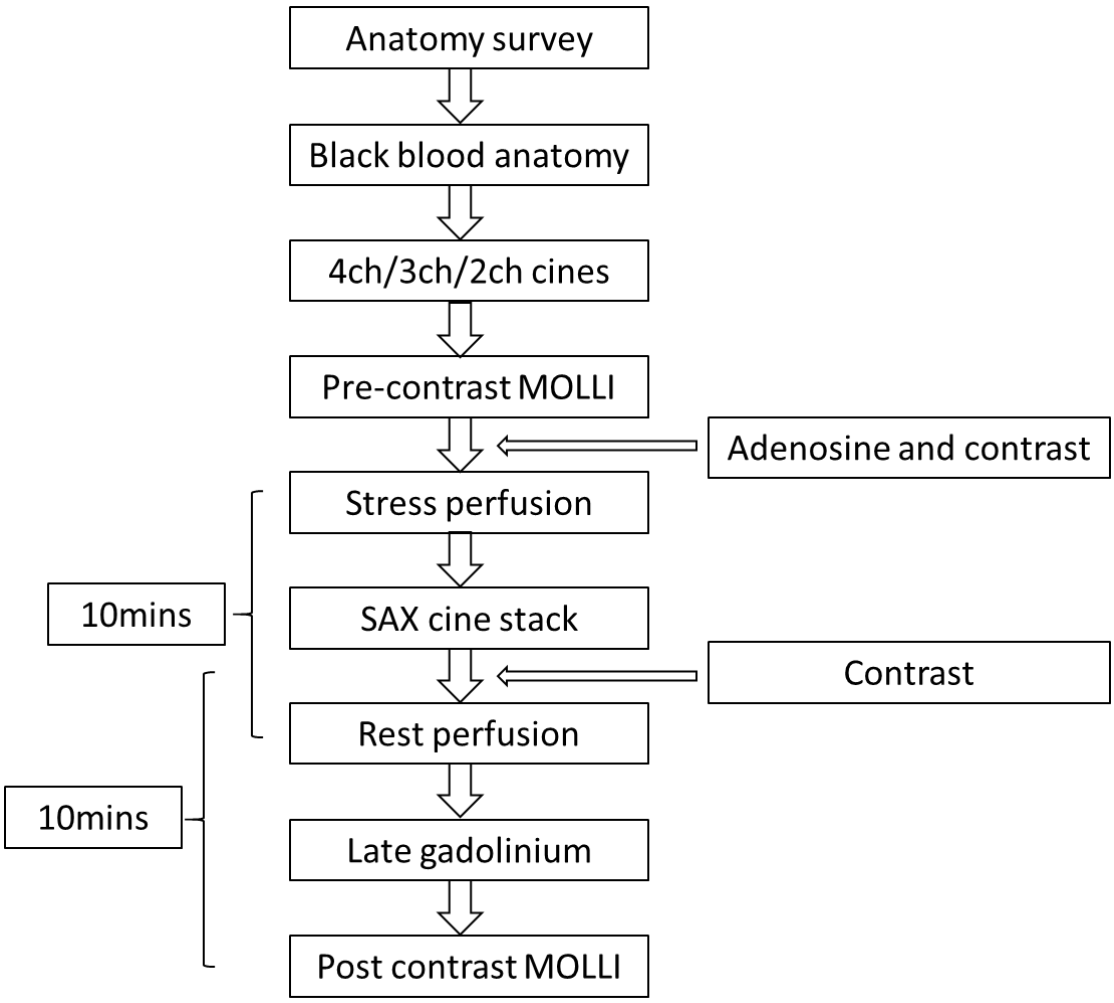


Figure 2-1 Standard Stress CMR protocol

Basic protocol for standard adenosine stress CMR scan. Additional sequences were included for some studies. All scans took ≤sixty minutes.

2.3.2 T1 mapping

T1 mapping used a breath held MOdified Look-Locker Inversion recovery (MOLLI) acquisition.

Three short axis slices acquired at the apex, mid-level and base of the left ventricle using the '3 of 5 technique'(136) were used for pre and post contrast T1 mapping using an electrocardiogram-triggered MOLLI scheme. The pre-contrast scheme used was a 5s(3s)3s acquisition. Scan parameters were: voxel size 1.5 x 1.5 x 8, flip angle 20° and field of view 320-420mm depending on patient size. Post contrast T1 maps were performed using the same slices 15 minutes after contrast administration.

2.3.3 Perfusion

Perfusion imaging used a dual sequence approach developed by Kellman et al(48) employing separately optimized sequences for the myocardium and blood pool signals in order to avoid blood pool signal saturation. Full details of the myocardial perfusion sequence have been previously described(48). Both sequences were ECG triggered saturation recovery prepared. The sequence used for imaging the LV blood pool to estimate the arterial input function (AIF) used a low flip angle FLASH low resolution protocol with 2 echoes such that the echo times were short to minimize T2* losses at high concentration, and so that remaining T2* losses could be estimated and corrected. Parameters for this protocol were: flip angle 5 degrees, matrix 48x34, parallel imaging factor 2, TEs 0.76 and 1.76ms, TR 2.45ms, slice thickness 10 mm, saturation preparation used 6-pulse sequence, saturation delay TS 24ms to k-space centre, imaging duration 42ms, total sequence duration 57ms acquired immediately following the R-wave trigger.

The myocardial imaging protocol in this study used a FLASH readout with typical imaging parameters: flip angle 14 degrees, spatial resolution 1.9x2.4 mm2, slice thickness 8.0 mm, TE/TR 1.0/2.1ms, matrix size 192x111, field of view 360x270 mm^2, parallel imaging acceleration factor 3, saturation recovery time (TS) 110ms to centre of k-space, trigger delay 72ms, imaging duration 59ms, saturation preparation use 5-pulse sequence, total duration including saturation 143ms per slice, enabling acquisition of 3-slices plus AIF sequence in less than 500ms permitting heart rates up to 120 bpm. Both AIF and myocardial imaging sequences included 3 measurements of proton density (PD) weighted images with flip angle of 5 degrees used for surface coil intensity correction.

In-line automatic reconstruction and post-processing were implemented within the Gadgetron software framework(48). Images were motion corrected and corrected for surface coil intensity variation based on the proton density weighted images. Signal intensity data were converted to Gd concentration (mmol/L) based on automatically generated look-up tables for the magnetization Bloch simulation. AIF data were extracted from the low-resolution Gd concentration images using automated segmentation of the LV cavity. MBF was calculated on a pixel-wise basis in the high-resolution images by blood tissue exchange (BTEX) model constrained deconvolution incorporating estimation of the delay time between bolus arrival in the left ventricle (LV) cavity and the tissue of interest. Details of the

reconstruction and processing including conversion to [Gd] concentration units, blood pool signal segmentation, and BTEX modelling are previously reported(48).

2.3.4 Late gadolinium enhancement imaging

LGE-imaging was performed 10-15 minutes after the final contrast injection using a modified look locker approach to determine optimal inversion time required to adequately null the LV myocardium. A motion corrected PSIR Trufi sequence was used with typical parameters: TE 1.2ms, TR 904ms, flip angle 50°, slice thickness 8mm.

2.4 Common CMR analysis

Images were evaluated offline using commercially available software: cvi42 (Circle Cardiovascular Imaging Inc, Calgary, Canada).

2.4.1 LV and RV ejection fraction

Left ventricular endocardial and epicardial, and right ventricular endocardial borders were manually contoured from the short axis cine stack at end diastole and end systole, generated right and left end diastolic and end systolic volumes according to the summation of discs methodology. Ejection fractions were derived from the equation EF=(EDV-ESV)/EDV x 100%. Trabeculations and papillary muscles were excluded.

2.4.2 Parametric mapping

T1, post contrast T1, rest and stress perfusion maps were analysed using the same methods. Endocardial and epicardial contours were manually contoured excluding trabeculations and papillary muscles. A 10% offset was applied to minimise partial volume effect. RV insertion points were marked, and 16 segment(25) bullseye plots were generated (Figure 2).

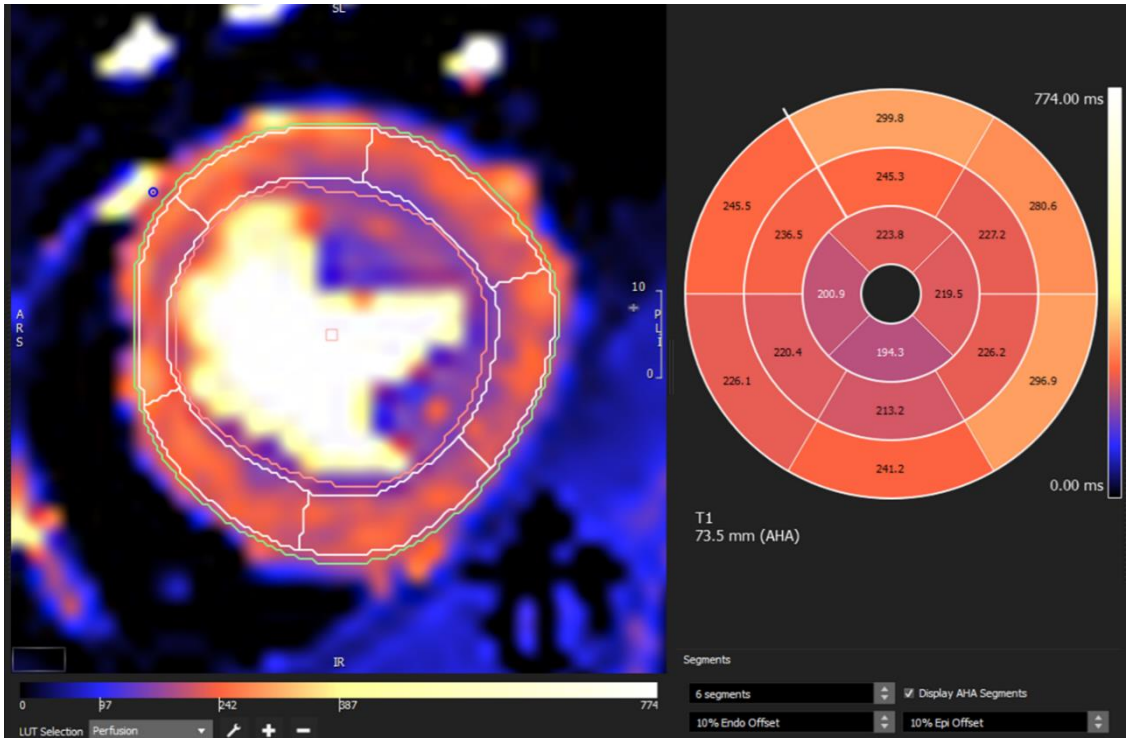


Figure 2-2 Contouring and bullseye plot in parametric mapping

Endocardial and epicardial borders were manually contoured and RV insertion points marked (left). From these images, a 16-segment bullseye plot generated (right).

Values were recorded for each of the 16 segments. Where the left ventricular outflow tract (LVOT) was included in the basal slice, or partial volume effect meant segments were too thin to contour, these segments were excluded from further analysis. The remaining segments were average to give values for the global myocardium. Analysis time was less than 5 minutes per set of maps.

Chapter 3

Fully automated, inline quantification of myocardial blood flow with cardiovascular magnetic resonance: repeatability of measurements in healthy subjects

3.1 Background

There is growing evidence that decision making regarding revascularisation in patients with coronary artery disease (CAD) should be supported by objective measurements of ischaemia rather than visual assessment(3,137,138). The current reference standard for non-invasive quantification of myocardial blood flow (MBF) and detection of ischaemia is positron emission tomography (PET). Quantitative perfusion cardiovascular magnetic resonance (CMR) offers an alternative to PET bringing the advantages of lack of ionising radiation and better spatial resolution with the added value of comprehensive assessment of left ventricular size, function and scar in a single study. Quantification of MBF by CMR has been validated in several small scale studies against microspheres, PET and invasive fractional flow reserve (FFR) measurements(33,46,139,140) but wider adoption of quantitative perfusion CMR has been limited due to the requirement for time-consuming, off-line processing and poor repeatability(141). Recently, a new myocardial perfusion CMR method with motion correction and Gadgetron-based automated in-line perfusion mapping has been proposed, allowing free breathing acquisition and pixel-wise quantification of myocardial blood flow (MBF)(48). This method has been shown to have good correlation with PET in patients with stable CAD(52), but needs further validation before it is used clinically and as a research surrogate endpoint. In this study, the repeatability of myocardial perfusion mapping by CMR was assessed in healthy volunteers.

3.2 Methods

3.2.1 Study population

Forty-two healthy volunteers were recruited (23(55%) female, median age 23yrs, IQR 22-29 yrs). Exclusion criteria included known cardiovascular disease, hypertension, hyperlipidaemia, diabetes mellitus, smoking and any contraindication to CMR, adenosine or gadolinium-based contrast agents.

3.2.2 Study protocol

The 42 volunteers underwent repeated CMR studies in two groups to allow assessment of intrastudy and interstudy repeatability of rest and stress MBF as well as myocardial perfusion reserve (MPR).

Group 1:

30 volunteers underwent CMR studies on two separate visits. Visit 1 consisted of three rest perfusion scans, visit 2 had two stress scans and one rest perfusion scan. Visit 1 allowed the assessment of short-term repeatability of rest MBF with minimal physiological variation, while the fourth rest scan acquired in visit 2 was used to evaluate long-term variability of rest MBF. The two stress scans in visit 2 assessed short-term variability of stress MBF.

Group 2:

20 volunteers (8 from the first cohort and 12 additional volunteers) had stress followed by rest perfusion scans in two separate visits to assess long-term repeatability of stress and MPR (Figure 1).

All CMR studies were carried out on a 3T scanner (Siemens Magnetom Prisma, Erlangen, Germany). A minimum of 7 days was allowed between each visit (mean 41, SD 40 days). Volunteers were advised to avoid caffeine for 24 hours before each scan.

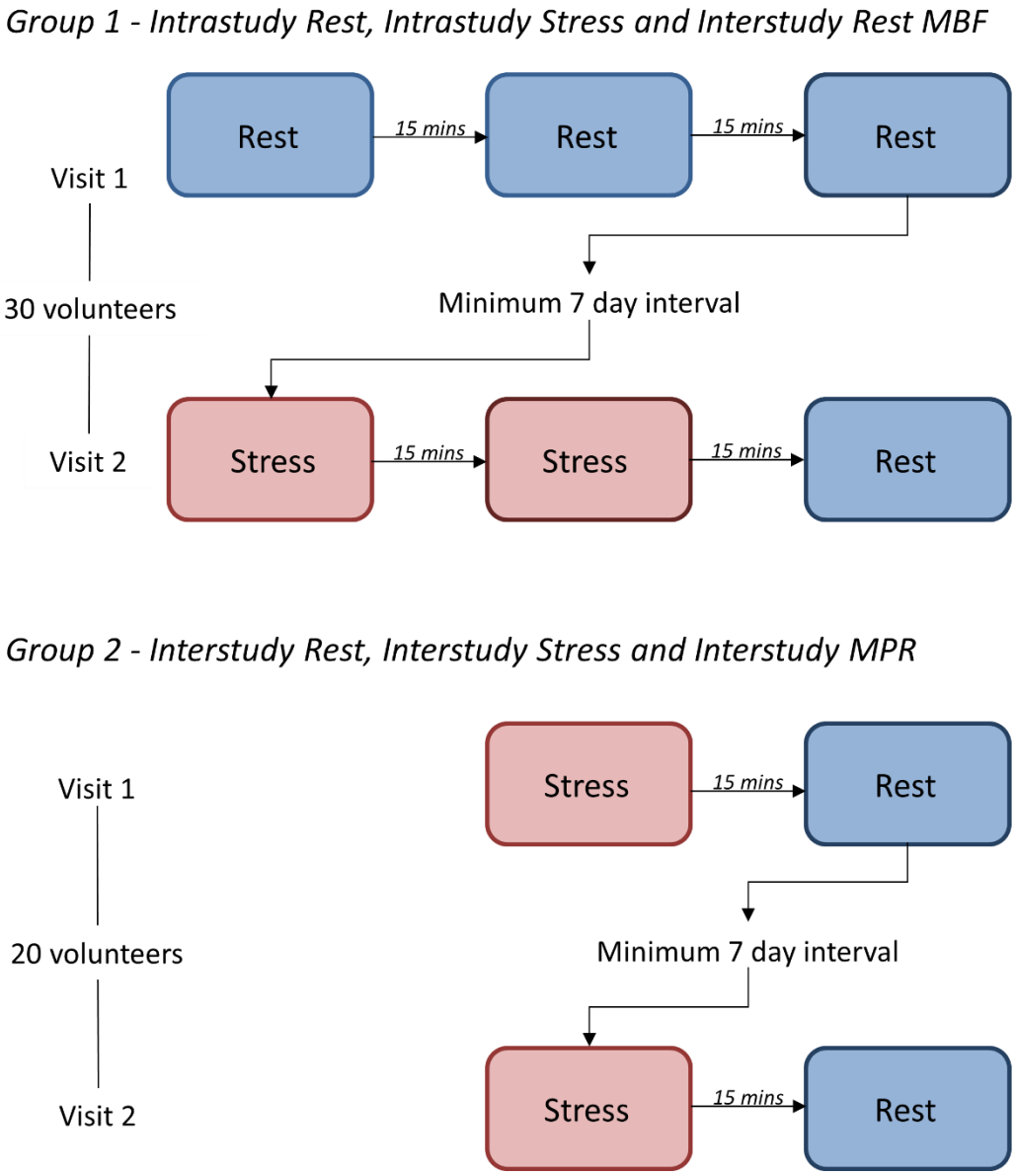


Figure 3-1 Study Protocol

Group one consisted of 30 volunteers, Group two included 8 volunteers from Group one, and an additional 12 healthy volunteers

MBF – myocardial blood flow

Pharmacological stress was achieved with adenosine infusion at 140 µg/kg/min for a minimum of 3 minutes. The dose was increased to a maximum of 210µg/kg/min if there was no haemodynamic or symptomatic response to adenosine(142). Volunteers were monitored for symptoms and heart rate (HR) throughout the

infusion, blood pressure (BP) and heart rate were recorded every 2 minutes during adenosine infusion. An intravenous bolus of 0.05mmol/kg gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5ml/s followed by a 20ml saline flush for each perfusion scan. A minimum gap of 15 minutes was maintained between each perfusion scan to allow for equilibrium of gadolinium kinetics from the previous series, and to ensure that the effects of adenosine had resolved.

Perfusion imaging used a dual sequence, free-breathing, saturation recovery pulse sequence with fast low angle shot (FLASH) readout, acquired over 60 heartbeats as previously described. In-line automatic reconstruction and post-processing were implemented within the Gadgetron software framework(48).

3.2.3 Quantitative analysis

The analysis of the quantitative maps was performed off-line on a separate workstation using cvi 42 software (Circle Cardiovascular Imaging, Calgary, Canada) as described in chapter 2. MBF was recorded for each of the 16 segments. Segmental values were averaged to give values for slice, coronary territory and global MBF. Coronary territories consisted of: left anterior descending (LAD) - segments 1, 2, 7, 8, 13 and 14, circumflex (Cx) - segments 5,6,11,12 and 16, and right coronary artery (RCA) – segments 3, 4, 9, 10 and 15.

RPP was calculated as systolic BP x heart rate. Correlation of MBF with HR and RPP were analysed, and where significant correlation was present, MBF values were corrected. Values for resting MBF were corrected for heart rate by dividing by scan heart rate and multiplying by the mean resting HR (62bpm) among all subjects. Interstudy repeatability was analysed on a regional basis for slices (basal, mid and apical) and coronary territories (LAD, Cx and RCA). MPR was calculated as a ratio of stress MBF:rest MBF.

3.2.4 Reproducibility of analysis

Intra- and inter-observer variability were assessed by repeating the analysis of 10 volunteer data sets for stress and rest after 1 month, by the same observer (LB) and by a second observer (SO). The second observer was blinded to the previous results.

3.2.5 Statistical analysis

Analysis was performed using SPSS 23 (IBM SPSS, Armonk, NY, USA). Normality of data distribution was assessed using Shapiro-Wilk test. Data are presented as mean ± standard deviation (SD). Repeatability was assessed using a wide range of methods to facilitate comparison with the inconsistent methods in the published literature. The three intrastudy rest scans were compared using repeated measures analysis of variance (ANOVA) with Bonferroni adjustment for post-hoc analysis. All other repeated mean MBF and inter- and intra- observer variability were compared using paired t tests. Coefficient of variation (CV) was calculated using the root mean square method(143). Reproducibility coefficient (RC) was calculated as 1.96*SD of difference and given as a percentage of the total mean and used to demonstrate bias and accuracy with Bland Altman plots. Reliability was assessed using intraclass correlation coefficient (ICC). All statistical tests were two-tailed and a p value <0.05 was considered significant.

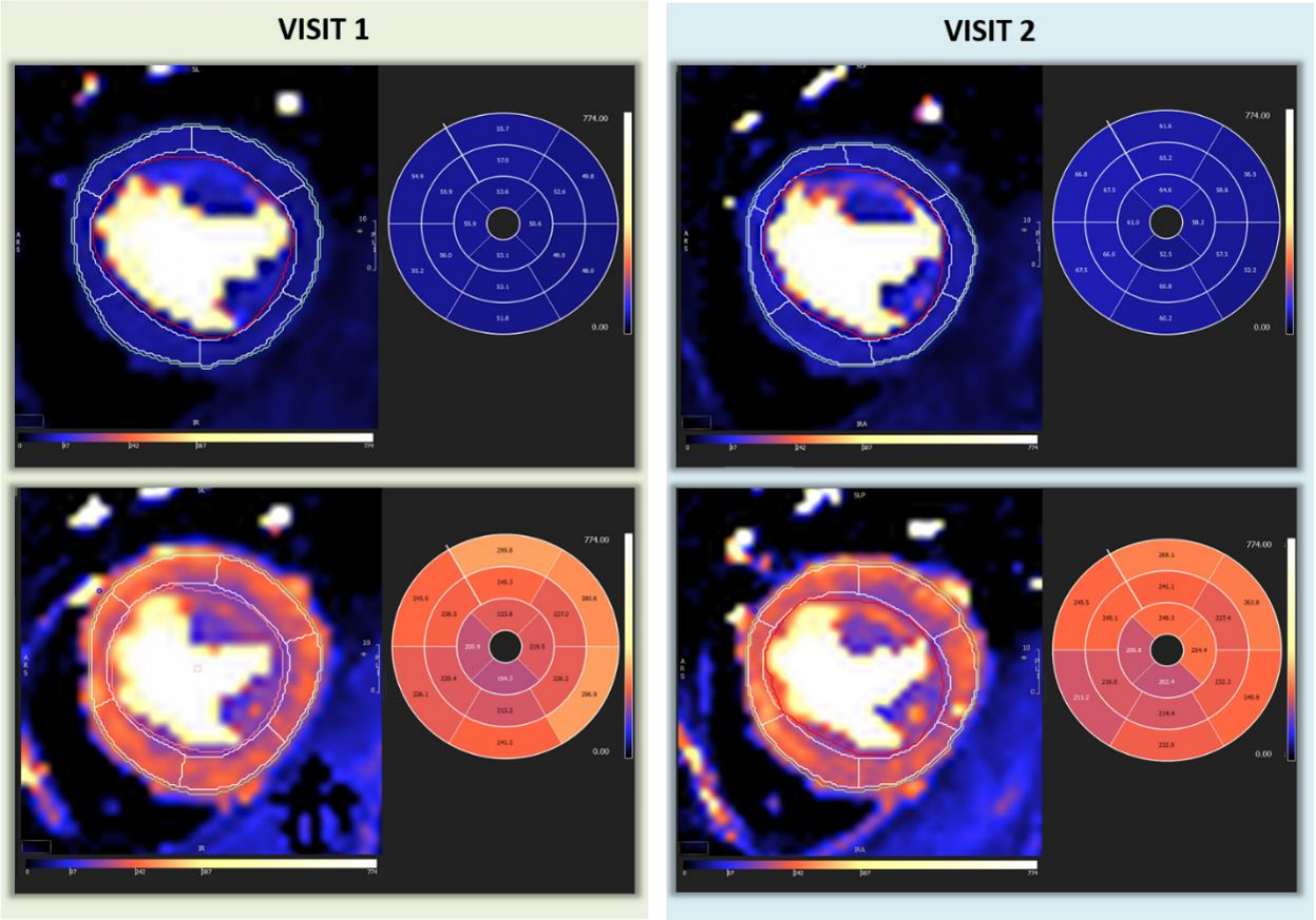


Figure 3-2 Rest and Stress MBF maps

Maps from visit 1 and visit 2 for the same volunteer. Values are displayed as ml/100g/min. MBF – myocardial blood flow

3.3 Results

All volunteers tolerated repeated CMR scans and adenosine stress well. One volunteer did not attend for the second visit for assessment of intrastudy rest repeatability, another was not included in intrastudy stress analysis due to triggering problems causing artefact on the MBF maps on one stress scan. One result was excluded from analysis of repeat stress MBF or MPR due to lack of stress response on one visit, confirmed by lack of symptoms despite increased adenosine dose, haemodynamic response and splenic switch off. One result was excluded from comparison of MPR due to severe artefact on rest perfusion maps.

3.3.1 Intrastudy repeatability

Twenty-nine studies were analysed for intrastudy repeatability of resting and hyperaemic MBF from the two separate visits of Group 1.

Mean global MBF at rest was 0.69 ±0.13 ml/g/min, 0.65 ±0.13 ml/g/min and 0.62 ±0.12ml/g/min for scans 1, 2 and 3 respectively (Table 1). There was a significant difference in mean MBF on the first rest scan compared to both the second ($p=0.01$) and third ($p=0.001$). There was no significant difference between the second and third scans (Figure 3). Coefficient of variation was 11-12% between the first scan and repeats, and 8% between second and third scans with good reliability (ICC = 0.8, RC 24%). Assessment of repeatability with Bland-Altman plots (Figure 4) showed a bias of -0.03ml/g/min (3.9% of the mean).

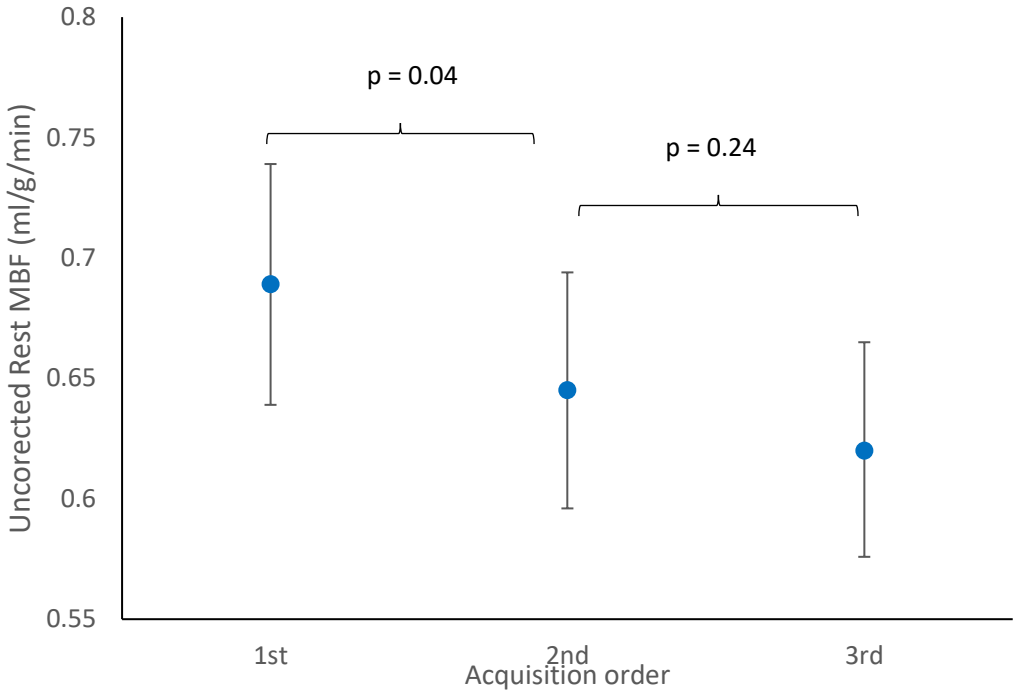
Table 3-1 – Intrastudy repeatability of global MBF measurements

	Test 1 (ml/g/min)	Test 2 (ml/g/min)	Difference in mean (ml/g/min)	p	RC	RC (%)	CV (%)	ICC
Rest				<0.01				
Rest 1 - Rest 2	0.69±0.13	0.65±0.13	-0.04±0.09	0.04	0.19	28.5	10.9	0.73
Rest 1 - Rest 3	0.69±0.13	0.62±0.12	-0.07±0.10	0.02	0.23	35.2	11.9	0.58
Rest 2 - Rest 3	0.65±0.13	0.62±0.12	-0.03±0.07	0.24	0.15	23.8	7.93	0.80
Stress								
Stress 1- Stress 2	2.89±0.56	2.83±0.64	-0.06±0.42	0.41	0.82	28.5	10.6	0.76

No significant difference was seen between 2nd and 3rd rest MBF values or stress MBF scans. Initial rest MBF was higher than subsequent rest values.

p – from repeated measures ANOVA and level of significance using Bonferroni post-hoc analysis for rest data, Student's T-test for stress values, RC - repeatability coefficient, RC (%) - repeatability coefficient as percentage of the mean, CV - within subject coefficient of variation, ICC - intraclass correlation coefficient, MBF - myocardial blood flow

A



B

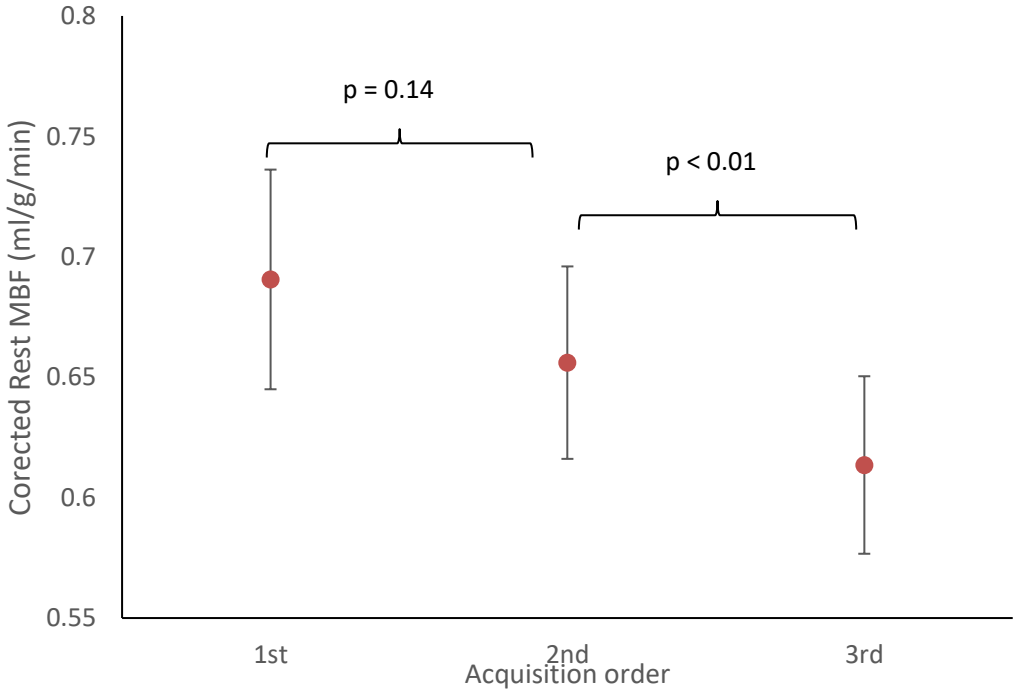
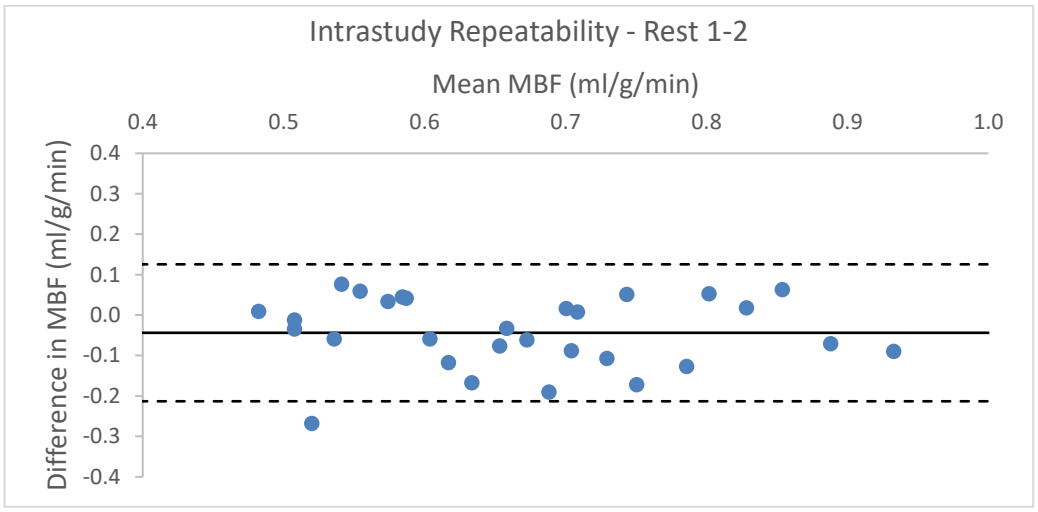


Figure 3-3 Global resting MBF on repeat throughout the same visit

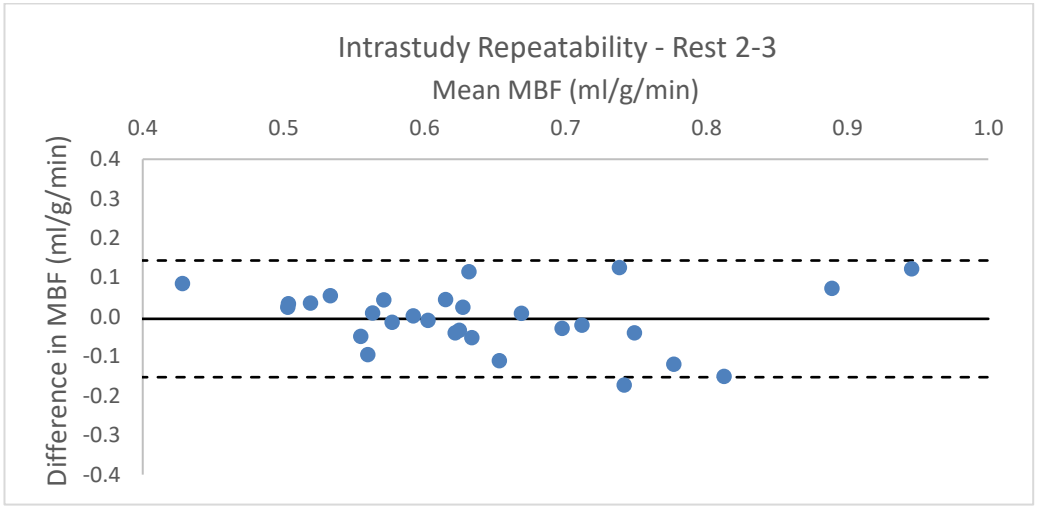
A – uncorrected values, B – corrected values (mean and 95% confidence interval of the mean). Rest MBF showed a trend to decrease on sequential measurements.

MBF – myocardial blood flow

A



B



C

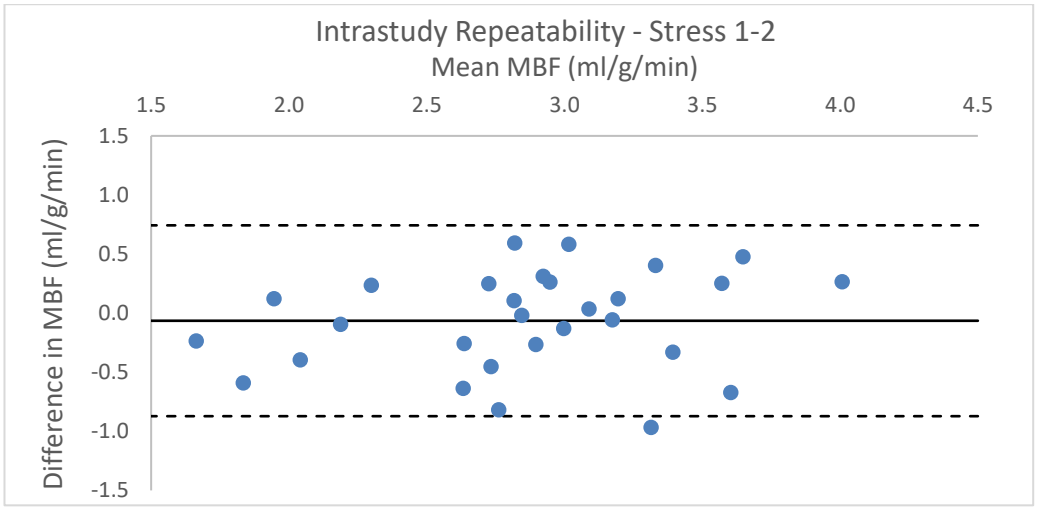


Figure 3-4 Intrastudy repeatability *(A) Rest 1-2 (B) Rest 2-3 (C) Stress 1-2*

Resting MBF correlated with heart rate ($r=0.49$, $p<0.01$), therefore MBF corrected for HR was also analysed (Table 2). A significant difference was still present between the scans ($p<0.01$) and the decrease with sequential scans remained (0.69 ml/g/min, 0.66 ml/g/min and 0.61ml/g/min for scans 1, 2 and 3). A significant difference was seen between scan 3 and the other two results ($p<0.01$). Whilst the level of significance differed when corrected for heart rate, both sets of values showed a trend to decrease with repeated measurement (Figure 3).

Stress MBF showed no significant difference between the two repeat acquisitions in visit 2 (mean difference -0.06 ±0.42ml/g/min, $p = 0.41$). Within subject coefficient of variation was 11% with good correlation and repeatability (ICC 0.76, RC 29%). One value was outside the limits of agreement on assessment with Bland-Altman plots, with a bias of 2.2% of the mean (Figure 4). Stress RPP was comparable between both studies (11202 ±2188 bpm.mmHg vs 10858 ±1877 bpm.mmHg, $p=0.09$) as was the percentage increase in heart rate (47.3±18.8% vs 44.4±18.4%, $p=0.24$) and RPP (51.1 ±21.7% vs 46.9 ±22.1%, $p=0.14$).

Table 3-2 – Global rest MBF corrected for heart rate

	Test 1 (ml/g/min)	Test 2 (ml/g/min)	Difference in mean (ml/g/min)	p	RC	RC (%)	CV (%)	ICC
Rest				<0.01				
Rest 1 - Rest 2	0.69±0.12	0.66±0.10	-0.03±0.09	0.14	0.18	27.3	9.9	0.66
Rest 1 - Rest 3	0.69±0.12	0.61±0.10	-0.07±0.07	<0.01	0.20	30.3	10.5	0.62
Rest 2 - Rest 3	0.66±0.10	0.61±0.10	-0.04±0.06	<0.01	0.15	23.7	8.3	0.72

p – from repeated measures ANOVA and Bonferroni post-hoc analysis, RC - repeatability coefficient, RC (%) -repeatability coefficient as percentage of the mean, CV - within subject coefficient of variation, ICC - intraclass correlation coefficient.

3.3.2 Interstudy repeatability

3.3.2.1 Global perfusion analysis

A total of 41 studies were analysed for interstudy repeatability of resting MBF with an average gap of 27 days between scans in visits 1 and 2. No significant difference was seen in MBF between scans (mean difference 0.004 ±0.1ml/g/min, p=0.8) (Table 3). Within subject coefficient of variation was 11%, RC 32% and bias was <1% of the mean (Figure 5).

Nineteen pairs of scans were analysed for interstudy stress and 18 for interstudy MPR in Group 2. The percentage increase in heart rate (52.1±26.6% vs 50.4±23.4%, p=0.7) and RPP (56.6±32.7% vs 52.1±26.6%, p=0.11) between rest and stress scans showed no significant difference between visits. Adenosine stress MBF had good repeatability with ICC 0.72 and RC 33%. CV was 12% and bias was -6% of the mean. Repeat MPR had a CV of 13.3 with no significant difference between the two tests. Weaker correlation was seen compared to stress and rest, although this remained significant (ICC 0.46, p<0.01).

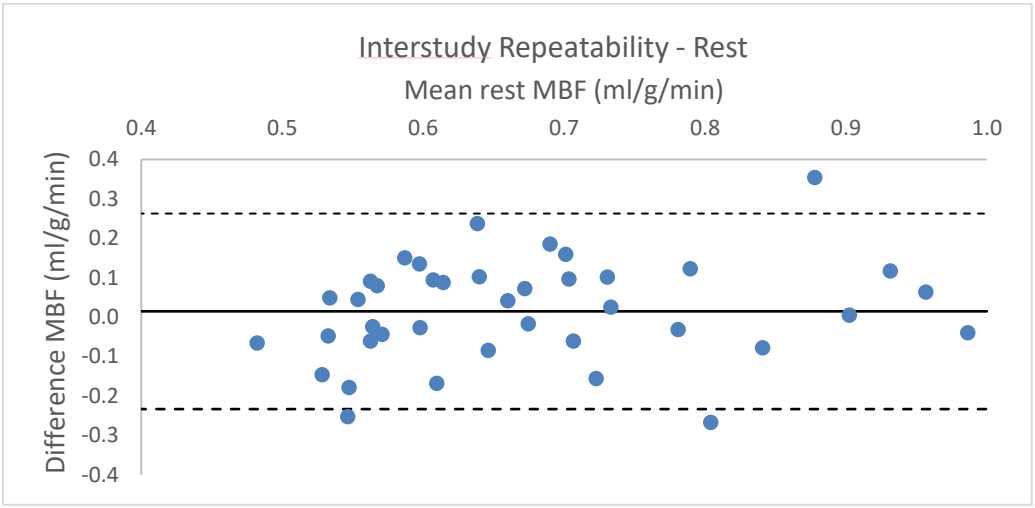
Table 3-3 - Interstudy repeatability of MBF measurements – by slice and coronary artery territory

	Test 1 (ml/g/min)	Test 2 (ml/g/min)	Difference in mean (ml/g/min)	p	RC	RC (%)	CV (%)	ICC
Rest								
Global	0.64±0.13	0.64±0.15	0.004±0.10	0.80	0.20	31.5	11.3	0.74
Basal	0.66±0.14	0.67±0.16	0.015±0.13	0.46	0.25	37.7	13.6	0.65
Mid	0.64±0.14	0.64±0.15	-0.003±0.10	0.87	0.20	31.0	11.2	0.76
Apex	0.60±0.13	0.60±0.15	-0.003±0.10	0.86	0.20	33.4	11.5	0.73
p	0.130	0.090						
LAD	0.69±0.16	0.68±0.16	-0.003±0.11	0.86	0.22	32.2	11.7	0.75
Cx	0.60±0.13	0.60±0.14	0.005±0.11	0.74	0.20	34.0	11.8	0.70
RCA	0.61±0.12	0.62±0.15	0.01±0.11	0.59	0.22	35.6	12.6	0.66
p	0.010	0.040						
Stress								
Global	2.71±0.61	2.55±0.57	-0.161±0.43	0.12	0.87	33.1	12.2	0.72
Basal	3.01±0.80	2.80±0.74	-0.209±0.51	0.09	1.05	36.1	13.2	0.76
Mid	2.48±0.55	2.39±0.51	-0.092±0.44	0.38	0.86	35.4	13.4	0.66
Apex	2.62±0.65	2.42±0.56	-0.201±0.62	0.17	1.24	49.2	16.3	0.48
p	0.05	0.07						

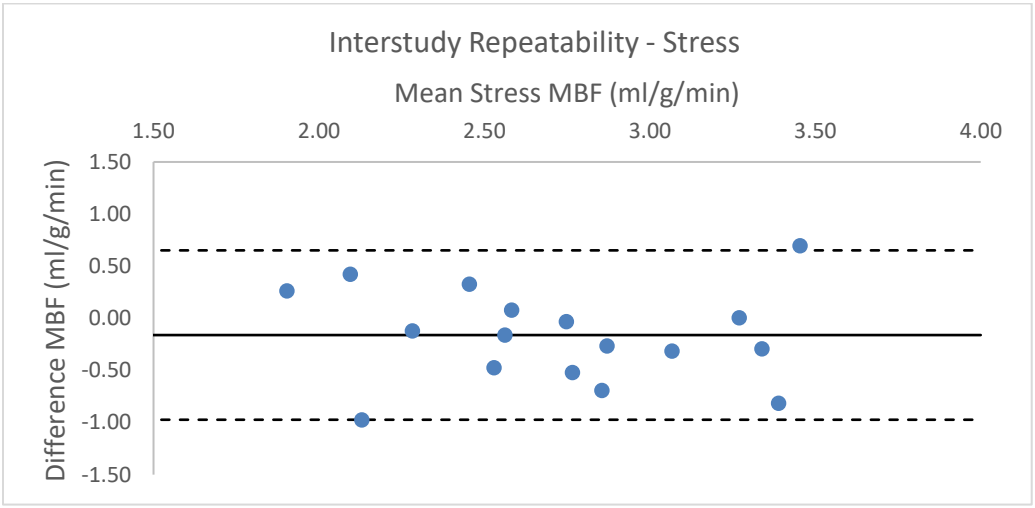
LAD	2.79±0.61	2.62±0.55	-0.167±0.39	0.08	0.82	30.3	10.8	0.75
Cx	2.69±0.61	2.51±0.66	-0.180±0.47	0.12	0.97	37.3	15.4	0.71
RCA	2.53±0.61	2.44±0.56	-0.096±0.47	0.39	0.92	37.0	13.6	0.68
p	0.440	0.630						
MPR								
Global	4.24±0.69	3.73±0.76	-0.214±0.76	0.25	1.46	36.4	13.3	0.46
Basal	4.53±0.90	4.27±1.00	-0.262±0.89	0.23	1.78	40.4	15.3	0.55
Mid	3.84±0.70	3.73±0.66	-0.113±0.83	0.57	1.60	42.4	15.5	0.26
Apex	4.42±0.90	4.12±0.76	-0.293±1.04	0.25	2.06	48.1	16.0	0.30
p	0.047	0.130						
LAD	3.96±0.58	3.83±0.81	-0.128±0.71	0.45	1.35	35.3	13.1	0.50
Cx	4.47±0.93	4.14±0.93	-0.325±0.86	0.13	1.76	40.8	15.7	0.54
RCA	4.06±0.76	3.91±0.66	-0.150±0.89	0.49	1.72	43.3	15.7	0.23
p	0.120	0.480						

p – level of significance using Student's T-test, RC - repeatability coefficient, RC (%) - repeatability coefficient as percentage of the mean, CV - within subject coefficient of variation, ICC - intraclass correlation coefficient. LAD – left anterior descending, Cx – circumflex artery, RCA – right coronary artery

A



B



C

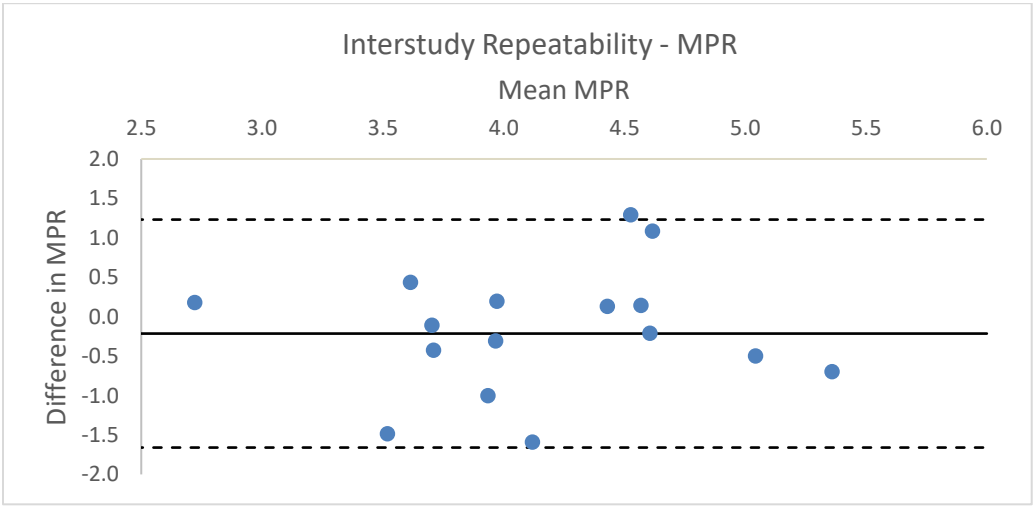


Figure 3-5 Interstudy repeatability *(A) Rest (B) Stress (C) MPR*

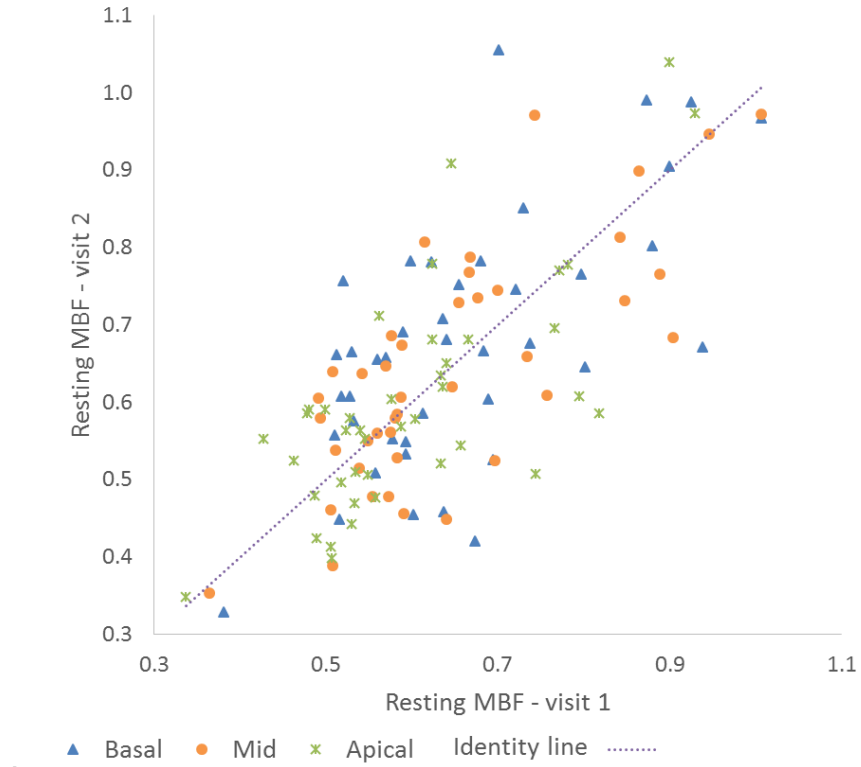
3.3.2.2 Regional perfusion analysis

At rest there was no significant difference between slices (p =0.13 and 0.09 for first and second scans) (Table 3). No significant difference was seen in individual slices between scans, and all showed good repeatability and correlation ($p<0.01$ in all slices) (Figure 6). Coefficients of variation were 13.6%, 11.2% and 11.5% for basal, mid and apical slices respectively.

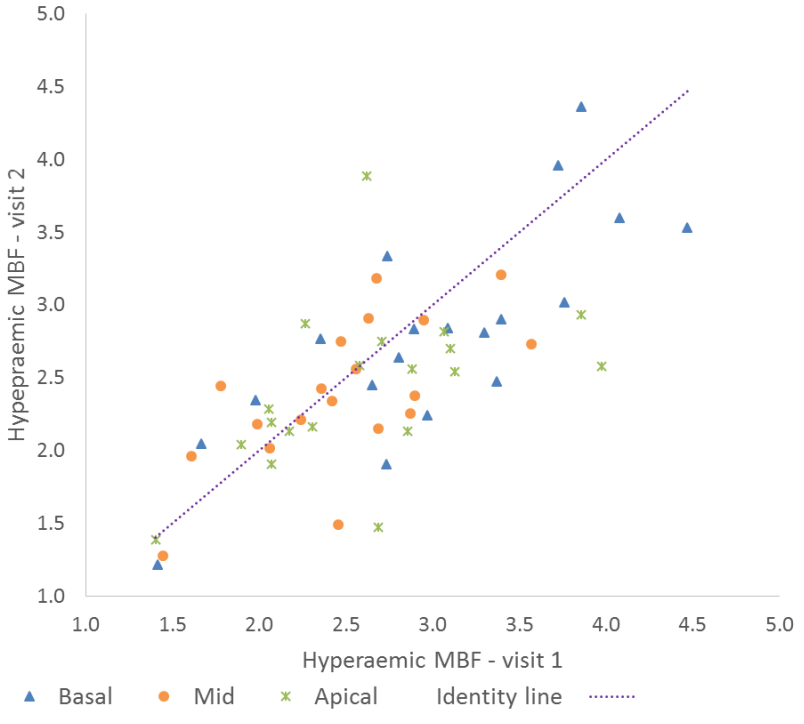
Mean MBF at stress was 3.01 ±0.8 ml/g/min, 2.48 ±0.55 ml/g/min and 2.62 ±0.65 ml/g/min on the first visit and 2.8 ±0.74 ml/g/min, 2.39 ±0.51 ml/g/min and 2.42 ±0.56 ml/g/min in basal, mid and apical slices respectively. No significant difference was seen between scans for any slice. The apical slice exhibited the lowest repeatability, ICC 0.46, RC 49% and within subject coefficient of variation 16%. Good correlation was seen in all slices (Fig 6).

No significant difference was seen in mean MPR between visits for any slice, RCs were 40%, 42% and 48% and CVs 15%, 15% and 16% for basal, mid and apical slices respectively.

Coronary territory flows were significantly different between vessel territories at rest on both visits. MBF in the LAD was higher than the Cx on both visits (mean difference 0.09 ml/g/min, p=0.01 on the first scan, and 0.08 ml/g/min, p=0.04 on the second). There was good correlation within all coronary territories between scans (Fig 7). All territories showed similar ICC (0.66-0.75) and repeatability coefficients (32-36%). CVs were very similar between territories (11.7%, 11.8% and 12.6%).



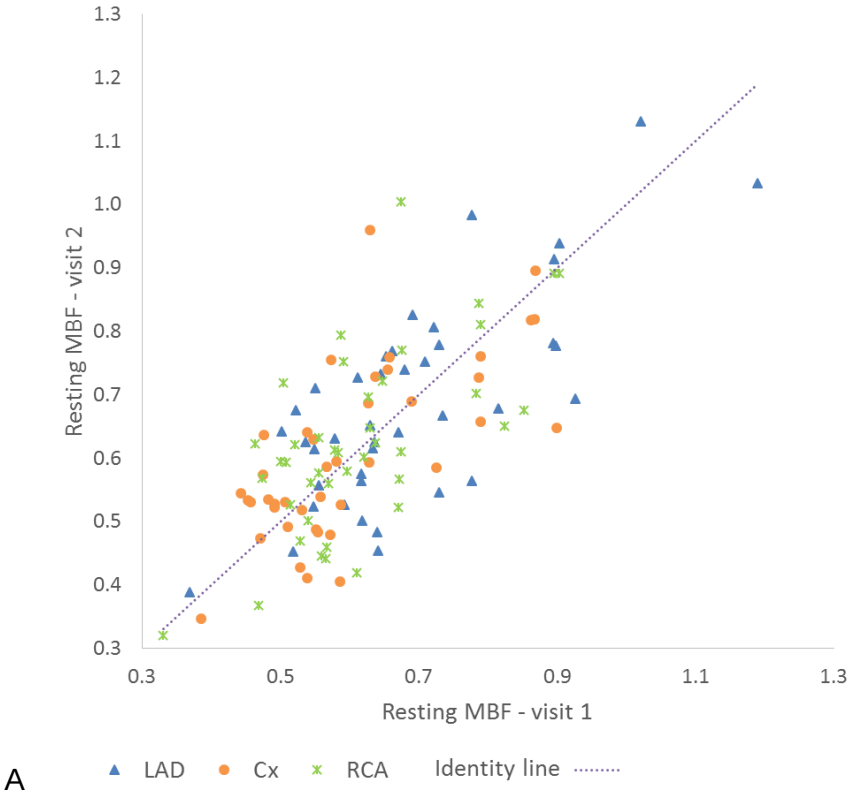
A



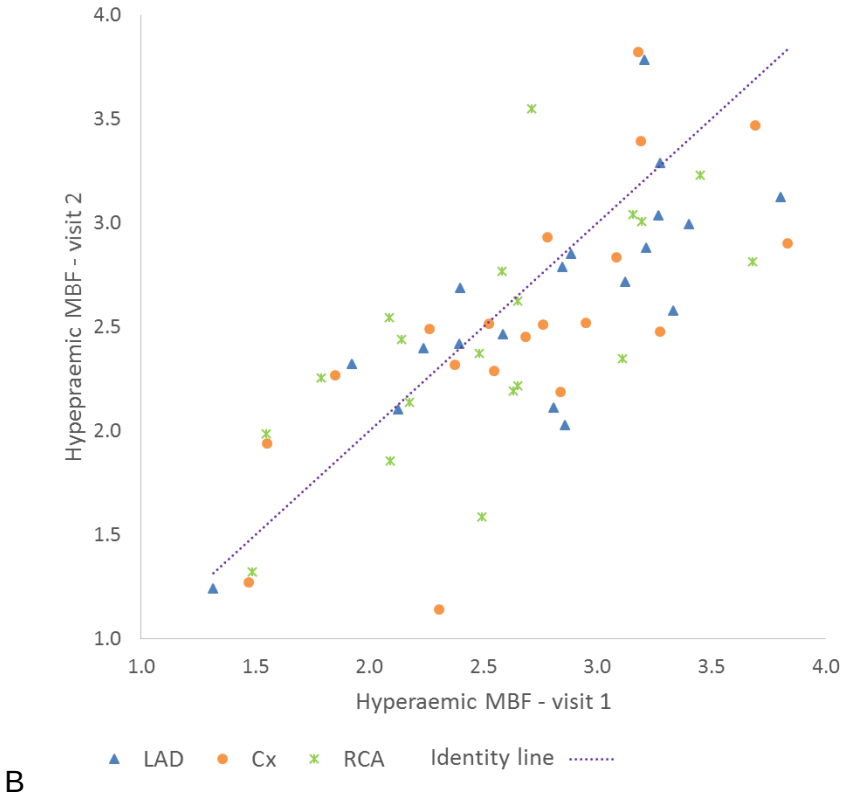
B

Figure 3-6 Correlation by slice

(A) rest (B) stress. Trend line represents line of perfect fit. MBF – myocardial blood flow



A



B

Figure 3-7 Correlation by coronary territory

(A) rest (B) stress. Trend line represents line of perfect fit. MBF – myocardial blood flow

No significant difference was seen between coronary territories in stress MBF, or in calculated MPR at either scan. All coronary territories showed good repeatability and correlation between scans. Coefficients of variation ranged between 10.8% and 15.4%, being highest in the circumflex territory.

No significant difference was seen in MPR in any coronary territory between visits. The LAD and Cx territories showed acceptable correlation and repeatability coefficients (LAD: ICC 0.5, RC 35%, Cx: ICC 0.54, RC 41%). The RCA territory did not show significant correlation between visits, ICC=0.23.

3.3.3 Interobserver and Intraobserver repeatability

Ten sets of perfusion maps were assessed for intraobserver variability at a minimum gap of 4 weeks between analysis, and for interobserver variability. There was excellent agreement for all measurements (Table 4). The highest coefficients of variation were seen in the apical slice.

Table 3-4 - Intra and inter-observer reproducibility

	Intra-observer		Inter-observer	
	CV (%)	ICC	CV (%)	ICC
Rest				
Global	0.7	0.999	0.8	0.999
Basal	1.1	0.998	1.2	0.998
Mid	1.0	0.999	0.6	0.999
Apical	3.0	0.990	3.7	0.980
LAD	1.1	0.997	1.3	0.996
Cx	1.4	0.997	1.3	0.995
RCA	1.8	0.996	1.8	0.991
Stress				
Global	2.0	0.995	2.4	0.995
Basal	2.0	0.996	3.0	0.993
Mid	2.6	0.999	3.3	0.994
Apical	6.3	0.963	8.0	0.959
LAD	2.8	0.990	2.6	0.990
Cx	2.5	0.992	4.3	0.986
RCA	1.9	0.992	1.9	0.993

Good interobserver and intraobserver repeatability was seen ion global and regional assessment.

CV - coefficient of variation, ICC – intraclass correlation coefficient LAD – left anterior descending, Cx – circumflex, RCA – right coronary artery

3.4 Discussion

In this study we have demonstrated good short- and long-term repeatability with in-line myocardial perfusion mapping by CMR. Global and regional assessment of MBF by coronary artery territory have good repeatability while MPR is a less reproducible assessment than MBF, particularly for regional assessment.

3.4.1 Global perfusion analysis

We have shown reliable repeatability of both global stress and rest MBF for automated perfusion mapping with CMR, both within one scan and with an interval between scans. Our results are in line with previous studies, using both invasive and non-invasive estimates of MBF and MPR.

Invasive assessment of coronary flow reserve has a coefficient of variation of 19% when repeated within minutes(144). A summary of the published data on repeatability of non-invasive MBF measurement is given in Table 5. In published literature repeatability with PET has ranged from RCs of 18-35% at rest and 18-41% during stress(145–148). In a recent study to assess the optimally repeatable kinetic model in PET the best variants had a combined repeatability coefficient of 15.8% for stress and rest(149). 120 volunteers underwent serial stress or rest scans in one of the largest studies of test-retest repeatability in PET to date; rest MBF CVs were 10.7% within test and 21.1% between tests, and stress MBF CVs were 9.6-10.6% within test and 19-21% between tests(150). As in the majority of the published literature, short-term repeatability had lower coefficients of variation and repeatability than delayed repeatability.

There are few published CMR studies of repeatability of MBF. The largest, a subset of the MESA study (151), reported repeatability coefficients of 30% and 41% for rest and stress MBF respectively, including 30 patients with an interval of almost a year between scans and producing similar values to PET studies. Another smaller study of 10 patients showed good correlation in interstudy repeatability at rest (r=0.77) and stress (r=0.9) with CVs of 23% at rest and 20% for stress, in keeping with previous PET literature(152). Other CMR studies have shown worse repeatability than PET; a more recent study of 11 subjects(141) showed repeatability coefficients of 45% at rest and 73% at stress, with coefficients of variation of 20% and 40% respectively.

Our data for automated in line perfusion CMR mapping are comparable to those achieved with PET and fit well with published CMR data. Compared with most CMR studies, the repeatability within the current study was better for global MBF both at rest and stress, and for short and long-term repeatability.

Table 3-5 – Summary of literature on MFB repeatability

					Rest					Stress					MPR			
	Author	**Year**	**n**		T test	r/ICC	RC (%)	CV (%)		T test	r/ICC	RC (%)	CV (%)		T test	r/ICC	RC (%)	CV (%)
Immediate (intrastudy)																		
PET	Nitzsche(153)	1996	15		0.33	0.99	33			0.16	0.97	13						
	Kaufmann(145)	1999	21		ns		18			ns		25			ns		33	
	Wyss(154)	2003	11		ns	0.77	21				0.77	27			ns	0.74	35	
	Schindler(155)	2007	20			0.72	29				0.76	20						
	Manabe(146)	2009	15		0.31		22			0.81		27			0.53		37	
	Kitkungvan(150)	2017	120		0.93			11		0.74			10					
	Ocneanu(149)	2017	12					21					15					
MRI	Keith†(156)	2017	10				53	13										
	This study				***0.08***	***0.8***	***24***	***8***		***0.41***	***0.76***	***29***	***11***					
Delayed (interstudy)																		

PET	Nagamachi(147)	1996	30		ns	0.63	31			ns	0.69	18					20	
	Schindler(155)	2007	20			0.75	30				0.71	23						
	Sdringola(148)	2011	48		p<0.05	0.68	35			ns	0.53	34			ns	0.47	38	
	Johnson(157)	2015	50		0.46		41			0.13		34			0.29		34	
	Kitkungvan(158)	2017	19		0.13					0.94			17		0.26			20
	Kitkungvan(150)	2017	120		0.13			21		0.81			19					
MRI	Jerosch-Herold(151)	2008	30		0.001		30			0.11		41						
	Larghat†(141)	2013	11		0.2		45	20		0.61		73	40		0.11		69	35
	Likhite(152)	2016	10			0.77										0.88		
	Keith†(156)	2017	10				61	16										
	This study				***0.8***	***0.74***	***32***	***11***		***0.12***	***0.72***	***33***	***12***		***0.25***	***0.44***	***36***	***13***

ns = not significant (p value not reported), r = Pearson correlation coefficient, RC = reproducibility coefficient (% of mean), CV = coefficient of variation.

*†Repeatability data is given for single mid-ventricular slice, all other studies, data is for global myocardium, averaged from multiple slices. Where RC was not published, but sufficient data was provided, this has been calculated using 1.96*SD of difference. Similarly, all RC are given as % for ease of comparison.*

3.4.2 Regional assessment

All coronary territories showed good repeatability for rest and stress MBF. Higher repeatability coefficients and coefficients of variation were seen than for global values, in keeping with the limited published literature in CMR and some of the PET literature. These differences are likely to reflect the smaller volume assessed in regional measurement, allowing a greater proportion of error. Early CMR studies of repeatability have included only the mid slice but have shown regional repeatability to be higher than global (RC 28% vs 21%)(159). PET literature is inconsistent regarding regional vs global repeatability. One PET study examined coronary territory MBF repeatability in 30 patients and reported similar repeatability coefficients to global flow at stress (RC 18% for global perfusion, 18-24% for regional perfusion) and at rest (RC 31% for global and 26%-46% for regional perfusion). A further study of 48 volunteers showed comparable values for global perfusion vs regional perfusion (quadrants), with regional rest values of RC 33-41% and stress 33-38% vs global values 35% and 34%(148). Other PET studies have shown worse repeatability in regional assessment compared to global values. A study of 21 subjects assessing repeatability of 15O PET had regional repeatability coefficients of 22-46% at rest and 41-59% at stress vs 18% and 25% globally(145). A smaller study of 11 showed regional repeatability coefficients of 38-55% at rest and 55-70% at stress vs 21% and 27% globally(154). Numerically the regional variability in our study was similar to or lower than in most PET studies. All coronary territories showed good correlation and repeatability with RCs of 32-36% at rest and 30-37% at stress.

In addition to coronary territories, we also compared variability between the three acquired slices. At stress the apical slice had the lowest ICC, showing only moderate reliability (ICC=0.48, p=0.02). It also had the largest inter- and intra-observer variability, with higher coefficients of variation than other regions. This illustrates the complexity in assessment and quantification within most apical slice; where the area of myocardium is small, and a larger partial volume effect can be expected due to this and the increased movement during stress.

3.4.3 MPR assessment

Within our study, the repeatability of MPR was lower than that of MBF, in particular for the analysis of coronary territories. Previous studies have shown similar values for repeatability of global MPR, with PET values of multiple studies within the range of 33-38%, in keeping with our results(145,146,148,154,157). One study of 30 volunteers showed a repeatability coefficient of 20% for MPR; however, these results included some studies repeated within the same day, which may account for the lower values. CMR data on inter-test reproducibility for MPR is limited, with a single study of 11 volunteers showing a RC of 69%(141) higher than both this study, and published PET data.

Where regional MPR has been compared to global values for repeatability, some studies have shown markedly worse repeatability. One study showed regional RC of 68-82% vs 33% for global values(145) another showed an increase from 35% for to 67-96%(154). Others have shown a small rise or comparable RC in a similar pattern to our data. The largest study, with 48 paired studies showed a RC of 38% globally with a maximum of 43% in the lateral wall(148).

The poorer repeatability seen in both the RCA territory and mid and apical slices, supports the use of stress MBF over MPR in clinical assessment where regional differences may be diagnostically important. Stress MBF correlates with the severity of stenosis in CAD(160), therefore the better reliability demonstrated in that measurement would support its use for this important diagnostic decision.

3.4.4 Variation in resting MBF

We have shown variation in resting MBF on short-term repeat, within the same scan, with a trend to decrease with serial measurement was not removed by correction for heart rate. Although correction for HR modified the level of significance in this difference, the trend for decrease with sequential repeat remained (Figure 3).

Published literature has not shown this decrease in MBF during the same scan. Studies have shown a decrease in resting MBF on repeat assessment between separate scans. The MESA study showed a decrease in resting MBF of 0.1ml/g/min, p=0.001 over a long time interval (mean 334 days)(151), this change was also accompanied by a significant change in heart rate, and this change was proposed to account for the drop. In another study of healthy volunteers with no

risk factors, with a median interval of 22 days between scans, a significant decrease of 0.05±0.13ml/g/min ($p<0.05$) was seen in rest MBF(148). This effect was not seen in stress perfusion, or in subjects with cardiovascular risk factors.

Although a number of these results differ from those in our study and the intervals between scans were different, they do provide more evidence of the susceptibility of rest MBF to physiological variation. Our study is the first to report more than 2 repeated measurements within one scan. The initial higher MBF seen within our study may be caused by an element of anxiety present at the beginning of the CMR scan in the volunteers examined. In addition to a rise in heart rate and blood pressure, stress is associated with vasodilation of the coronary microvasculature in healthy volunteers(161) which would lead to higher MBF. Within our study, even after correction for heart rate the decrease in MBF remained, suggesting that vasodilation rather than cardiac work is the cause of the higher values on the first scans. This hypothesis is supported by the lack of difference between sequential stress perfusion, where maximal vasodilatation is reached so that differences in anxiety should have no additional impact. The absence of significant difference in sequential stress scans also supports that this change is likely to be physiological rather than an effect of residual Gd from the previous series. The lack of significant difference between the second perfusion acquisition and subsequent assessment is reassuring clinically, as this would imply that stress followed by rest perfusion assessment, the commonly used protocol, would produce repeatable values for both rest and stress.

3.4.5 In-line perfusion mapping

The development of automated inline perfusion mapping overcomes one of the main previous limitations of quantitative perfusion CMR by removing the previously time-consuming analysis and the need for specialist knowledge to implement this.

We have demonstrated repeatability of MBF measurements that is comparable to PET, the current reference method, and at least as good as previous, manual, CMR analysis methods supporting use of this method as part of CMR analysis. Assessment using perfusion maps allows objective assessment of MBF, providing simpler and faster analysis and may have clinical advantages of detecting disease with global decrease in MBF such as microvascular and multivessel disease.

3.4.6 Study limitations

Our data are influenced by physiological variation as well as variation within the model and analysis. Although we attempted to minimise physiological variation as much as possible, some effects may not have been controlled for. Caffeine has been demonstrated to affect coronary vasomotor tone at rest(162) and adenosine stress perfusion CMR(163). Although we asked our volunteers to avoid caffeine for 24hrs prior to the scan, previous studies have demonstrated that up to 20% may still have detectable caffeine levels(164). In addition, dosing of adenosine was determined clinically according to symptoms and response rather than a direct repeat from previous scan. This would mimic clinical practice, and any difference in doses required may result in physiological variants in response. We can be confident that adequate stress was achieved; having seen appropriate increases in heart rate and RPP together with symptomatic response, however, the degree of hyperaemia may vary from maximal and account for the increased variation seen in stress MBF. All studies were performed using a FLASH perfusion sequence at 3T, results for MBF may vary using other sequences or field strengths.

3.4.7 Conclusion

Quantitative perfusion CMR using automated perfusion mapping achieves estimates of MBF and MPR with repeatability similar to the reference standard method PET. In this study rest and stress MBF, rather than MPR were a more reproducible assessment, particularly in regional analysis. The degree of physiological variation emphasises the importance in establishing normal ranges to allow for accurate diagnostic use.

Chapter 4

Sex and age-specific normal values for automated quantitative pixel wise myocardial perfusion cardiovascular magnetic resonance

4.1 Introduction

There is increasing recommendation that clinical decision making for patients with stable coronary artery disease (CAD) should be based on quantitative rather than visual assessment to determine the functional significance of coronary stenosis(3,4,137,138,165). The invasive reference standard for functional assessment of coronary stenosis is fractional flow reserve (FFR) while positron emission tomography (PET) is considered the reference standard for non-invasive quantitative assessment of myocardial blood flow (MBF) and myocardial perfusion reserve (MPR)(166). Cardiovascular magnetic resonance (CMR) myocardial perfusion imaging can also be used to estimate MBF and MPR and compared with PET, CMR has the advantages of not exposing patients to ionising radiation, more widespread availability, higher in-plane spatial resolution and the ability to provide additional assessment of cardiac structure and function within the same study. In the past quantitative myocardial perfusion CMR has required time-consuming, manual, offline processing, which restricted its use to expert centres and prevented wider clinical application. Recently developed motion corrected myocardial perfusion CMR with automated in-line perfusion mapping allows the generation of pixel-wise MBF maps(48) during free-breathing acquisition and without user interaction. This method offers the enticing potential of making largely user independent quantitative myocardial perfusion analysis available in routine clinical care.

CMR myocardial perfusion mapping has been shown to provide comparable MBF and MPR values to PET both in assessment of CAD and in repeatability of measurements(52,167). Adoption of the method in clinical practice requires the definition of a specific range of normal values. This study looked to establish sex-specific normal values for CMR myocardial perfusion mapping in healthy volunteers and over a wide range of age groups, representative of patients seen in clinical care.

4.2 Methods

4.2.1 Study population

151 healthy volunteers with no history of cardiac disease or major risk factors, were recruited in two cardiac centres, (Leeds Teaching Hospitals Trust, Leeds, UK and University Hospitals of Leicester NHS Trust, Leicester, UK). Exclusion criteria included hypertension, hypercholesterolaemia, diabetes mellitus, smoking, previous coronary artery disease or revascularisation, and contraindications to adenosine or gadolinium-based contrast or MRI.

4.2.2 Study protocol

All scans were performed at 3 Tesla (Prisma (Leeds) or Skyra (Leicester), Siemens Healthcare, Erlangen, Germany) according to a standard protocol including cine imaging, adenosine stress and rest perfusion, and late gadolinium enhancement. Patients were asked to abstain from caffeine for 24 hours before the scan. All patients underwent adenosine stress according to a standard clinical protocol.

Pharmacological stress was achieved with adenosine infusion at 140 µg/kg/min for a minimum of 3 minutes. The dose was increased to 210 µg/kg/min after 2 minutes if there was no symptomatic or haemodynamic response. Volunteers were monitored for symptoms throughout the scan. Blood pressure (BP) and heart rate (HR) were recorded during adenosine infusion. An intravenous bolus of 0.05 or 0.075mmol/kg of gadolinium-based contrast was administered.

Data acquisition used a multi-slice, free-breathing, saturation recovery pulse sequence with fast low angle shot (FLASH) readout, acquired over 60 heartbeats. In the first three beats proton density weighted images (without saturation preparation) were acquired. AIF data were obtained from interleaved low-resolution images (dual-sequence method) in a single slice with dual-echo acquisition to allow correction of T2* related signal loss(48).

At a delay of at least 7 minutes from stress perfusion, rest perfusion images were obtained using the same contrast and acquisition regime as for stress perfusion.

4.2.3 In-line processing and quantitative analysis of perfusion data

In-line automatic reconstruction and post-processing of the perfusion data were implemented within the Gadgetron software framework(48,168). Images were motion corrected and then corrected for surface coil intensity variation based on the proton density weighted images. Signal intensity data were converted to Gadolinium concentration based on automatically generated look-up tables. AIF data were extracted from the low-resolution Gadolinium concentration images using automated segmentation of the left ventricular (LV) cavity. MBF was calculated on a pixel-wise basis in the high-resolution images by blood tissue exchange model constrained deconvolution incorporating estimation of the delay time between bolus arrival in the LV cavity and the tissue of interest. Automatic segmentation of the left ventricular cavity and myocardium was performed by the artificial intelligence tool, excluding myocardial fat and papillary muscles. AHA segment data was then further delineated and segmental MBF was calculated automatically as an average of all pixels(169). Values for each of the AHA 16 segments were recorded for stress and rest MBF, and global values were calculated as an average of these. Segments including LVOT or significant artefact were excluded from analysis.

Rate pressure product (RPP) was calculated for each perfusion acquisition (HR x systolic BP). MBF values were corrected for RPP by dividing by the individual value for each patient and multiplying by 10,000 in keeping with previously established practice(170).

Age and sex matched cohorts were compared between centres and between Gd dosing to ensure that they could reasonably be combined as a study population. Age was matched to within 2 years to allow a reasonable sample size for comparison.

Myocardial perfusion reserve (MPR) was calculated for each segment as stress MBF/rest MBF.

4.2.4 Statistical analysis

Outliers were identified using the Tukey robust approach and removed from analysis if either <Q1-1.5IQR or >Q3+1.5IQR. Proposed normal ranges were described as the 95% reference range of the cohort, using previously published methods(171).

Analysis was performed using SPSS 23 (IBM SPSS, Armonk, NY, USA). The Shapiro Wilk test was used to assess normality of data. Data are presented as mean ±standard deviation (SD) or median (IQR). Groups were compared using Student's t-test or Mann-Whitney U test for non-parametric variables. Differences between coronary territories were compared using repeated measures ANOVA. Coefficient of variation (CV) was calculated as SD/mean as a standardised measurement of dispersion. Correlation was assessed using Pearson's correlation coefficient. All statistical tests were two-tailed and $p<0.05$ was considered significant.

4.3 Results

151 volunteers were recruited and underwent adenosine stress CMR as described above.

150 volunteers were included in the final analysis. One set of data was excluded due to poor quality of data due to arrhythmia during the scan. 5 volunteers only had either stress or rest perfusion data available and a further 9 results were classed as outliers (6 rest MBF and 3 stress MBF). Single data sets were analysed from these participants, with no MPR available. Median age was 49(IQR 24-59) ±18.8 with 62 (41%) females.

Haemodynamic data and response are seen in Table 1. BP was not recorded during rest perfusion in 11 volunteers. Mean HR and RPP were significantly higher at stress than rest ($p<0.001$).

Table 4-1 - Haemodynamic characteristics of participants

	All n=150	**Male** n=88	**Female** n=62	**p (M:F)**
Age (yrs)	49 (24-59)	52 (25-59)	35 (23-57)	0.105
Rest HR (bpm)	63±9.5	62±9.5	65±9.3	0.111
Rest BP (mmHg)	122±19	124±17	119±20	0.111
Rest RPP (mmHg.bpm)	7775±1733	7765±1646	7791±1880	0.935
Stress HR (bpm)	91±17	86±14	98±18	<0.001
Stress BP (mmHg)	123±17	125±17	120±18	0.118
Stress RPP (mmHg.bpm)	11189±2609	10777±2371	11778±2836	0.027
Change HR (bpm)	26±12	23±11	31±13	<0.001
% change HR	41.8±21	37.6±18	48.3±23	0.003
Change bp (mmHg)	1.5±9	1.3±9	1.7±10	0.800
% change bp	1.6±7	1.3±7	2.0±9	0.616
Change RPP (mmHg.bpm)	3270±1753	3005±1649	3692±1846	0.029
% change RPP	44.0±25	40.2±23	49.9±28	0.033

Higher heart rates and RPP were seen in females at stress compared to males. They also showed a higher increased in HR and RPP.

HR - heart rate, RPP - rate pressure product, SBP - systolic blood pressure.

4.3.1 Myocardial Blood Flow

Mean MBF at rest was 0.62±0.13ml/g/min and 2.24±0.53ml/g/min during adenosine stress. Mean MPR was 3.742±1.00. Mean corrected rest MBF was 0.83±0.21 ml/g/min.

There was no significant difference between stress or rest MBF measurements in age and sex matched cohorts from different centres (Table 2) or when using 0.05mmol and 0.075mmol contrast agent boluses (Table 3).

Table 4-2 - Comparison between CMR centres

	Centre 1 n=30	**Centre 2** n=30	**p**
Age (yrs)	57.2±9.5	58.2±9.4	0.695
Male	23 (77%)	23 (77%)	1
Rest MBF (ml/g/min)	0.59±0.12	0.60±0.14	0.827
Stress MBF (ml/g/min)	2.01±0.55	1.93±0.50	0.508

No significant difference was seen between MBF between the two centres. MBF – myocardial blood flow

Table 4-3 - Comparison between contrast dosing

	0.05mmol/kg GBCA n=17	**0.075mml/kg GBCA** n=17	**p**
Age (yrs)	52.6±7.6	52.6±7.5	1
Male	11 (65%)	11 (65%)	1
Rest MBF (ml/g/min)	0.62±0.11	0.66±0.13	0.210
Stress MBF (ml/g/min)	2.09±0.52	2.23±0.39	0.360

No significant difference was seen between MBF acquired using different GBCA doses.

MBF – myocardial blood flow, GBCA – gadolinium-based contrast agent

When coronary artery territories were compared, both rest and stress flow were highest in the LAD territory, with significant differences between the three territories (Table 4). Mean stress and rest MBF measured on a segmental basis were highest in AHA segment 1 (basal anterior) 2.63±0.73 ml/g/min and 0.69±0.12ml/g/min, and lowest in segment 15 (apical inferior) 1.90±0.46ml/g/min and 0.53±0.12ml/g/min. Full data for all segments are provided in tables 7-9 at the end of this chapter.

Table 4-4 - Global and coronary artery territory MBF

	Rest MBF (ml/g/min)		**Stress MBF** (ml/g/min)		**MPR**	
	Mean	**CV (%)**	**Mean**	**CV (%)**	**Mean**	CV (%)
Global	0.621±0.13	20.9	2.243±0.53	23.6	3.742±1.00	26.7
Coronary territories						
LAD	0.668±0.15	22.5	2.360±0.57	24.2	3.630±0.96	26.4
Cx	0.591±0.14	23.7	2.247±0.56	24.9	3.950±1.17	29.62
RCA	0.593±0.12	20.2	2.100±0.50	23.8	3.66±0.98	26.8
	p<0.001		p<0.001		p<0.001	

There was a significant difference in MBF between coronary artery territories. Rest and stress MBF were highest in the LAD territory.

MBF – myocardial blood flow, LAD – left anterior descending, Cx – circumflex, RCA – right coronary artery, CV – coefficient of variation.

4.3.2 Sex

Haemodynamic and MBF values divided by sex are given in Tables 1 and 5.

Both rest and stress MBF were significantly higher in females compared to males (Figure 1). Resting and stress HR were higher in females compared to males. The absolute increase in HR and RPP between rest and stress was also significantly higher in females (Table 1). Rest RPP was shown to correlate with rest MBF (r=0.41, p<0.001), therefore values of rest MBF, corrected for RPP were

calculated. Resting MBF remained significantly higher in females (Table 5). No significant difference was seen in MPR between sexes.

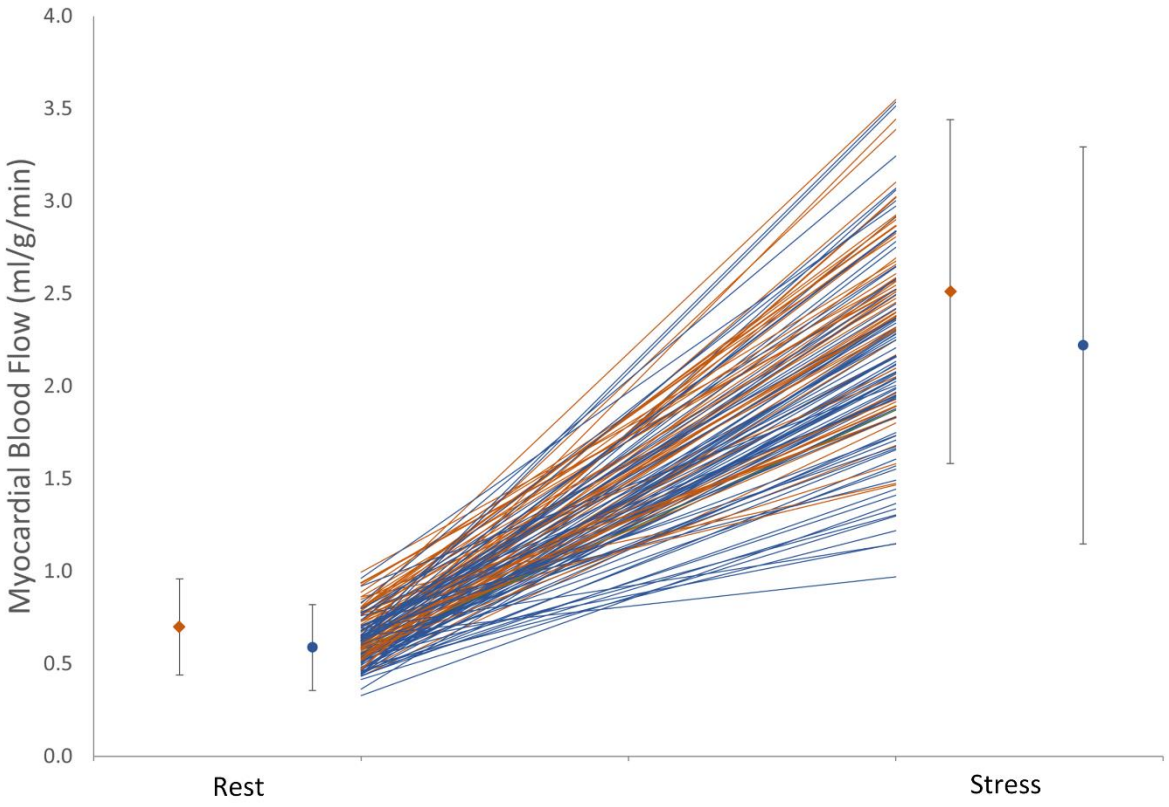


Figure 4-1 Rest and Stress MBF in both sexes

Global myocardial blood flow was significantly lower in males both at rest (0.58±0.12 vs 0.69±0.13ml/g/min, p<0.001) and during adenosine stress (2.13 ±0.54 vs 2.41±0.47ml/g/min, p0.001).

● *female* ● *male*

Table 4-5 – Myocardial Blood Flow and Perfusion Reserve values in different sexes

	All n=150	**Male** n=88	**Female** n=63	**p (M:F)**
Rest MBF (ml/g/min)	0.621±0.13	0.577±0.12	0.688±0.13	<0.001
Stress MBF (ml/g/min)	2.243±0.53	2.125±0.54	2.412±0.47	0.001
MPR	3.742±1.00	3.786±1.00	3.674±1.00	0.522
RPP corrected rest MBF (ml/g/min)	0.829±0.21	0.768±0.18	0.924±0.21	<0.001

Stress and rest MBF were significantly higher in females. Rest MBF remained higher in females when corrected for RPP.

MBF = myocardial blood flow, MPR = myocardial blood flow, RPP = rate pressure product.

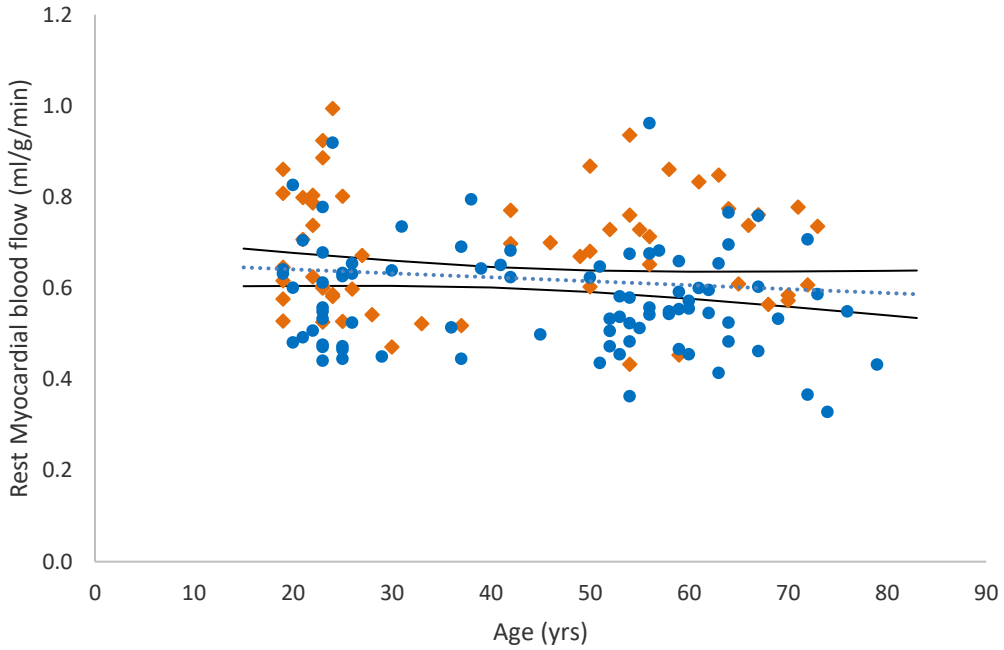
4.3.3 Age

Ages of volunteers ranged from 19 to 79 years. No significant difference was seen between males and females (52 (25-59) vs 35 (23-57), p=0.105). Increasing age negatively correlated with stress MBF (r=-0.434, p<0.001) and MPR (r=-0.339, p<0.01). No correlation was seen between age and rest MBF (Figure 2).

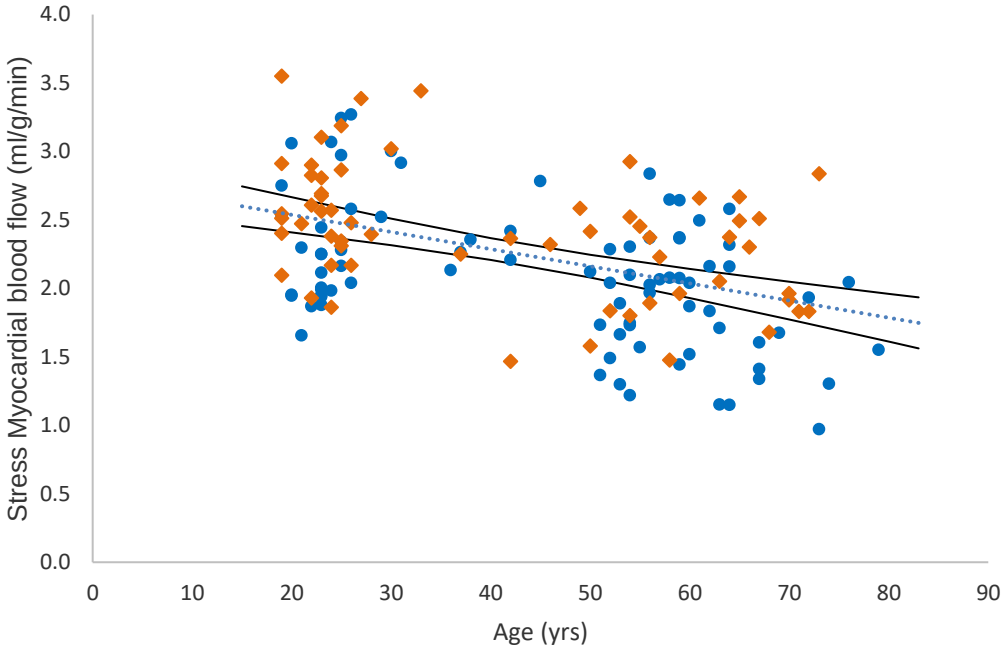
Rest RPP correlated with age (r=0.247, p=0.004), when rest MBF was corrected for RPP, there was a negative correlation with age (r=-0.337, p<0.001) (Figure 2).

Normal ranges for MBF and MPR, with 95% confidence intervals are shown in Figure 3.

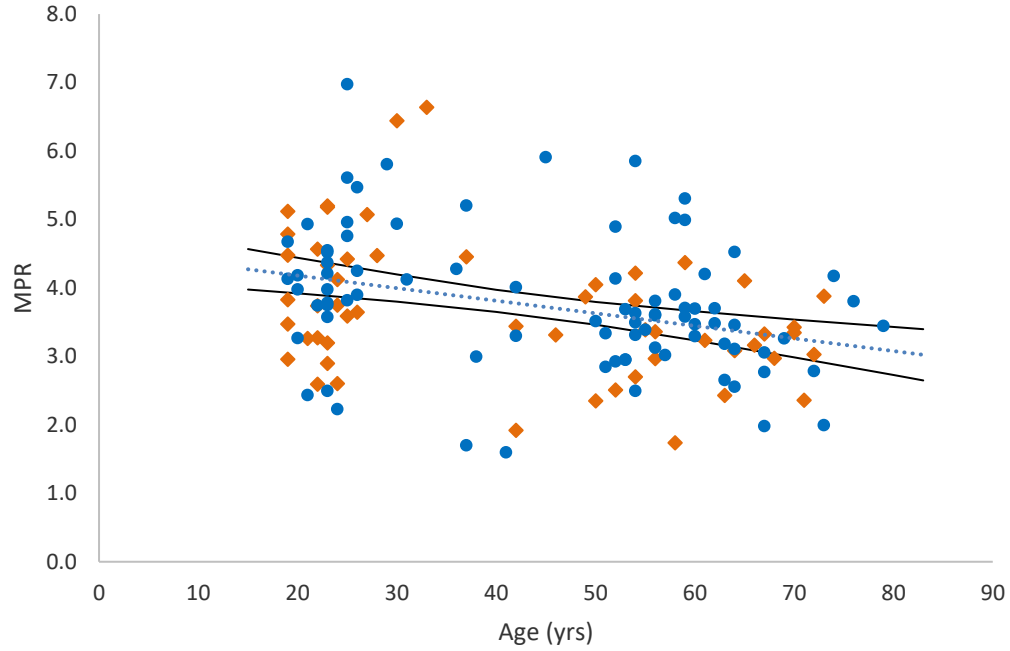
A


Rest Myocardial blood flow (ml/g/min)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
90
Age (yrs)

B


Stress Myocardial blood flow (ml/g/min)
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
90
Age (yrs)

C



D

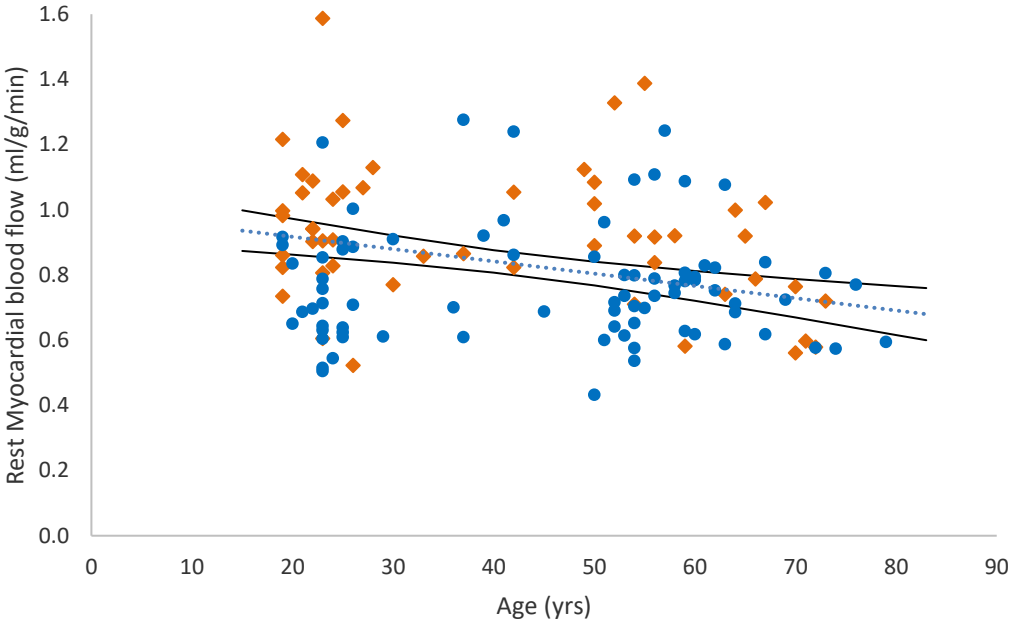
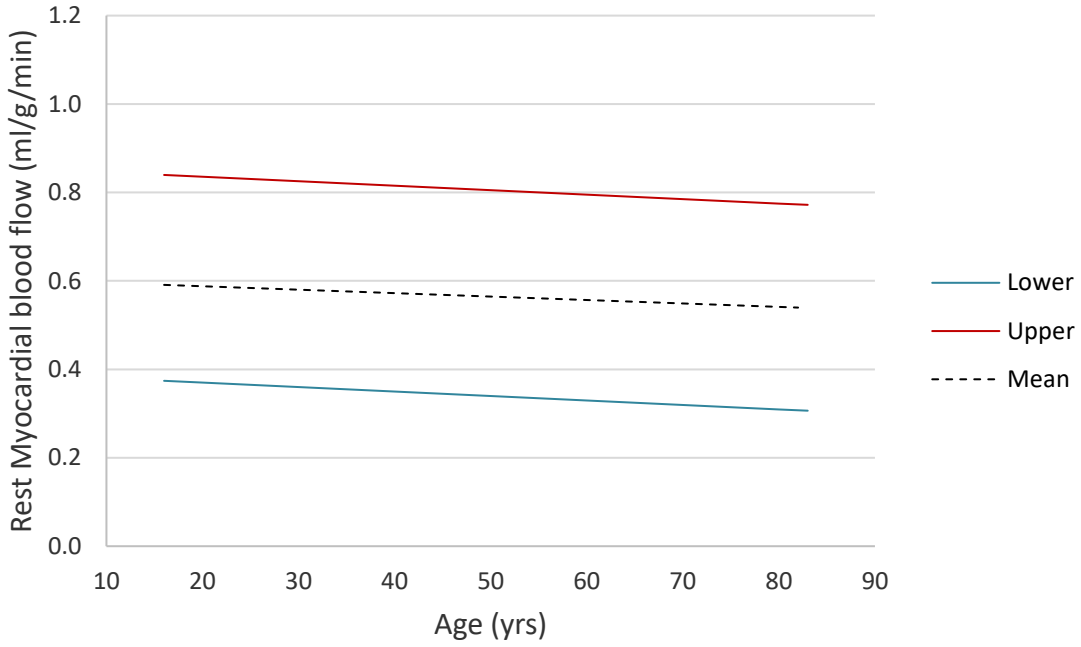


Figure 4-2 Correlations between MBF, MPR and age

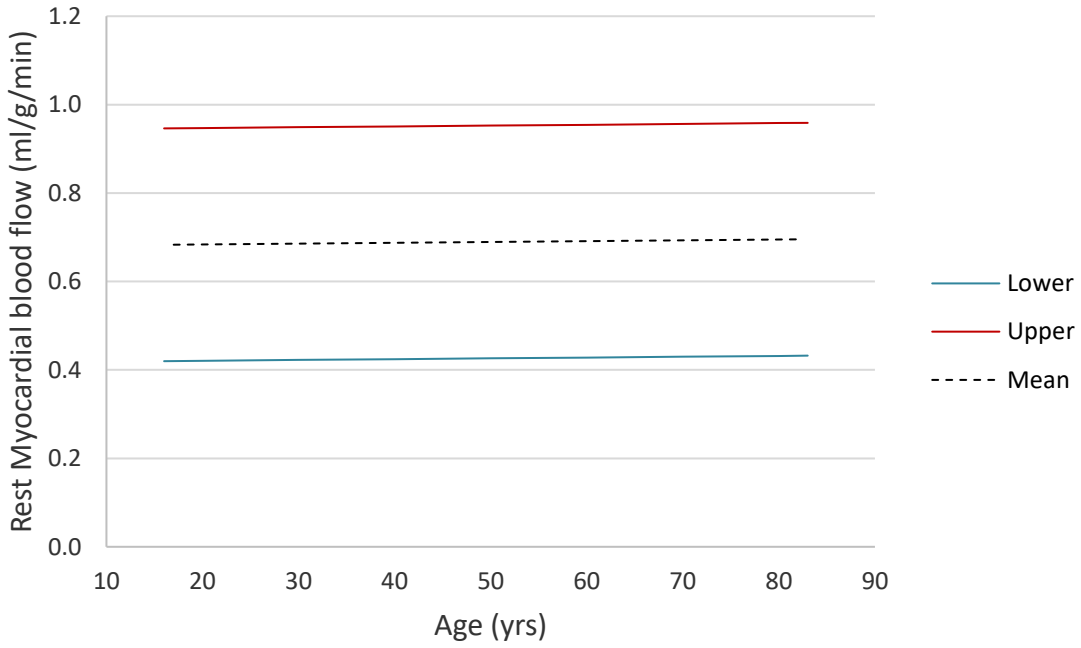
Rest MBF (A) showed no correlation between MBF and age. Stress MBF (B) and MPR (C) demonstrate a significant negative correlation with age. Stress MBF r=-0.434, p<0.001, MPR r = -0.339, p<0.001. Rest MBF corrected for RPP (D) demonstrates a significant correlation with age, r=-0.337, p<0.001.

♦ *Female* ● *Male*

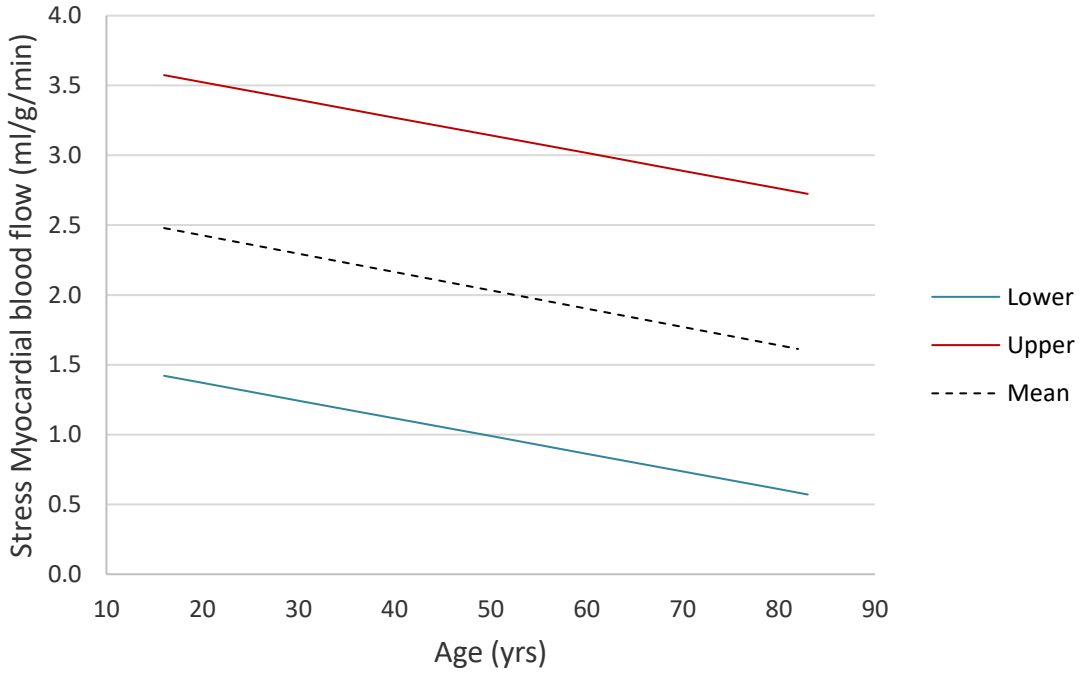
A - Rest MBF - Male



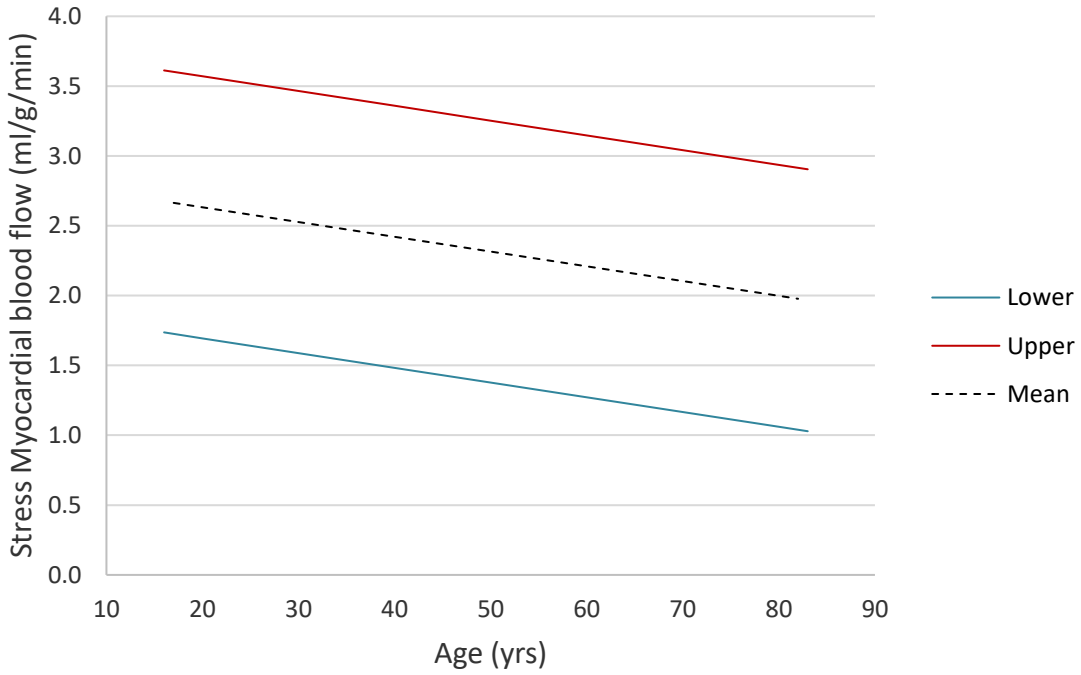
B - Rest MBF - Female



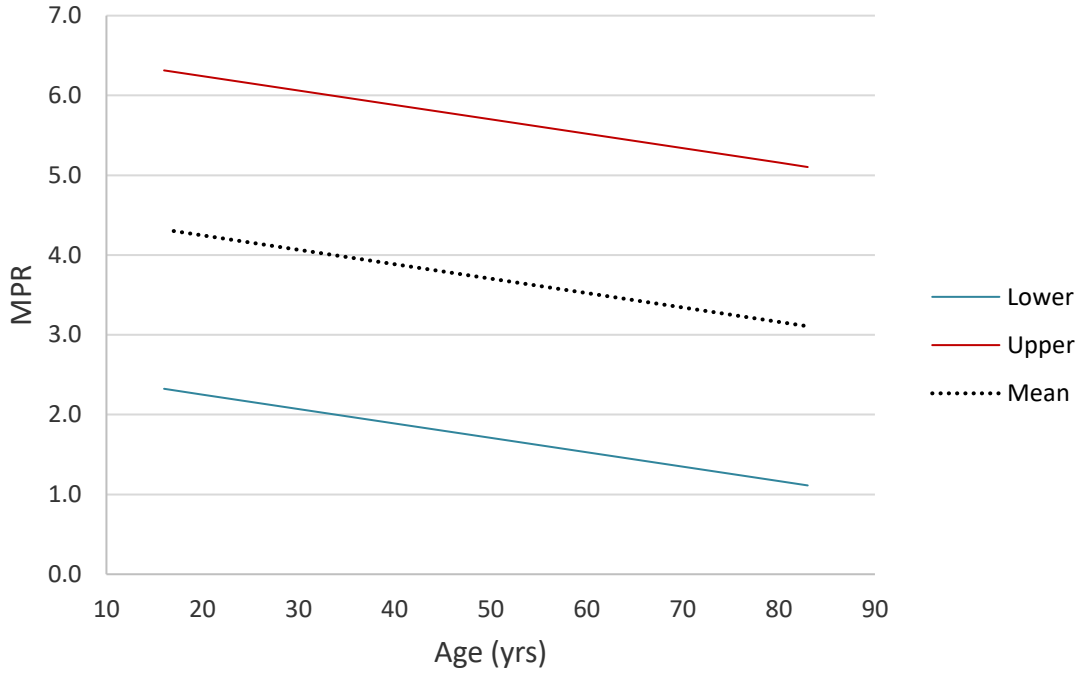
C - Stress MBF - Male



D - Stress MBF - Female



E - MPR – Male



F - MPR – Female

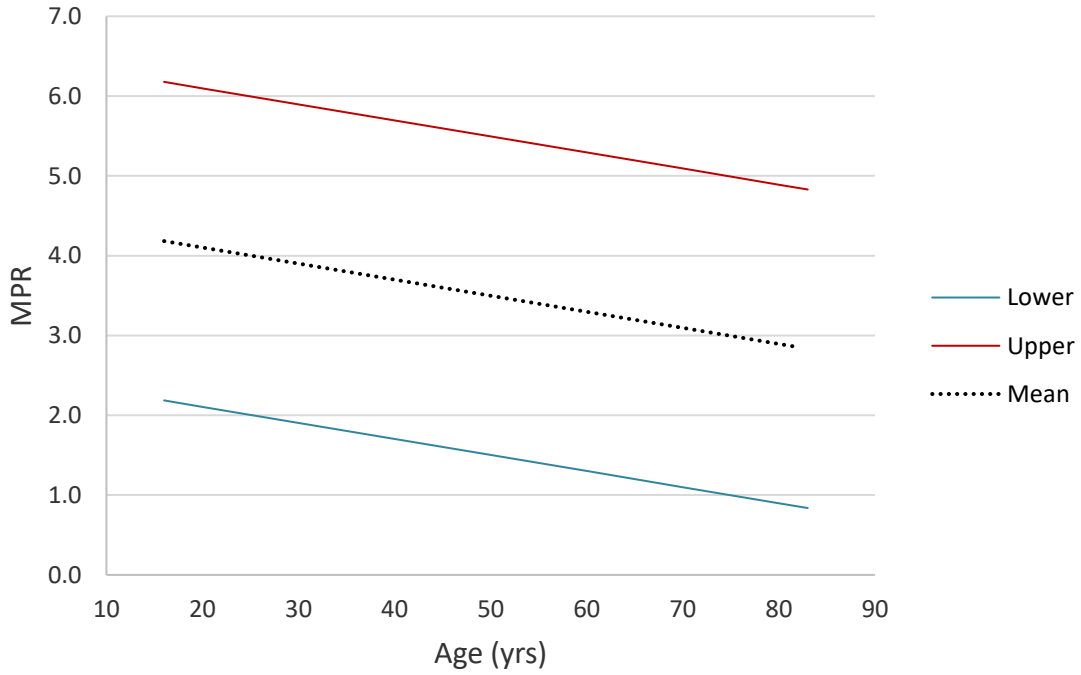


Figure 4-3 Normal reference ranges for MBF and MPR

Figures show mean values and the 95% confidence interval for the range, allowing for changes related to age.

MBF – myocardial blood flow, MPR – myocardial perfusion reserve

4.4 Discussion

This study reports the largest normal range of MBF and MPR for myocardial perfusion CMR in healthy volunteers, acquired with automated in-line perfusion mapping. We present the first normal values for myocardial perfusion CMR stratified by age and sex and report differences between coronary territories.

4.4.1 Global MBF

Previously published normal ranges of MBF and MPR obtained using PET and CMR (Table 6) illustrate the range of values obtained with these methods. Previous CMR studies have reported rest MBF values between 0.76 ±0.1ml/g/min and 1.24 ±0.19ml/g/min(172–176) with CV of up to 41%. In general, MBF estimates obtained by CMR have been higher than those for PET, although no previous large study of normal, healthy volunteers exists for CMR.

Reported rest MBF in PET have varied depending on the PET tracer(153,177), kinetic model(149) and methodology(178) used. The large number of confounders makes comparison between published data and modalities challenging. Values have ranged from 0.62 ±0.14ml/g/min to 1.10 ±0.2ml/g/min with CV between 15-23%. The most recent PET studies including healthy volunteers have reported rest MBF of 0.68 ±0.2ml/g/min(179) and 0.71 ±0.11ml/g/min(175), both more similar to our results.

Stress MBF by CMR has been reported to vary from 2.78 ±0.61ml/g/min to 4.50 ±0.91ml/g/min in previous smaller studies with CVs between 20 and 35%(172,174,176,180). Within PET studies, a wider range of normal values have been reported ranging from 1.97 ±0.45ml/g/min to 4.40 ±0.9ml/g/min and CV varying between 17 and 31%(175,181–183).

Previous values of MPR with CMR have ranged from 2.7 ±0.3 to 4.2 ±1.0 with CV between 11 and 32%(172,175,180) and PET values have varied between 3.75±1.24 and 4.46 ±1.43 with CV between 15 and 33%(183–185). The findings in the current study are in keeping with these previous values.

Table 4-6 - Summary of previous published data with larger groups of healthy volunteers

Author	Year	N	Age	Rest		Stress		MPR	
				MBF (ml/g/min)	CV (%)	MBF (ml/g/min)	CV (%)		CV (%)
CMR									
Vasu(172)	2013	15	21 ±3.5	1.04 (±0.24)	23	2.78 (±0.61)	22	2.7 (±0.3)	11
Fairbairn(174)	2014	19	22±4	0.97 (±0.4)	41	3.4 (±1.2)	35		
Tomiyama(175)	2015	20	28±8.9	0.76 (±0.1)	13	3.04 (±0.82)	27	4.13 (±1.33)	32
Motwani(176)	2015	30	22±2	1.24 (±0.19)	15	4.5 (±0.91)	20	3.63 (±0.95)	26
Knott(186)	2019	24	37	0.86		3.07			
Nickander(180)	2020	41	26±5	0.88±0.19	22	3.62±0.71	20	4.2±1.0	24
PET									
Chan(182)	1992	20	34±16	1.10 (±0.2)	18	4.40 (±0.9)	20		
Nagamachi(147)	1996	21	34±15	0.62 (±0.14)	23	1.97 (±0.45)	23		
Muzik(183)	1998	20	44±11	0.67 (±0.11)	17	2.85 (±0.49)	17	4.28 (±0.65)	15

Kaufmann(187)	2000	61	45±7	0.87 (±0.14)	16	3.63 (±1.02)	28	4.23 (±1.29)	30
Chareonthaitawee(184)	2001	169	46±12	0.99 (±0.23)	23	3.54 (±1.01)	29	3.75 (±1.24)	33
Sdringola(148)	2011	107	29±5	0.70 (±0.15)	21	2.75 (±0.58)	21	4.03 (±0.84)	21
Tomiyama(175)	2015	20	28±8.9	0.71 (±0.11)	15	3.09 (±0.97)	31	4.46 (±1.43)	32
Combined		567	36	0.89		3.32		3.92	
This Study		***150***	***49 (IQR 24-59)***	***0.62 (±0.13)***	***25***	***2.24 (±0.53)***	***26***	***3.74(±1.00)***	***29***

*Values are expressed as mean (±standard deviation). CV is calculated as standard deviation/mean *100(%) for ease of comparison between studies.*

CMR = cardiac magnetic resonance, CV = coefficient of variation, MBF = myocardial blood flow, MPR = myocardial perfusion reserve, PET = positron emission tomography.

Both stress MBF and MPR values have merit in assessing ischaemia. In our study, MPR showed greater variability between individuals than absolute stress values, which was expected as MPR is calculated as the ratio of two parameters, compounding variability from both of its constituents. In clinical PET studies, stress MBF has accurately detected the presence of significant coronary stenosis(188), with comparable accuracy as MPR to detect ischaemia(189). This, together with the better reproducibility of stress MBF demonstrated in both CMR and PET studies(148,167), supports the use of stress MBF rather than MPR for the diagnosis of ischaemia when using a single method of MBF quantification. When evaluating between modalities and methods however, MPR may be a more useful comparator, minimising the effect of different reference ranges between techniques – as seen in published studies (Table 6), the range of MPR seen in normal volunteers is considerably lower than that in either stress or rest MBF.

4.4.2 Regional MBF

We have demonstrated higher resting MBF within the LAD territory compared to the other coronary territories. This finding is consistent with some, but not all of the previous literature. PET data are usually interpreted on a segmental basis, dividing the myocardium into 4 quadrants (anterior, lateral, inferior, and septal). One large study (n=169) showed a significant difference in corrected rest MBF between regions, which was due to higher flow in the anterior (1.443 ±0.4113ml/g/min) and lateral segments (1.405 ±0.385ml/g/min), both attributable to the LAD territory, vs the inferior segment (1.232 ±0.322ml/g/min, $p<0.0001$ for both)(184).

Several other PET studies have shown highest flow values in the anterior, and lowest values in the inferior segments but have not undertaken statistical comparison (145,148,190). Invasive assessment of coronary flow has shown a higher rest flow within the LAD compared to the Cx coronary artery ($p=0.007$), and showed a similar difference during stress(191). The difference in invasive measurements was related to vessel diameter but not flow rate. Conversely, several other studies have shown no significant difference between regions(154,181,192), including previous CMR studies(176).

We also provide data on regional blood flow and MPR in AHA segments, (Supplementary Tables 1-3). To the best of our knowledge, segmental MBF for all 16 segments has not previously been published. These values have been provided as they may be integrated in future analysis algorithms for the diagnosis

of coronary disease. Whilst there is slightly more variance in values of segmental flow, as may be expected when comparing data from smaller regions, CV for both stress and rest MBF was less than 30% at segmental level.

4.4.3 Sex

Multiple studies have shown differences in MBF between sexes, consistently showing MBF at rest to be higher in females compared to males as in our data. This has been seen in both large CMR and PET studies including a MESA sub study of 222 asymptomatic patients, and a large PET study of 169 healthy volunteers, supported by other smaller and more recent data (180,184,193–195) .Invasively measured coronary flow has also been reported to show sex differences. In a study of 28 patients with angiographically normal coronary arteries, coronary flow indexed to LV mass was higher in females (0.996 ±0.236ml/g vs 0.854 ±0.337ml/g) although this difference was not assessed for significance (191).

We have also demonstrated a heightened haemodynamic response to adenosine, and an increased stress MBF in females compared to males, findings that have been previously reported in studies with larger proportions of female participants (180,193,194).

One large PET study showed no significant difference in hyperaemic blood flow between sexes, but the proportion of females was only 22%, limiting the study's ability to differentiate reliably between sexes. Others have shown no significance difference between MBF in both sexes at either rest or stress(170,196).

Our findings are in keeping with the previous studies that showed higher MBF at both stress and rest and an increased physiological response to pharmacological stress in females. The underlying cause for this difference is unclear. Although females had higher resting HR, when we corrected MBF for either HR or RPP, a significant difference remained and other mechanisms such as oestrogen levels, which can mediate coronary tone, may be at play(197,198). However, in the MESA study(193), correction of MBF for menopause status and hormone use did not remove differences in MBF between the sexes. The studies we quote that have compared MBF and seen no difference between the sexes comprise relatively small numbers (n=22(170) and n=14(196)). It is likely that intrinsic variability of MBF requires larger numbers to establish significant difference between groups.

These differences support the use of separate normal ranges for the sexes, narrowing the limits compared to a less homogeneous group.

4.4.4 Age

We have shown a decrease in both stress MBF and MPR with age, while no difference was seen in rest MBF. The findings are consistent with previous studies showing lower values in older age groups(199), or a decline in stress MBF with age (184,193,195). Findings from the MESA sub-study reported increasing rest RPP with age, a correlation that we have also demonstrated, suggesting a higher level of cardiac work required to maintain the same resting flow. No related correlation was seen between RPP at stress, potentially demonstrating a blunted stress response in age which is likely multifactorial including adenosine response and change in vascular structure and function associated with age. The correlations support the use of age-related values when establishing normal limits. The values for stress MBF in this study are at the lower end of those previously published, and it may be that the inclusion of a larger proportion of older volunteers accounts for some of this difference.

4.4.5 Study limitations

All normal data sets of values for MBF are influenced by physiological variation as well as variation within the model and analysis. While we aimed to minimise physiological effect as much as possible, not all factors may have been controlled for. Caffeine can affect coronary vasomotor tone at rest(162) and response to adenosine stress(163). Although we advised our volunteers to avoid caffeine for 24hrs prior to the scan, previous studies have demonstrated that up to 20% may still have detectable caffeine levels(164). We can however be confident that adequate stress was achieved through clinical monitoring and haemodynamic response. Volunteers did not undergo coronary angiography, therefore coronary arteries may not have been truly normal. All studies were performed using a FLASH pulse sequence and results for MBF may vary using other pulse sequences.

4.4.6 Conclusion

We have shown that quantitative CMR myocardial perfusion mapping produces values similar to those of the reference method PET and with a similar degree of variation. We have also demonstrated a significant difference in rest MBF

between sexes advocating the use of sex-specific reference ranges in diagnostic use and a reduction of stress MBF and MPR with advancing age.

Table 4-7 – Segmental rest myocardial blood flow values

	Overall		Male		Female	
AHA Segment	**MBF** (ml/g/min)	**CV** (%)	**MBF** (ml/g/min)	**CV** (%)	MBF (ml/g/min)	**CV** (%)
1	0.691±0.17	24.6	0.640±0.15	23.4	0.770±0.17	22.1
2	0.695±0.16	23.0	0.647±0.15	23.2	0.766±0.15	19.6
3	0.649±0.15	23.1	0.612±0.15	24.5	0.705±0.14	19.9
4	0.575±0.13	22.6	0.537±0.12	22.3	0.632±0.13	20.6
5	0.538±0.13	24.2	0.504±0.12	23.8	0.590±0.13	22.0
6	0.640±0.17	26.6	0.593±0.15	25.3	0.712±0.17	23.9
7	0.689±0.16	23.2	0.628±0.14	22.3	0.779±0.16	20.5
8	0.670±0.15	22.4	0.618±0.13	21.0	0.750±0.14	18.7
9	0.626±0.14	22.4	0.586±0.13	22.2	0.657±0.13	19.8
10	0.590±0.13	22.0	0.547±0.11	20.1	0.656±0.13	19.8
11	0.583±0.14	24.0	0.535±0.12	22.4	0.657±0.15	22.8
12	0.650±0.16	24.6	0.598±0.13	21.7	0.729±0.17	23.3
13	0.456±0.16	35.1	0.594±0.13	21.9	0.726±0.16	22.0
14	0.620±0.15	24.2	0.577±0.13	22.5	0.657±0.15	22.8
15	0.526±0.12	22.8	0.501±0.11	22.0	0.564±0.12	21.3
16	0.542±0.15	27.7	0.511±0.13	25.4	0.589±0.16	27.2

AHA =American Heart Association, CV = coefficient of variation, MBF = myocardial blood flow.

Table 4-8 – Segmental stress myocardial blood flow values

	Overall		Male		Female	
AHA segment	**MBF** (ml/g/min)	**CV** (%)	**MBF** (ml/g/min)	**CV** (%)	**MBF** (ml/g/min)	**CV** (%)
1	2.626±0.73	27.8	2.439±0.71	29.1	2.896±0.68	23.5
2	2.452±0.70	28.5	2.331±0.71	30.5	2.623±0.66	25.2
3	2.154±0.60	27.9	2.087±0.60	28.7	2.250±0.58	25.8
4	2.240±0.61	27.2	2.127±0.61	28.7	2.402±0.57	23.7
5	2.118±0.59	27.9	1.964±0.67	34.1	2.339±0.56	23.9
6	2.553±0.73	28.6	2.375±0.71	29.9	2.808±0.67	23.9
7	2.359±0.64	27.1	2.204±0.63	28.6	2.582±0.58	22.5
8	2.258±0.57	25.2	2.157±0.61	28.3	2.404±0.47	19.6
9	2.136±0.52	24.3	2.045±0.51	24.9	2.266±0.51	22.5
10	2.059±0.53	25.7	1.939±0.52	26.8	2.230±0.49	22.0
11	2.134±0.56	26.2	2.008±0.57	28.4	2.314±0.51	22.0
12	2.283±0.62	27.2	2.155±0.64	29.7	2.467±0.56	22.7
13	2.339±0.63	26.9	2.240±0.63	28.1	2.485±0.61	24.5
14	2.109±0.54	25.6	1.999±0.53	26.5	2.272±0.50	22.0
15	1.900±0.47	25.3	1.857±0.50	26.9	1.964±0.41	20.9
16	2.142±0.61	28.5	2.052±0.58	28.3	2.274±0.63	27.7

AHA =American Heart Association, CV = coefficient of variation, MBF = myocardial blood flow.

Table 4-9 – Segmental Myocardial perfusion reserve

	Overall		Male		Female	
AHA Segment	**MPR**	**CV (%)**	**MPR**	**CV (%)**	**MPR**	**CV (%)**
1	3.895±1.10	28.2	3.900±1.13	29.0	3.889±1.08	27.8
2	3.656±1.08	29.5	3.692±1.07	29.0	3.602±1.09	30.3
3	3.461±1.05	30.3	3.528±1.05	29.8	3.358±1.05	31.3
4	4.022±1.19	29.6	4.093±1.20	29.3	3.911±1.17	29.9
5	4.089±1.28	31.3	4.052±1.26	31.1	4.145±1.32	31.8
6	4.178±1.42	34.0	4.178±1.45	34.7	4.175±1.37	32.8
7	3.528±1.02	28.9	3.560±0.97	27.2	3.479±1.12	32.2
8	3.448±0.92	26.7	3.530±0.93	26.3	3.320±0.89	26.8
9	3.509±0.94	26.8	3.568±0.94	26.3	3.419±0.94	27.5
10	3.602±1.05	29.2	3.645±1.13	31.0	3.536±0.94	26.6
11	3.785±1.21	32.0	3.870±1.28	33.1	3.653±1.08	29.6
12	3.621±1.11	30.7	3.671±1.09	29.7	3.543±1.13	31.9
13	3.768±1.20	31.8	3.877±1.23	31.7	3.595±1.13	31.4
14	3.519±1.03	29.3	3.536±0.99	28.0	3.492±1.10	31.5
15	3.774±1.08	28.6	3.837±1.05	27.4	3.674±1.14	31.0
16	4.118±1.37	33.3	4.149±1.32	31.8	4.069±1.46	35.9

AHA =American Heart Association, CV = coefficient of variation, MBF = myocardial blood flow.

Chapter 5

A comparison of standard and high dose adenosine protocols in routine stress CMR: dosage affects hyperaemic myocardial blood flow in patients with severe LV systolic impairment.

5.1 Introduction

Stress perfusion cardiovascular magnetic resonance (CMR) is an accurate, non-invasive technique for the detection of myocardial ischaemia(23,200). The method is widely used in the assessment of patients with suspected or known coronary artery disease (CAD); either to detect ischaemia, or in the context of cardiac dysfunction to detect an underlying ischaemic cause.

Intravenous adenosine has been shown to induce near maximal hyperaemia(201) and is used for assessment of ischaemia in both invasive measurements such as fractional flow reserve (FFR) and non-invasive techniques including CMR and positron emission tomography (PET). For perfusion CMR, adenosine is the most commonly used pharmacological stress agent(22). Accepted protocols recommend administration of adenosine at a dose of 140 µg/kg/min with an increase up to 210 µg/kg/min if required to achieve adequate stress(142). The duration of adenosine infusion is standardised and usually given for at least 3 minutes prior to data acquisition, but it is not known if a longer duration or higher dose may produce a better response.

Conventionally, adequate stress is defined by a heart rate (HR) rise of ≥10bpm or a systolic blood pressure (SBP) fall of >10mmHg(142), based on the assumption that adenosine infusion leads to systemic vasodilation and reflex tachycardia. Recent studies have suggested however, that these peripheral measurements may not be a true reflection of myocardial hyperaemia and should not be used to assess adenosine response(202,203). In addition, certain patient groups including those with heart failure and diabetes mellitus (DM) have a blunted haemodynamic response to intravenous adenosine(204–206) and it is unclear to what extent this reduced response is reflected in coronary vasodilation.

Recently developed techniques of inline myocardial perfusion mapping with CMR provide accurate, reproducible assessment of rest and vasodilator stress myocardial blood flow (MBF) following adenosine administration(48,167,168). This

study looked to compare stress MBF with different dosing regimens of adenosine during stress perfusion CMR in patients with suspected CAD, heart failure and healthy controls.

5.2 Methods

5.2.1 Study population

Twenty healthy volunteers were recruited as controls to compare 3 adenosine perfusion protocols. Exclusion criteria were any known cardiovascular disease, hypertension, hyperlipidaemia, diabetes mellitus, smoking, BMI >30 and any contraindication to CMR, adenosine or gadolinium-based contrast agents.

Sixty patients with symptoms of angina or heart failure were recruited prospectively from CMR or coronary angiography waiting lists, for comparison of 2 adenosine protocols determined from the results in volunteers. Patients were divided into 3 groups for analysis consisting of; Group 1 - Patients with coronary disease and left ventricular ejection fraction (EF) ≥40%. Group 2 – Mild to moderate HF, EF ≥ 40% and no evidence of coronary disease, and Group 3 - Moderate to severe HF, EF <40% and no evidence of coronary disease. Exclusion criteria for patients were the presence of any contraindication to CMR, adenosine or gadolinium-based contrast agents. Coronary disease was defined by either ischaemic (subendocardial) LGE on CMR, at least moderate stenosis on coronary angiography, previous percutaneous coronary intervention or previous coronary arterial bypass grafting.

5.2.2 Study protocol

All CMR studies were undertaken on a 3T system (Siemens Magnetom Prisma, Erlangen, Germany). Participants were advised to avoid caffeine for 24 hours before the study. The protocol consisted of cine imaging, stress and rest perfusion, and late gadolinium enhancement as described in chapter 2.

For perfusion imaging, adenosine was infused at a set dose for a preassigned time. Healthy volunteers had 3 stress perfusion acquisitions: standard dose (140 µg/kg/min adenosine for 4 minutes), high dose (210 µg/kg/min for 4 minutes) and long dose (140 µg/kg/min for 8 minutes). Patients received standard dose and high dose for 4 minutes each. Doses were given in random order. A ten-minute

interval was kept between perfusion acquisitions, rest perfusion images were acquired 10 minutes after the final stress perfusion sequence in all participants.

Participants were monitored for symptoms throughout the scan. BP and HR were recorded prior to starting adenosine infusion and before acquisition. For each perfusion acquisition, an intravenous bolus of 0.05mmol/kg gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5ml/s followed by a 20ml saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer)..

5.2.3 Qualitative analysis

Ischaemic segments were identified on visual assessment of perfusion images. Splenic switch off was assessed by visually comparing enhancement of splenic tissue at stress and at rest. Splenic perfusion was defined as switched off if there was visually lower enhancement on stress images compared to rest, or failed switch off if there was similar enhancement(207) or if splenic enhancement was low during stress(208).

5.2.4 Quantitative analysis

Perfusion maps were analysed using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada) as described in chapter 2. Segments with ischaemic (subendocardial) LGE were also excluded from analysis of MBF. MBF values for all remaining segments were averaged to provide a value for global MBF.

5.2.5 Subgroup analyses

Subgroup analysis was carried out comparing patients with a HR change of <10bpm (non-responders) compared to those with a rise of ≥10bpm (responders). Within Group 1, segments with ischaemia on visual assessment were compared with non-ischaemic segments.

5.2.6 Statistical analysis

Analysis was performed using SPSS 23 (IBM SPSS, Armonk, NY, USA). Normality of distribution was assessed using Shapiro-Wilk test. Different dosing regimens were analysed using paired t-tests or Wilcoxson Signed Rank test in patients and analysis of variance (ANOVA) with repeated measures and post-hoc Bonferroni correction, or the Kruskal Wallis H test in healthy controls. Categorical data was analysed using chi-square test or Fisher's exact test when expected numbers were <5. All statistical tests were two-tailed and $p<0.05$ was considered significant.

Myocardial perfusion reserve (MPR) was calculated as stress MBF:rest MBF. Inadequate HR response was defined as <10bpm in keeping with Society for Cardiovascular Magnetic Resonance guidelines(142).

5.3 Results

5.3.1 Healthy controls

Twenty healthy control subjects were recruited, mean age 25 ±2.7 years, mean EF 57 ±3.3%. One volunteer withdrew after the first dose of adenosine had been given. Haemodynamic data were available for all three dosing regimens for 19 volunteers. In one case artefact on perfusion maps at standard dose meant these were not included in analysis of MBF response. The final cohort therefore consisted of 18 subjects.

5.3.1.1 Haemodynamic Response

Haemodynamic data are shown in Table 1. For all doses there was a significant rise in HR at stress from rest ($p<0.001$). In both standard and long dose protocols there was a significant increase in stress SBP (systolic blood pressure) from rest ($p<0.01$). There was no significant change in SBP between rest and high dose adenosine. Only one participant had a SBP decrease of ≥10mmHg at standard and high dose adenosine, and none had this degree of change with long dose.

There was a significant difference in stress HR between standard and high dose adenosine, but no difference between standard and long dose. No significant difference was seen in SBP or change in SBP between doses.

5.3.1.2 MBF

Global stress MBF was 2.50 ±0.74 ml/g/min with standard dose adenosine, with values of 2.66 ±0.59ml/g/min after high dose and 2.59 ±0.64ml/g/min with long dose, overall there was no significant difference between the three groups (p=0.323) (Figure 1).

Although there was no significant difference in MBF, the largest increases in HR were seen following the high dose regimen (Table 1), therefore this regime was chosen for comparison with standard dose in patients.

Table 5-1 – Haemodynamic response and MBF in healthy volunteers

	Adenosine dosing			
	140µg/kg/min 4 minute duration	**210µg/kg/min 4 minute duration**	**140µg/kg/min 8 minute duration**	**p**
Rest HR (bpm)	71.3 ±14.9	71.0 ±14.1	70.9 ±13.0	0.962
Rest sBP (mmHg)	117 ±11.8	118 ±13.5	117 ±13.4	0.481
Stress HR (bpm)	101.7 ±18.8	107.9 ±16.0	105.9 ±18.7	0.017
Stress sBP (mmHg)	121 ±15.6	121 ±16.4	124 ±17.3	0.195
Change in HR (bpm)	30.4 ±12.2	36.9 ±11.3	35.0 ±12.8	0.045
Change in sBP (mmHg)	4.7 ±6.3	2.5 ±8.5	7.1 ±11.0	0.072
Stress MBF (ml/g/min)	2.50 ±0.74	2.66 ±0.59	2.59 ±0.64	0.323
MPR	3.52 ±0.93	3.82 ±0.83	3.72 ±1.00	0.191

A significant difference was seen in stress HR and change in stress HR.

HR – heart rate, sBP – systolic blood pressure, MBF – myocardial blood flow.

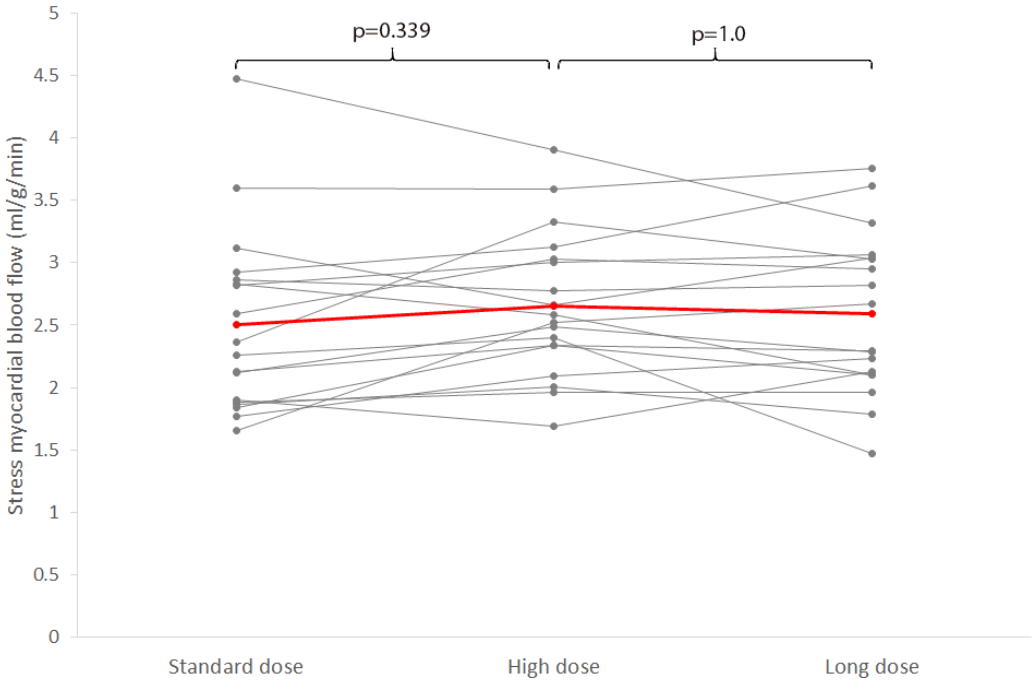


Figure 5-1 Results in healthy control group

No significant differences in MBF were seen between doses of adenosine despite significantly higher heart rate with high dose adenosine and long dose adenosine.

MBF – myocardial blood flow

5.3.2 Patients

Sixty patients took part in the study divided into 20 in Group 1 (coronary artery disease), 16 in Group 2 (mild to moderate HF, EF ≥40%) and 24 in Group 3 (moderate to severe HF, EF <40%). No significant differences were seen between the groups in incidence of diabetes mellitus, beta-blocker usage, age or sex (Table 2).

Table 5-2 – Characteristics of patient groups

	Group 1 n=20	**Group 2** n=16	**Group 3** n=24	**p**
Sex - male	17 (85%)	8 (50%)	17 (71%)	0.074
Age	62.9 ± 8.7	63.5 ± 16.3	65.1 ± 12.7	0.344
Diabetes mellitus	4 (20%)	0	6 (25%)	0.102
Beta blocker usage	14 (70%)	12 (75%)	17 (71%)	0.940
LV ejection fraction	57.5 ± 7.9	48.1 ± 5.2	26.2 ± 7.0	<0.001

Other than ejection fraction, no significant difference was seen between the groups in factors previously reported to affect adenosine response.

Group 1 - Patients with coronary disease and left ventricular ejection fraction (EF) >40%. Group 2 – Mild to moderate HF, EF ≥ 40% and no evidence of coronary disease. Group 3 - moderate to severe HF, EF <40% and no evidence of coronary disease.

5.3.2.1 Haemodynamic Response

Haemodynamic data are shown in Table 3. Mean stress HR increased significantly from mean rest HR in all groups and following both standard and high dose adenosine ($p<0.01$). There was no significant change between stress and rest SBP in any group. In total, 36 (60%) patients had a HR rise ≥10bpm with standard dose adenosine, and 42 (70%) with high dose. One (2%) patient had a SBP decrease of ≥10mmHg with standard dose, and 2 (3%) with high dose. In each group, there was a similar proportion of non-responders.

Within group 1, stress HR was significantly higher following high dose compared with standard dose adenosine (77.5 ±12.5bpm vs 74.6 ±13.0bpm, $p=0.025$), but no significant difference was seen in groups 2 or 3 (Figure 2). Stress SBP was significantly lower following high dose adenosine compared to standard dose in group 2 (119.5 ±12.6mmHg vs 124.1 ±11.3mmHg, $p=0.005$). There was no significant difference in stress SBP between doses in the other groups.

No significant difference was seen in absolute HR rise between the groups and no significant correlation was seen between EF and HR rise across all 60 patients ($r=0.122$, $p=0.353$).

Table 5-3 - Haemodynamics and response to adenosine

	CAD n = 20			**HF EF≥40%** n=16			**HF EF<40** n=24			**Comparison between groups** (p)	
	Standard dose	High dose	p	Standard dose	High dose	p	Standard dose	High dose	p	Standard dose	High dose
Rest HR (bpm)	62.3 ± 10.1	62.9 ± 8.8	0.534	64.7 ± 14.3	64.7 ± 12.9	1.000	75.2 ± 12.4	76.5 ± 13.7	0.547	0.002	0.001
Rest sBP (mmHg)	131.7 ± 16.9	131.1 ± 16.3	0.735	124.2 ± 16.1	123.9 ± 16.8	0.320	119.0 ± 16.0	119.0 ± 16.4	0.526	0.066	0.089
Stress HR (bpm)	74.6 ± 13.0	77.5 ± 12.5	0.027	78.6 ± 20.3	79.8 ± 17.3	0.659	87.3 ± 16.0	89.6 ± 18.2	0.418	0.039	0.038
Stress sBP (mmHg)	123.5 ±14.9	121.1 ±12.1	0.219	124.1 ±11.3	119.5 ±12.6	0.005	121.5 ±18.0	123.5 ± 18.5	0.391	0.597	0.970
Change in HR (bpm)	12.3 ± 9.7	14.6 ± 8.3	0.112	13.9 ± 13.7	15.1 ± 8.7	0.637	12.1 ± 11.7	13.1 ± 18.8	0.751	0.875	0.889
Change in SBP (mmHg)	-2.1 ± 9.7	-6.1 ± 11.2	0.455	-1.6 ± 12.7	-1.9 ± 11.4	0.139	2.4 ± 8.4	3.2 ± 12.9	0.700	0.377	0.072

Non-responders (<10bpm)	8 (40%)	4 (20%)	0.168	6 (38%)	3 (19%)	0.433	10 (42%)	11 (46%)	0.771	0.966	0.097
Failed splenic switch off	2 (10%)	0	0.487	1 (6%)	0	1	2 (8%)	2 (8%)	1	1.0	0.334

Resting HR was significantly higher in the moderate-severe HF group, no difference was seen between groups in rate of non-responders or absolute measures of haemodynamic response to adenosine.

Group 1 - Patients with coronary disease and left ventricular ejection fraction (EF) >40%. Group 2 – Mild to moderate HF, EF ≥ 40% and no evidence of coronary disease. Group 3 - moderate to severe HF, EF <40% and no evidence of coronary disease. Standard dose – 140 µg/kg/min. High dose – 210 µg/kg/min. HR – heart rate, sBP – systolic blood pressure.

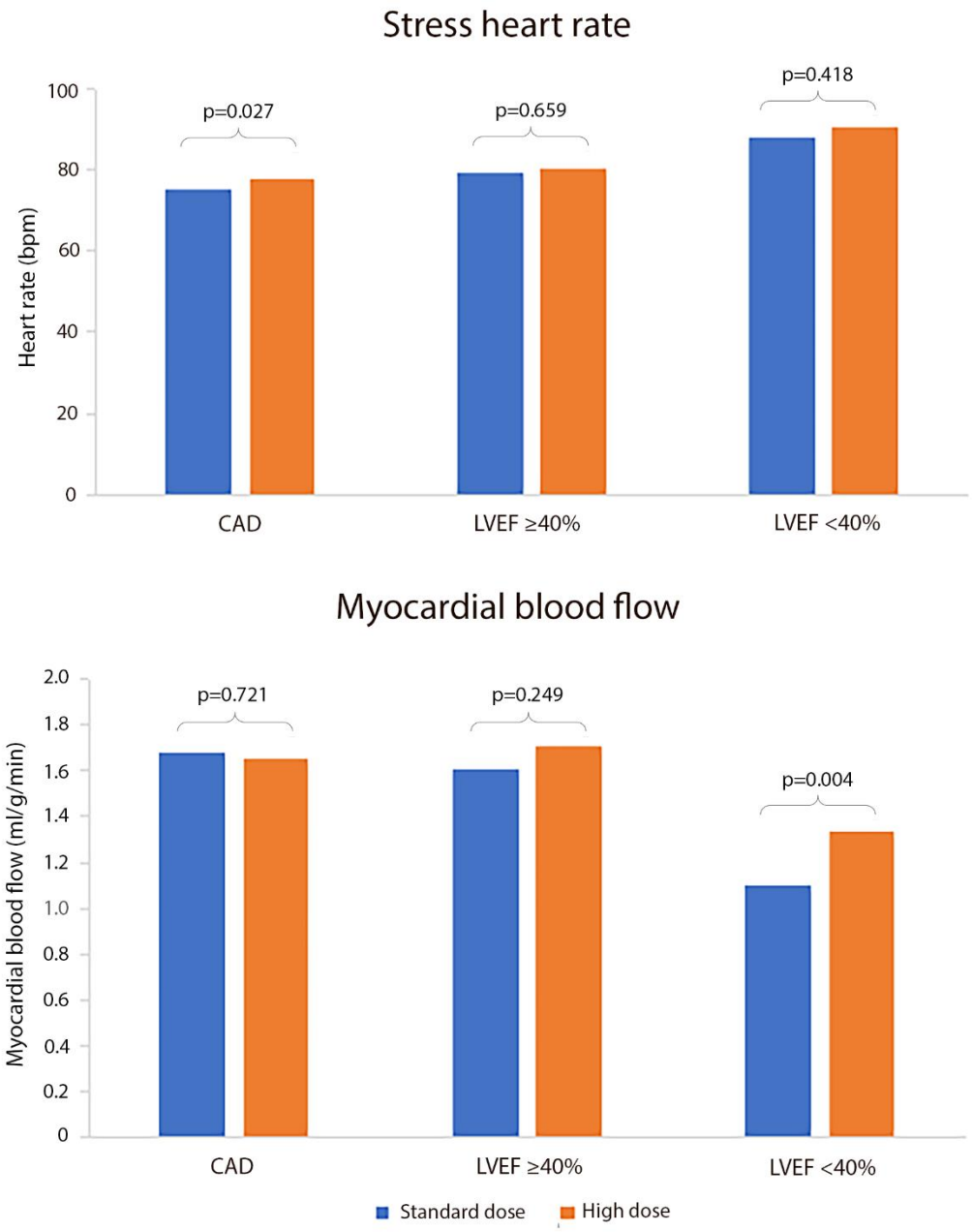


Figure 5-2 Stress heart rate and myocardial blood flow

No difference was seen in stress heart rate between doses. In those with moderate to severe heart failure, stress MBF was higher with higher dose adenosine.

CAD – coronary artery disease, LVEF – left ventricular ejection fraction

5.3.2.2 MBF and MPR

No significant difference was seen in MBF or MPR between different adenosine doses in either Group 1 or 2. Within Group 3, MBF was significantly higher following high dose than after standard dose adenosine (standard dose 1.10 ±0.47ml/g/min vs high dose 1.33 ±0.46 ml/g/min, p=0.04). MPR demonstrated the same pattern (standard dose 1.90 ±0.88 vs high dose 2.26 ±0.9, p=0.04) (Figure 2 and 3).

Bland Altman plots (Figure 4) show the spread of differences in MBF between adenosine doses and those with and without adequate response to adenosine at standard dose.

There were weak correlations between increase in HR and MPR both with standard (r=0.266, p=0.045) and high dose adenosine (r=0.535, p<0.001). HR response with standard dose adenosine did not correlate with an increase in MBF or MPR with high dose adenosine.

5 patients demonstrated failed splenic switch off with standard dose adenosine (Table 3). Analysis was repeated, excluding those with failed splenic switch off. These showed the same pattern with no difference in stress MBF or MPR between standard and high dose adenosine in Group 1 and Group 2, but higher stress MBF (1.15 ±0.46ml/g/min vs 1.38 ±0.45ml/g/min, p=0.009) and MPR (1.99 ±0.88 vs 2.34 ±0.91, p=0.009) in Group 3.

5.3.3 Subgroup analyses

5.3.3.1 Heart rate response to adenosine

Patients were divided into groups of non-responders (n=24) and responders (n=36) based on a heart rate increase of <10bpm or ≥10bpm. There was no significant difference in EF, age or incidence of diabetes or beta blocker usage between the two groups. Stress HR was significantly different between groups both with standard and high dose adenosine (69.8 ±14.0bpm vs 88.1 ± 14.8bpm, p=<0.001 at standard dose and 74.5 ±12.0bpm vs 88.6 ± 17.5bpm, p=0.001 at high dose). There was no significant difference in rest heart rate or stress MBF between the 2 groups at either adenosine dose.

In the non-responder group, stress HR was significantly higher with high dose adenosine than with standard dose (Table 4). Those with adequate HR response

to standard dose did not have a significant increase in stress HR between doses. No significant difference was seen in stress MBF between standard and high dose adenosine regardless of adequate HR response to standard dose adenosine.

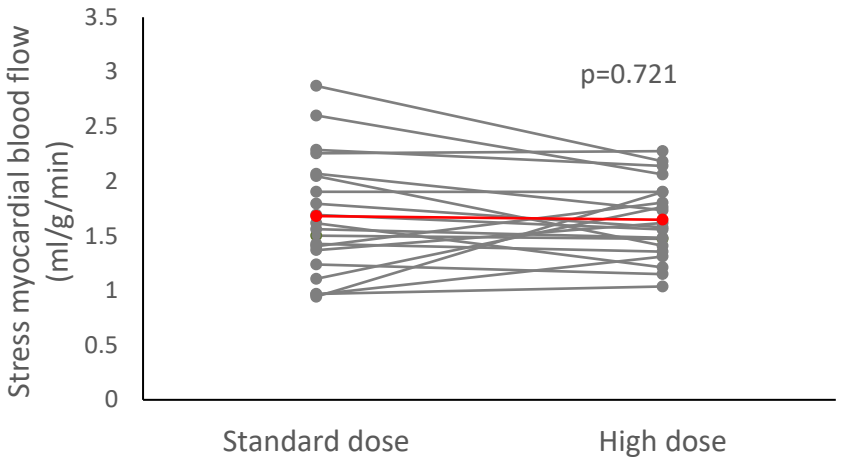
Table 5-4 - Difference in response to adenosine doses divided by HR response to standard dose

	Standard Dose	High Dose	p
Non-responders (n=24)			
Rest HR (bpm)	67.1 ±15.6	67.5 ±14.4	0.789
Stress HR (bpm)	69.8 ±14.4	74.5 ±12.0	0.034
Increase in HR (bpm)	2.63 ±5.71	6.96 ±11.2	0.053
Stress MBF (ml/g/min)	1.351 ±0.494	1.447 ±0.463	0.188
MPR	1.927 ±0.77	2.046 ±0.70	0.215
Adequate HR response (n=36)			
Rest HR (bpm)	68.7 ±11.9	69.7 ±12.9	0.461
Stress HR (bpm)	88.1 ±14.8	88.6 ±17.5	0.762
Increase in HR (bpm)	19.3 ±9.3	18.9 ±12.8	0.838
Stress MBF (ml/g/min)	1.479 ±0.598	1.592 ±0.429	0.103
MPR	2.324 ±0.856	2.527 ±0.634	0.073

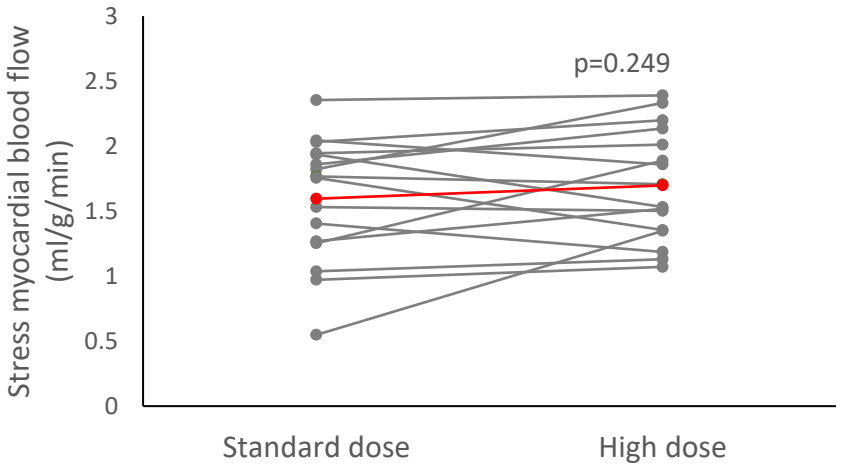
Stress HR was significantly higher with high dose adenosine in the non-responder group only. Non-responders and responders were defined by heart rate increase with adenosine of <10bpm or ≥10bpm. There data are from patients only. No difference was seen in stress MBF in either group.

HR – heart rate, MBF – myocardial blood flow, MPR – myocardial perfusion reserve.

Group 1 – Coronary artery disease



Group 2 – Heart failure, EF ≥40%



Group 3 – Heart failure, EF <40%

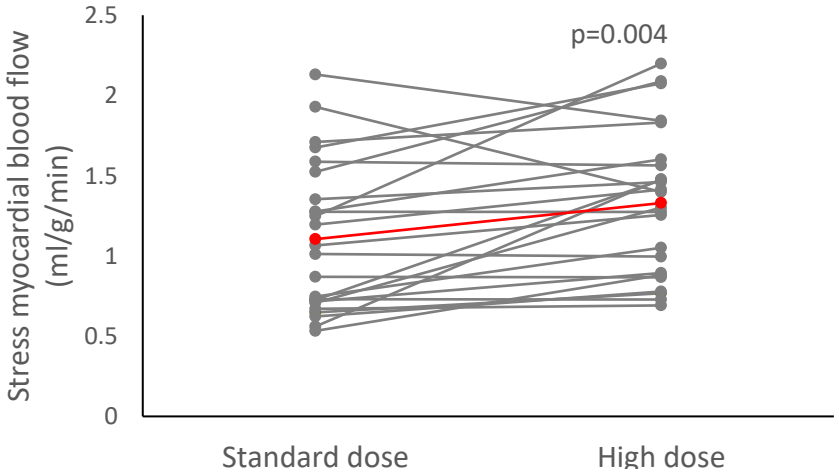


Figure 5-3 Difference in stress MBF between doses

Within Group 3 (moderate-severe HF) stress MBF was significantly higher with high dose adenosine compared to standard dose.

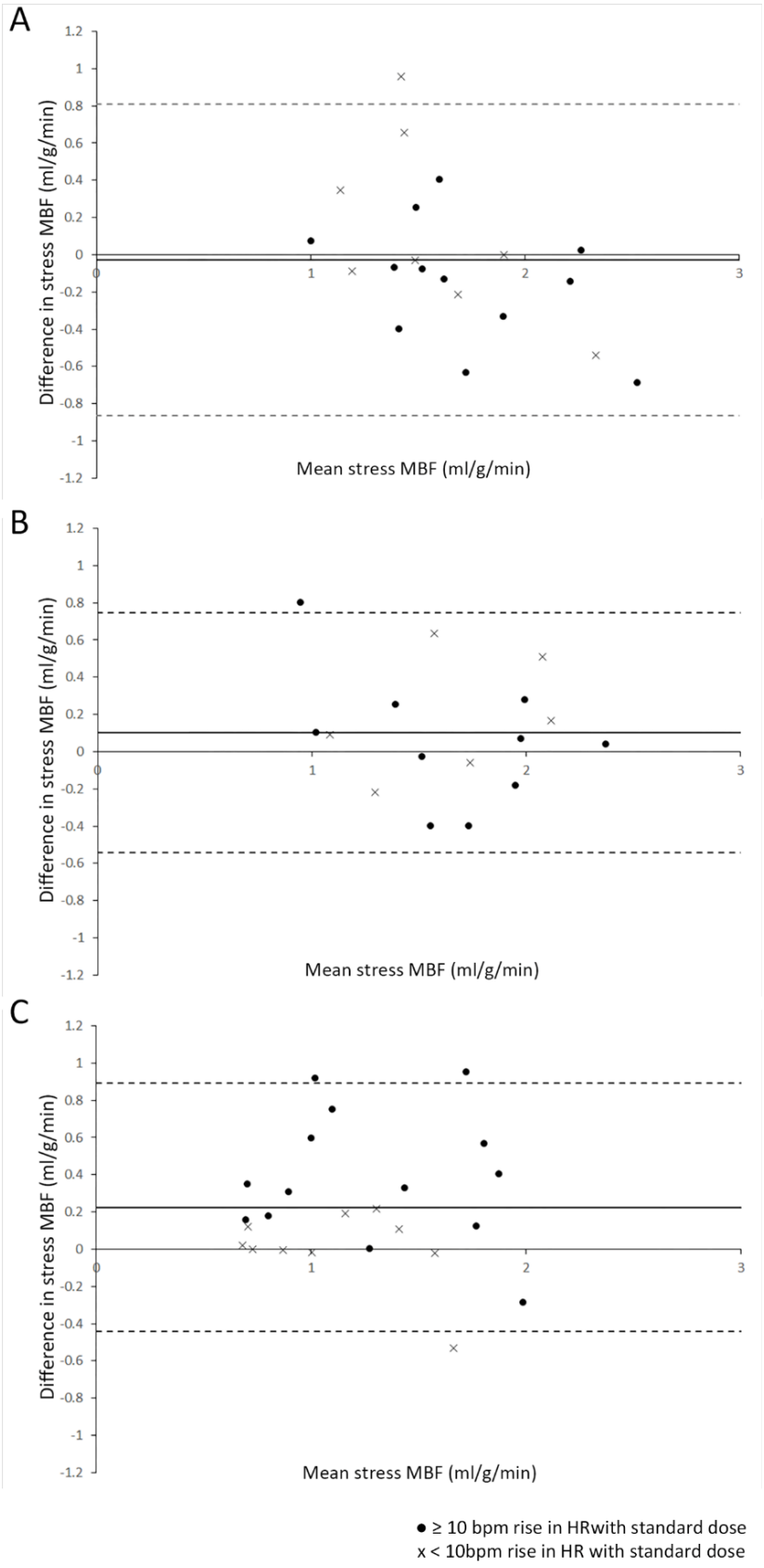


Figure 5-4 Difference in MBF between doses

In Group 3, stress MBF is significantly higher following high dose adenosine, this effect does not appear to be related to HR response at standard dose.

5.3.3.2 Ischaemia

Within group 1, eleven patients had evidence of inducible ischaemia. On visual analysis, ischaemia was seen in the same coronary territories between the adenosine doses. A total of 60 ischaemic segments were visually identified following standard dose adenosine, and 63 segments following high dose. No significant difference was seen in MBF in between standard and high dose adenosine in either the ischaemic (1.461 ±0.295ml/g/min vs 1.477 ±0.402 ml/g/min, p= 0.697) or non-ischaemic segments (1.716 ±0.568ml/g/min vs 1.768 ±0.524 ml/g/min, p=0.130) in these patients.

5.4 Discussion

The Society for Cardiovascular Magnetic Resonance Standardized CMR imaging protocols for stress perfusion recommend an adenosine dose of 140μg/kg body weight/min for 2–4 min with an increase in the dose if there is inadequate HR and BP response(142). Our results inform several aspects of this recommendation: the duration of adenosine infusion, the dose of adenosine and the use of HR and BP as indicators of adequate response. In healthy controls, increased dose or extended duration adenosine were not associated with significant changes in stress MBF compared with standard dose adenosine. Equally, in patients with normal or mildly impaired LV function there was no effect of higher dose adenosine, but in those with moderate to severe heart failure (EF ≤ 40%), higher dose adenosine produced higher stress MBF. We further show that HR and BP are unreliable markers of haemodynamic response.

5.4.1 Duration of adenosine infusion

The duration of adenosine infusion has not previously been studied for stress perfusion CMR but has been the subject of studies in nuclear cardiology. In Single Photon Emission Tomography, a 3-minute adenosine infusion showed better tolerability with similar diagnostic performance compared with a 6-minute protocol(209). A PET study using Rb-82 compared several adenosine regimes in 127 subjects and found that a 6-minute adenosine infusion protocol with Rb-82 activation at 3 minutes was associated with 11.4% higher stress MBF and 15.7% higher coronary flow reserve (CFR) than a 4-minute adenosine infusion with Rb-82 activation at 2 minutes (185). Further extension of the adenosine infusion time prior to Rb-82 activation did not increase increased stress MBF or CFR further.

These results are not directly applicable to CMR due to the differences in tracer kinetics and data capture between PET and CMR. Our data show that in myocardial perfusion CMR, there is no significant difference in haemodynamic response and no change in quantitative MBF between a 4 minute and an extended 8 minute adenosine protocol in healthy volunteers. These results suggest that the shorter duration protocols that are in current clinical use and recommended in current guidance are adequate for myocardial perfusion CMR.

5.4.2 Dose of adenosine infusion

The dose of adenosine infusion has been studied more extensively, using multiple modalities. Early invasive studies using intracoronary Doppler assessment of coronary blood flow velocity and total coronary resistance showed that intravenous adenosine at doses of 140 µg/kg/min resulted in maximal hyperaemia, defined by papaverine response, in 84% of subjects(201). Several invasive studies have assessed the effect of adenosine dose on fractional flow reserve (FFR) with higher doses showing no significant change in FFR compared with lower doses(210–212). In a CMR study, Karamitsos et al showed that a stepwise increase in the adenosine dose from 140 µg/kg/min to 210 µg/kg/min is safe and increases the rate of patients with an adequate haemodynamic response(213). However, the MBF response to different adenosine doses has not previously compared using quantitative myocardial perfusion CMR. In addition to studying the same individuals repeatedly, we obtained MBF values at different adenosine doses in the same imaging session. This approach overcomes the potential confounders of day-to-day physiological variation in haemodynamic response and allows direct comparison of dose effects. Our data show no significant difference in MBF following standard and high dose adenosine in healthy volunteers and patients with coronary disease or heart failure with EF ≥40%, suggesting that irrespective of haemodynamic response, standard dose adenosine in these groups reliably induces maximal hyperaemia.

5.4.3 Impaired LV function

In patients with severe systolic impairment, previous studies have shown a blunted heart rate response to adenosine, with an increase in adenosine from 140 µg/kg/min to 210 µg/kg/min more commonly required to achieve a sufficient haemodynamic response(204). In CMR, a previous study reported LV EF< 57% as an independent predictor of inadequate haemodynamic response to standard

adenosine dose(213). Within our patient cohort, there was no correlation between EF and HR rise, and no difference in HR rise between the patient groups, or any significant difference in the rate of non-responders. However, our study showed for the first time that among patients with heart failure and significant LV systolic impairment (EF≤40%), stress MBF increases with higher doses of adenosine, suggesting that standard dose regimes fail to induce maximal hyperaemia and are not appropriate in these patients. A pattern of decreased response to adenosine in heart failure requiring higher doses to achieve stress has previously been suggested(204). Potential mechanisms for the lower adenosine effect include the downregulation of gene expression of both adenosine receptors and adenosine deaminase (ADA) in impaired myocardium, together with a decrease in the activity of ADA(214,215). Increased levels of cardiac adenosine have also been measured in chronic heart failure patients, and this higher endogenous level may explain the requirement for higher exogenous doses to achieve the anticipated vasodilation required in stress testing(214,215).

We found that both with standard and high dose adenosine, failed splenic switch off was relatively common. It was observed more at both doses in HF patients. Our overall findings of higher MBF with higher dose adenosine in HF patients was observed regardless of the presence of splenic switch off. These findings raise questions of the mechanism of the apparent reduced adenosine sensitivity in HF patients but also the reliability of splenic switch off as a marker of adenosine response in the presence of LV impairment.

5.4.4 Haemodynamic response

In non-invasive testing, response to adenosine and the achievement of hyperaemia is commonly assessed using haemodynamic response relating to peripheral vasodilation. Conventionally, an increase in HR by >10 bpm and a fall in systolic BP by >10mmHg are considered markers of adequate hyperaemia(142). A small previous PET perfusion study suggested a correlation between HR response and stress MBF(185), but confounders such as ejection fraction were not explicitly considered. In an earlier larger PET study, change in HR correlated poorly with stress MBF, and not with coronary flow reserve (CFR), leading the authors to suggest that peripheral haemodynamic changes could not be used to assess the adequacy of response to adenosine(202). No CMR studies have previously looked at haemodynamic response and change in quantitated MBF.

Within our study, 61% of those patients with EF >40% reached the threshold of 10bpm, comparable to published results in other studies with a similar patient group(204). This relatively low response rate may be due to the presence of medications and other co-morbidities in our patient cohort. Blunted haemodynamic response to adenosine has been reported in DM, beta-blocker usage and CAD as well as impaired LVEF (204–206,213,216). Increase in heart rate correlated only weakly with MPR and no significant difference was seen in stress MBF between groups classified by heart rate response.

This study showed no significant relationship between rise in HR at standard dose adenosine and an increase in stress MBF or MPR with high dose adenosine. This indicates that in patients with a low HR response at standard dose adenosine, a higher dose does not increase myocardial perfusion – a finding that questions the validity of current guideline recommendations and widely used clinical practice.

Further, we saw no significant difference in BP change between groups of patients, or adenosine doses and <3% of patients had a decrease in SBP of ≥10mmHg as described in standard protocols. Systolic blood pressure even increased in healthy controls over baseline. These data suggest, in keeping with previous studies, that in particular BP response cannot be used as a marker of adequate vasodilator response, possibly due to an adrenergic response to adenosine symptoms, which overcomes the vasodilator effects on BP.

5.4.5 Clinical implications

Our data suggest that those with poor EF should have higher dose adenosine to achieve maximal hyperaemia, regardless of haemodynamic response. Our data also question the use of HR and BP response to standard dose adenosine as criteria to increase the adenosine dose as it does not appear to increase MBF. However, due to low numbers of patients with inducible ischaemia in this study we have not been able to assess the diagnostic impact of our observations although we observed a small, non-significant, increase in the number of ischaemic segments identified following high dose adenosine.

5.4.6 Study limitations

Our data may be influenced by physiological variation, although we have tried to minimise this. It is possible some effects may not have been controlled for, although we have previously demonstrated no significant difference in serial

measurements of stress MBF within a CMR study(167). Caffeine has been demonstrated to affect adenosine stress perfusion CMR(163). Although we advised our volunteers to avoid caffeine for 24hrs prior to the scan, previous studies have demonstrated that up to 20% may still have detectable caffeine levels(164) and we cannot account for how these may be distributed between our patient groups in this study. The age range of our healthy volunteers was considerably lower than those of the patient groups, if age influences the response to adenosine then the results from these volunteers may not be applied to our patient groups. There was no significant difference in mean ages between the patient groups. Our data cannot exclude that a higher dose of adenosine than 210µg/kg/min might further increase stress MBF in those with LVEF <40%, but our data also cannot be extrapolated to support this possibility. Although the use of doses in excess of 210 µg/kg/min are not used in routine practice, future studies should explore higher doses in particular in HF patients.

5.4.7 Conclusions

Increasing adenosine dose is well tolerated and related to increased stress myocardial blood flow in patients with significant LV impairment. Achievement of adequate myocardial vasodilator response, assessed by quantitative perfusion, is not significantly related to peripheral haemodynamic measurements BP and HR. These observations may impact future practice guidelines for stress perfusion CMR. Dosage of adenosine in clinical perfusion assessment should be carefully considered and may need to be increased in subsets of patients, in particular those with severely impaired LV function, or alternative stress agents considered.

Chapter 6
Aetiology of heart failure with mid-range ejection fraction: Deep phenotyping using CMR

6.1 Introduction

Heart failure has historically been classified based on left ventricular (LV) ejection fraction (EF) and divided into two groups: Heart failure with reduced ejection fraction (HFrEF) and heart failure with preserved ejection fraction (HFpEF). The 2016 ESC Heart Failure Guidelines defined a new, middle, category: heart failure with mid-range ejection fraction (HFmrEF) of 40-50% between HFrEF (EF<40%) and HFpEF (EF≥50%)(217).

Both HFrEF and HFpEF have been shown to have different patient characteristics, outcomes and therapeutic response(218,219). Within clinical trials, the HFmrEF group have often been excluded, split or grouped with HFpEF (219,220). The ESC guidelines highlight this new category as an area with a gap in evidence and advocate research into underlying characteristics, pathophysiology and diagnosis.

Since the publication of these guidelines, the concept of a categorical classification of heart failure based solely on LVEF has been challenged reflecting overlap in epidemiology, pathophysiology and clinical manifestation across the spectrum of heart failure and the currently defined subtypes. Large epidemiological registry studies have shown HFmrEF to most closely resemble HFrEF in terms of age, gender and presence of ischaemic heart disease, while in the majority of other characteristics, it has been an intermediate group(221–223).

Cardiovascular magnetic resonance (CMR) tissue characterisation has previously been used in HF to identify high risk features such as ischaemic scar, focal and diffuse fibrosis. These characteristics have not previously been described for HFmrEF. However, across the HF spectrum they have been shown to be potential markers of prognosis and may be helpful in guiding therapy.

We aimed to use CMR to define the cardiac phenotype of presumed non-ischaemic HFmrEF specifically comparing the prevalence of occult ischaemic heart disease and tissue characteristics to HFpEF and HFrEF.

6.2 Methods

6.2.1 Study population

300 patients, seen in outpatient cardiology clinics and referred for a CMR scan following a new, clinical diagnosis of heart failure were prospectively recruited. Patients were excluded if they had a known history of coronary artery disease (stenosis >70% on angiography, myocardial infarction, previous percutaneous coronary intervention or coronary artery bypass grafting) or symptoms of angina. Other exclusion criteria included hypertrophic cardiomyopathy, amyloidosis, congenital heart disease, suspected acute pathology such as myocarditis, advanced renal failure, or contraindication to CMR or gadolinium-based contrast agents. In addition, 25 healthy controls were recruited who underwent CMR with an identical protocol. Patients were divided using into classifications of HFpEF (LVEF ≥50%), HFmrEF (LVEF 40-50%) and HFrEF (LVEF <40%) using the LVEF measured from their CMR scan.

6.2.2 Patient characteristics

Patients underwent clinical assessment on the day of their CMR appointment, including medical history, New York Heart Association (NYHA) functional class, risk factors and medical management. In patients with a LVEF >50%, a H2FPEF score(224) was calculated using previous echocardiogram results, where available, and medical history. Haematocrit (Hct) was measured from a blood sample taken at the time of the CMR scan.

6.2.3 Study protocol

All CMR studies were undertaken on a 3T system (Siemens Magnetom Prisma, Erlangen, Germany). Participants were advised to avoid caffeine for 24 hours before the study. To gain a complete phenotype, the protocol (Figure 1) consisted of cine imaging, T1 mapping using a MOdified Look Locker Inversion recovery (MOLLI) sequence (native 5(3)3; post-contrast 4(1)3(1)2), stress and rest perfusion using free breathing, motion corrected (MOCO) automated in-line perfusion mapping(48), and MOCO bright blood late gadolinium enhancement (LGE).

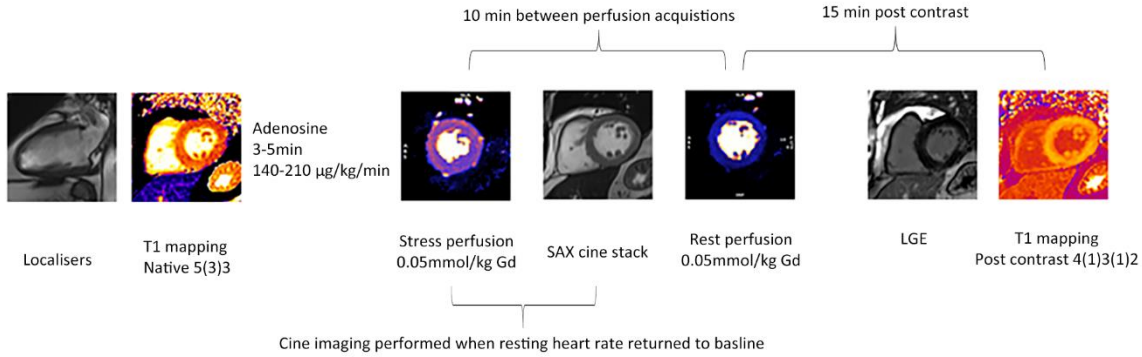


Figure 6-1 CMR protocol

Study protocol consisted of parametric mapping of native T1 and post-contrast T1, and adenosine stress and rest myocardial perfusion. Long axis cines and a short axis stack were used to obtain volumes and myocardial mass. LGE imaging identified ischaemic and non-ischaemic scar.

LGE images were acquired as a SAX stack and in 4 and 2 chamber views. When it was unclear if enhancement seen on bright blood LGE was ischaemic a dark blood LGE stack was also acquired(225).

For perfusion imaging, adenosine was infused for a minimum of 3 minutes, at a rate of 140 µg/kg/min and increased up to a maximum of 210 µg/kg/min according to haemodynamic and symptomatic response. Images were acquired over 90 dynamics to allow for poor ventricular function. A minimum ten-minute interval was kept between perfusion acquisitions.

Blood pressure and heart rate were recorded during adenosine infusion. For perfusion imaging, an intravenous bolus of 0.05mmol/kg gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5ml/s followed by a 20ml saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer). Perfusion mapping was performed and implemented on the scanner using the Gadgetron streaming software image reconstruction framework(48).

6.2.4 Qualitative analysis

LGE was reported if enhancement was identified on two orthogonal planes or where available on both bright and dark blood LGE images. Ischaemic LGE was defined as involving the subendocardium in a typical coronary distribution while non-ischaemic LGE did not involve the subendocardium. Inducible ischaemia was defined as a visual perfusion defect affecting >1 segment present at stress, but not at rest or matching infarct on LGE imaging, in a coronary distribution.

6.2.5 Quantitative analysis

T1 and perfusion maps were analysed using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada) as described in Chapter 2. In order to report global microvascular function (rather than the effects of occult coronary artery disease or replacement fibrosis) segments with ischaemia or late gadolinium enhancement were also excluded from analysis. T1 times and MBF values for all remaining segments were averaged to provide a global value.

Myocardial perfusion reserve (MPR) was calculated as stress:rest MBF. ECV was calculated using the formula “myocardial ECV = (1 − Hct) × (ΔR1myocardium/ΔR1blood), where R1 = 1/T1”. These were calculated for each segment and averaged to provide a global value.

6.2.6 Statistical analysis

Analysis was performed using SPSS 23 (IBM SPSS, Armonk, NY, USA). Normality of distribution was assessed using the Shapiro-Wilk test. Data is presented as mean (±standard deviation) for continuous data and number (percentage) for categorical data,

Continuous variables were analysed using analysis of variance (ANOVA) with post-hoc Bonferroni correction, Kruskal Wallis H test or Mann-Whitney U test. Categorical data was analysed using chi-square test. Correlation was assessed using Pearson r correlation. Statistical tests were two-tailed and $p<0.05$ was considered significant.

6.3 Results

Of the 300 patients that were prospectively recruited, 13 were excluded from analysis because of adenosine contraindications (n=4), data that could not be

analysed (n=4,) or because of discovered exclusion criteria (n=5), (CONSORT diagram, Figure 2).

Of the 287 patients included in the final analysis, 134 (46.7%) met criteria for HFrEF, 93 (32.4%) had HFmrEF and 60 (20.9%) had HFpEF (HFpEF score median 3, IQR 2-5).

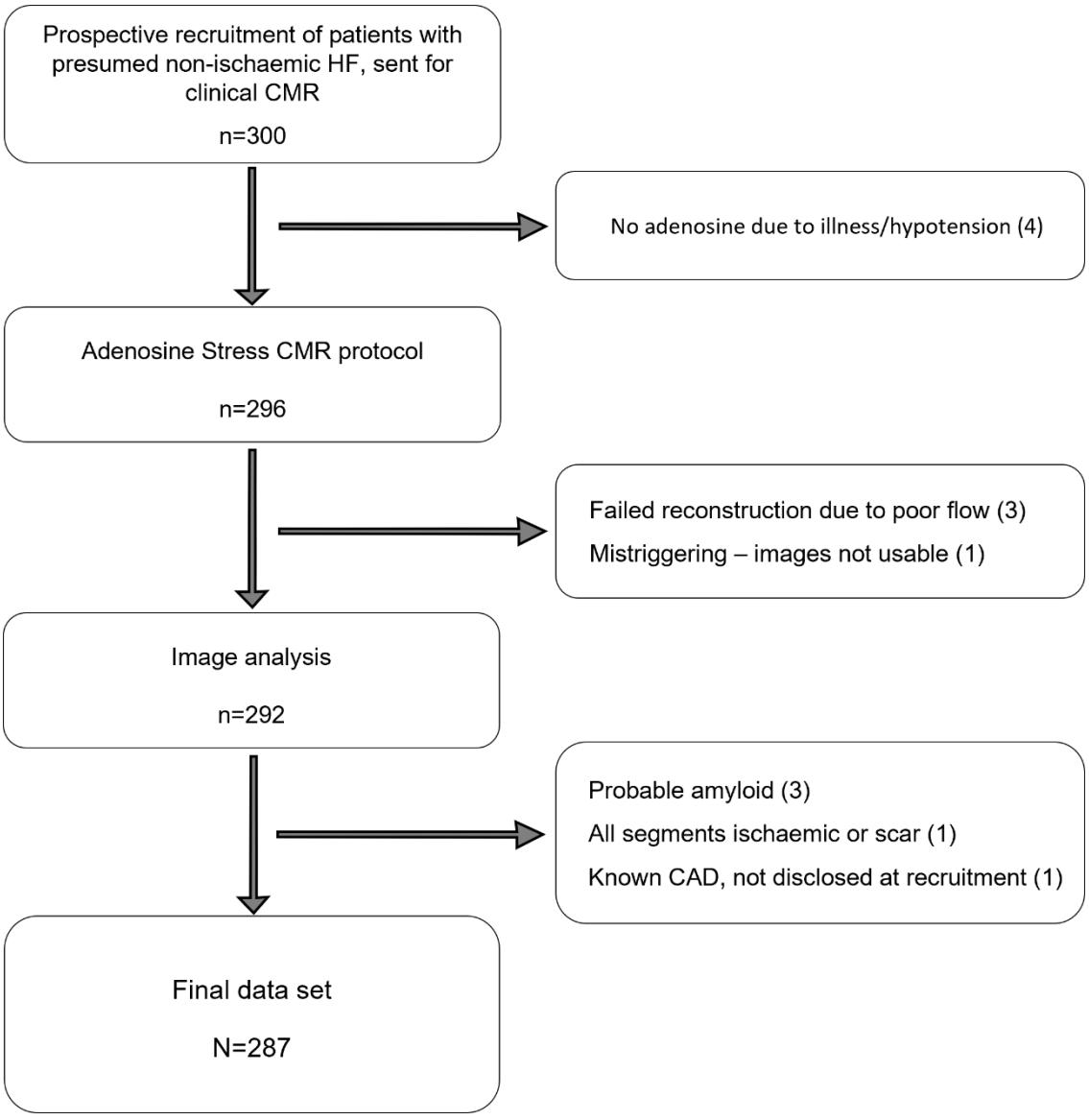


Figure 6-2 Recruitment into study

300 patients were prospectively recruited prior to clinical CMR. 287 were included in final analysis.

CAD – coronary artery disease, HF - heart failure

6.3.1 Clinical characteristics

Age and sex of HFmrEF patients fell between HFpEF and HFrEF but no significant differences were seen in cardiovascular risk factors between HFmrEF patients and other groups (Table 1). Groups were comparable for co-morbidities other than atrial fibrillation (AF) which was more prevalent in both HFmrEF and HFrEF compared to HFpEF. There were no differences in symptoms or functional class between the groups. HFmrEF patients reported symptoms of breathlessness in similar proportions to HFpEF patients, and less than HFrEF patients. Diuretic and mineralocorticoid antagonist was lowest in HFpEF and highest in HFrEF.

HFmrEF fell between HFpEF and HFrEF in assessment of left and right ventricular volumes and mass (Table 2).

6.3.2 Prevalence of occult ischaemic heart disease

Ischaemic scar was seen in 58 patients (20%) and was more frequent in both HFmrEF and HFrEF (17% and 31%) than in HFpEF (2%), $p<0.001$. Inducible ischaemia was seen in 20 (7%) overall, most commonly in HFmrEF (9.7%) and HFrEF (7.5%) and less often in HFpEF (1.7%), $p=0.157$. Non-ischaemic scar was detected in 91 patients (31.7%), but the prevalence was not significantly different between groups, $p=0.096$. The presence of occult ischaemic heart disease (defined as either inducible ischaemia or ischaemic LGE) was 20% in HFmrEF and 33% in HFrEF but only 3% in the HFpEF group ($p<0.001$ for trend), (Table 3).

6.3.3 Tissue characteristics

Native T1 and ECV were both increased between patients and healthy controls (Table 4).

Native T1 was highest in HFrEF and with no significant difference between HFmrEF and HFpEF: (HFpEF 1310±33ms, HFmrEF 1311±32ms and HFrEF 1340±45ms, HFpEF vs HFmrEF $p=1$, HFmrEF vs HFrEF $p<0.001$.

ECV showed the same pattern with highest ECV in HFrEF (implying more interstitial expansion): HFpEF 25.0±2.6%, HFmrEF 24.6±3.2% and HFrEF 26.3±3.1%, HFpEF vs HFmrEF $p=0.316$, HFmrEF vs HFrEF $p<0.001$.

When EF was considered as a continuous variable, there were significant negative correlations between EF and T1 (r=-0.393, p<0.001), and EF and ECV (r=-0.263, p<0.001).

Stress MBF was lower in all patient groups than healthy controls, with no difference between HFmrEF and HFpEF, stress MBF was lower in HFrEF group: HFpEF 1.93±0.57ml/g/min, HFmrEF 1.89±0.62ml/g/min and HFrEF 1.51±0.50ml/g/min, HFpEF vs HFmrEF p=0.597, HFmrEF vs HFrEF p<0.001.

There was a significant correlation between stress MBF and EF (r=0.338, p<0.001) Figure 3. These differences and correlations remained present when patients with CMR discovered ischaemic heart disease were excluded from analysis.

Rest MBF was significantly higher in patients than healthy controls (Table 4). Within HF patients, rest MBF was significantly lower in HFmrEF compared to HFpEF with no difference between HFmrEF and HFrEF: HFpEF 0.79±0.25ml/g/min, HFmrEF 0.71±0.19ml/g/min and HFrEF 0.70±0.22ml/g/min, HFpEF vs HFmrEF p=0.019, HFmrEF vs HFrEF p=0.515.

There was no significant correlation between EF and rest MBF (Figure 3).

MPR was not different between HFmrEF and HFpEF (p=0.165) but was lower in HFrEF compared to HFmrEF (2.28±0.84ml/g/min vs 2.75±0.84ml/g/min, p<0.001) (Table 4). These findings remained when those with ischaemic heart disease were removed from analysis.

Table 6-1 – Clinical features

	All Patients 287	HFpEF 60	HFmrEF 93	HFrEF 134	p	HFpEF: HFmrEF	HFmrEF: HFrEF
Age (yrs)	62.4±12.5	58.7±11.0	61.2±14.3	64.9±12.3	0.003	0.638	0.081
Female	104 (36.2)	34 (56.7)	37 (39.8)	33 (24.6)	<0.001	0.029	0.032
Symptoms							
NYHA I	183 (63.8)	43 (71.7)	65 (69.9)	75 (56.0)	0.075	0.717	0.091
II	90 (31.4)	13 (21.7)	24 (25.8)	53 (39.6)			
III	14 (4.9)	4 (6.7)	4 (4.3)	6 (4.5)			
SOBOE	106 (36.9)	18 (30.0)	27 (29.0)	61 (45.5)	0.016	0.989	0.026
Orthopnoea	48 (16.7)	9 (15.0)	11 (11.8)	28 (20.9)	0.182	0.570	0.075
Peripheral oedema	45 (15.7)	9 (15.0)	13 (14.0)	23 (17.2)	0.799	0.993	0.782
Risk factors							
Diabetes	52 (18.1)	7 (11.7)	17 (18.3)	28 (20.9)	0.304	0.349	0.410
HbA1c (mmol/mol)	43.4±12.6	41.2±1.8	42.2±1.7	45.5±0.88	<0.001	0.912	<0.001
Hypertension	122 (42.5)	22 (36.7)	40 (43.0)	60 (44.8)	0.569	0.435	0.792
Systolic BP (mmHg)	124±19.3	129±2.6	125±2.1	121±1.7	0.008	0.345	0.096
Hypercholesterolaemia	72 (25.1)	12 (20.0)	29 (31.2)	31 (23.1)	0.230	0.127	0.176
Stroke	38 (13.2)	7 (11.7)	8 (8.6)	23 (17.2)	0.160	0.534	0.065
Atrial fibrillation	112 (39)	15 (25.0)	40 (43.0)	57 (42.5)	0.043	0.026	0.943

Smoking history	159 (55.4)	30 (50.0)	51 (54.8)	78 (58.2)	0.563	0.474	0.817
BMI (kg/m^2)	27.8 ±4.9	27.8 ±5.3	28.1 ±4.9	27.6 ±4.9	0.809	1.000	1.000
Medications							
Antiplatelet	55 (19.2)	13 (21.1)	16 (17.2)	26 (19.4)	0.787	0.492	0.675
Beta blocker	225 (78.4)	43 (71.7)	75 (80.6)	107 (79.9)	0.359	0.144	0.627
Statin	119 (41.5)	17 (28.3)	36 (38.7)	66 (49.3)	0.019	0.236	0.065
ACEi/ARB	241 (84.0)	43 (71.7)	81 (87.1)	117 (87.3)	0.014	0.017	0.962
MRA	64 (22.3)	5 (8.3)	14 (15.1)	45 (33.6)	<0.001	0.218	0.002
Diuretic	116 (40.4)	11 (18.3)	27 (29.0)	78 (58.2)	<0.001	0.135	<0.001

NYHA – New York Heart Association, SOBOE – shortness of breath on exertion, BMI – body mass index, ACE – angiotensin converting enzyme inhibitor, ARB – angiotensin receptor blocker, MRA – mineralocorticoid receptor antagonist

Table 6-2 - Volumetrics

	All Patients 287	**HFpEF** 60	**HFmrEF** 93	**HFrEF** 134	**p**	**HFpEF: HFmrEF**	**HFmrEF: HFrEF**
LVEF (%)	39.8 ±12	55.7±4.5	45.2±2.8	28.9±8.0	<0.001	<0.001	<0.001
LVEDVi (ml/m^2)	111±36	84.3±15	101±25	130±39	<0.001	<0.001	<0.001
RVEDVi (ml/m^2)	77.8±22	71.7±17	75.7±20	81.9±25	0.018	0.448	0.063
LV mass indexed (g/m^2)	67.6±19	56.8±14	63.2±18	75.5±18	<0.001	0.033	<0.001
RVEF (%)	49.6±13	57.3±9.9	52.5±10	44.1±13	<0.001	0.006	<0.001
MCR (g/ml)	0.63±0.15	0.68±0.15	0.64±0.14	0.61±0.15	0.001	0.088	0.0.34
LV:RV	1.48±0.59	1.21±0.24	1.36±0.28	1.69±0.76	<0.001	0.001	<0.001

LVEDVi – left ventricular end-diastolic volume indexed to body surface area, RVEDVi – right ventricular end-diastolic volume indexed to body surface area, RVEF – right ventricular ejection fraction, MCR – mass to cavity ratio, LV:RV – left ventricular to right ventricular end diastolic volume ratio.

Table 6-3 - Presence of ischaemic heart disease or late gadolinium enhancement

	HFpEF 60	HFmrEF 93	HFrEF 134	p	HFpEF: HFmrEF	HFmrEF: HFrEF
Regional ischaemia	1 (1.7)	9 (9.7)	10 (7.5)	0.157	0.050	0.553
Ischaemic LGE	1 (1.7)	16 (17.2)	41 (30.6)	<0.001	0.003	0.022
IHD	2 (3.3)	19 (20.4)	44 (32.8)	<0.001	0.003	0.004
Non-ischaemic LGE	16 (26.7)	25 (26.9)	50 (37.3)	0.161	0.977	0.100

The incidence of both ischaemic heart disease and non – ischaemic LGE were highest in the HFrEF group. Ischaemic heart disease was seen in a significantly higher proportion of patients with HFmrEF compared to HFpEF, and lower compared to HFrEF.

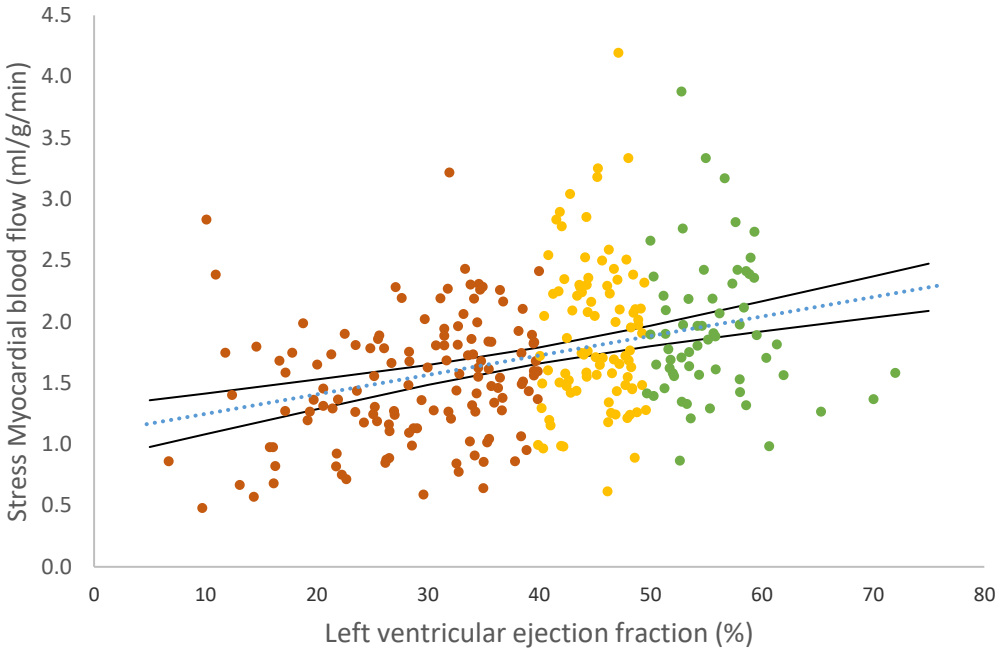
LGE – late gadolinium enhancement, IHD – ischaemic heart disease.

Table 6-4 - Parametric data

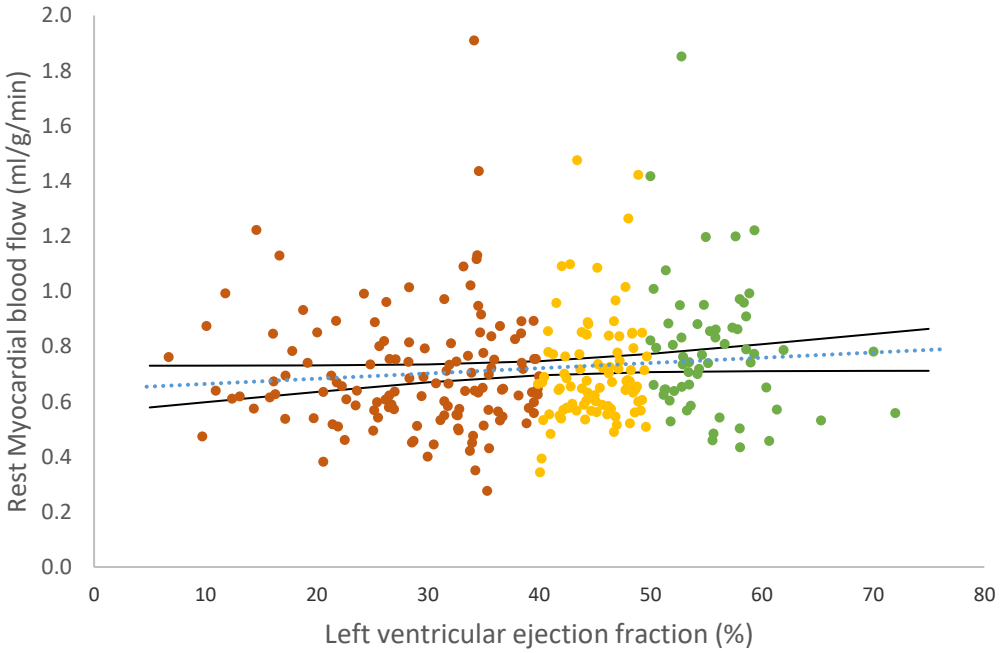
	Controls 25	**p (Controls: Patients)**	**HFpEF** 60	**HFmrEF** 93	**HFrEF** 134	**p (all HF)**	**HFpEF: HFmrEF**	**HFmrEF: HFrEF**
Native T1 (ms)	1252.4±21.2	<0.001	1310±33	1311±32	1340 ±45	<0.001	1	<0.001
ECV (%)	22.7±1.7	<0.001	25.0±2.6	24.6±3.2	26.3±3.1	<0.001	0.316	<0.001
Stress MBF (ml/g/min)	2.23±0.69	<0.001	1.93±0.57	1.89±0.62	1.51±0.50	<0.001	0.597	<0.001
Rest MBF (ml/g/min)	0.60±0.15	0.003	0.79±0.25	0.71±0.19	0.70±0.22	0.014	0.019	0.515
MPR	3.85±1.23	<0.001	2.56±0.71	2.75±0.84	2.28±0.84	<0.001	0.165	<0.001

ECV – extracellular volume, MBF – myocardial blood flow, MPR – myocardial perfusion reserve

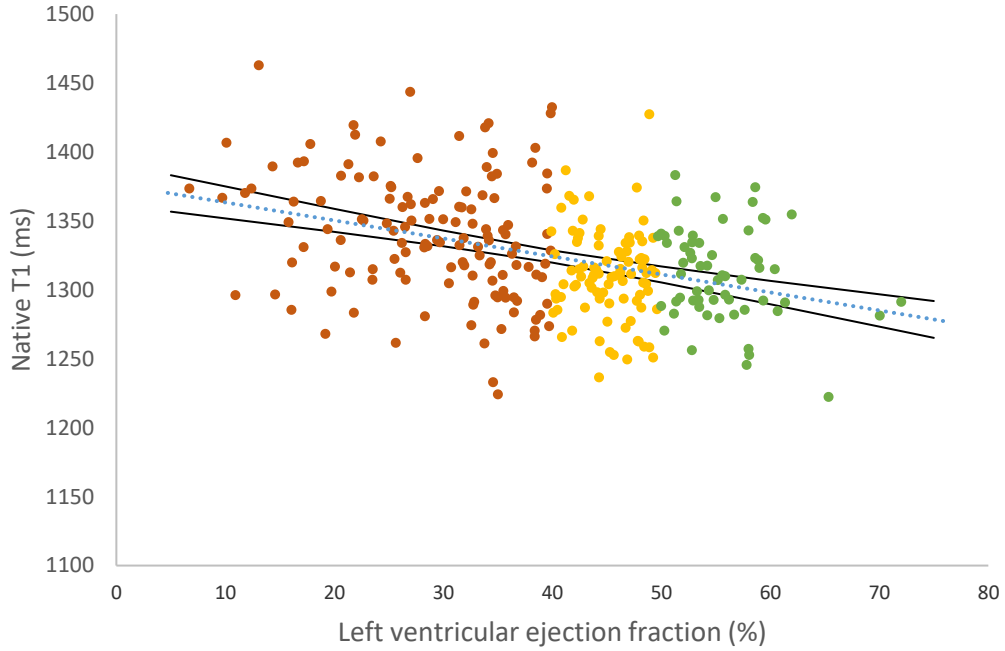
A


Stress Myocardial blood flow (ml/g/min)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
60
70
80
Left ventricular ejection fraction (%)

B


Rest Myocardial blood flow (ml/g/min)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
70
80
Left ventricular ejection fraction (%)

C



D

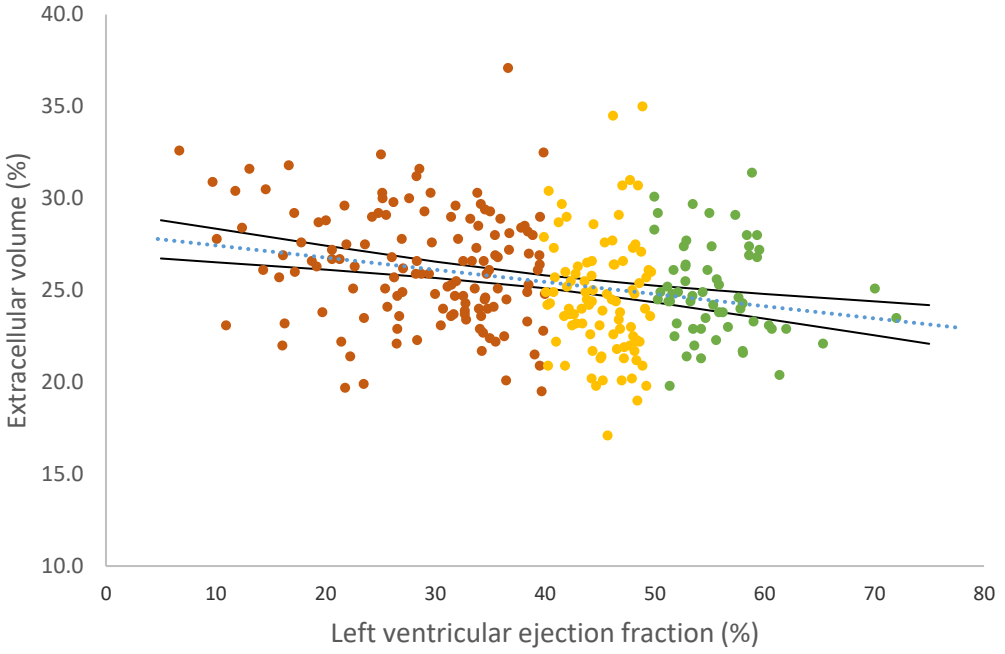


Figure 6-3 Correlation with ejection fraction

Significant correlation was seen between left ventricular ejection fraction (LVEF) and stress myocardial blood flow (MBF) (A), native T1 (C) and extracellular volume (D). There was no significant correlation between rest MBF and LVEF in patients. A r=0.338, p<0.01, B r=0.107, p=0.07, C r=-0.393, p<0.01, D r=-0.263, p<0.01.

• *HFrEF* • *HFmrEF* • *HFpEF*

6.4 Discussion

This study has shown that among 287 patients being investigated for the aetiology of heart failure, those with HFmrEF had tissue characteristics, markers of diffuse fibrosis and microvascular function similar to HFpEF. Conversely HFmrEF patients had a 20% incidence of occult coronary artery disease (CAD) which was similar to HFrEF

6.4.1 Silent ischaemia and infarction

Unselected heart failure studies have shown an incidence of IHD in HFmrEF between 41 and 61% (221,226) with higher rates of IHD in HFmrEF than in HFpEF, and similar rates compared to HFrEF(221,222,226–229). Evaluation of IHD is a common indication for CMR and in order to avoid selection bias we only recruited patients without symptoms or history of IHD. Our study reported the novel finding that patients with HFmrEF have an increased level of occult ischaemic heart disease than in HFpEF and comparable to HFrEF group. Previous CMR studies of higher risk groups have identified a similar prevalence of myocardial infarction in both older adults(230) and those with type 2 diabetes(119).

20% of the HFmrEF group had evidence of either previous MI or inducible ischaemia as the underlying aetiology for their LV dysfunction. The importance of underlying diagnosis lies in its ability to direct either further investigation, such as angiography in the case of ischemia where revascularisation may be considered, or in directing medical therapy where the presence of coronary artery disease supports therapy with antiplatelet agents and statins.

Within our study, 26% of the HFmrEF population with IHD diagnosed on CMR were not receiving either antiplatelet agents or anticoagulation – a potential group for therapy change to reduce the risk of future cardiovascular events. In the same cohort, only 40% of patients were on statin therapy, again, recommended in ESC guidelines for all patients with chronic coronary syndromes for the prevention of future cardiovascular events(4).

The presence of ischaemic scar is known to be associated with adverse cardiovascular outcomes(74,81,230). Silent ischaemia or infarction are both associated with an increased risk of adverse cardiac events (75,231). The subset of patients with ischaemic HFmrEF potentially have a worse prognosis although it remains to be established whether they respond differently to medical or device

therapy. Identification of this subgroup supports the role of CMR in recognising occult IHD in patients with HFmrEF.

6.4.2 Non-ischaemic heart failure

6.4.2.1 Diffuse Fibrosis

HFmrEF patients showed elevated native T1 and ECV values, markers of diffuse fibrosis(232,233), compared to healthy controls. Although values in HFmrEF were more similar to HFpEF and significantly less elevated than in HFrEF when considered as discrete groups, overall, there was a correlation between EF and both T1 and ECV suggesting a degree of continuity over the spectrum of ejection fractions.

A recent study by Doeblin et al, examined tissue characterization in HFmrEF, demonstrating significantly higher values of native T1 in HFmrEF compared to HFpEF, and no significant difference between HFmrEF and HFrEF. No significant difference was seen between the groups in ECV(234). This study included 17 patients with HFpEF and HFrEF and 18 with HFmrEF, and absolute differences in ECV and T1 are relatively small, which may account for the differences from our results. Similarly, to our results, there were negative correlations between T1 and ECV, and LVEF.

Previous studies have examined the significance of ECV in both preserved and reduced LV ejection fraction. In HFpEF, ECV has been demonstrated to be significantly different to controls, as in our study, and a predictor of disease severity such as LV stiffness(235) and baseline BNP(236), as well as associated with adverse outcomes including hospitalisation for heart failure and death(236,237).

CMR registry data have shown that increased ECV is associated with increased risk of heart failure hospitalisation and mortality across the spectrum of ejection fraction, including patients with HFmrEF(120,238–241). In addition it has been suggested that ECV may also be a predictor of recovery of LVEF in some DCM(242).

The evidence for ECV as a prognostic marker in both HFpEF and HFrEF makes it likely that it would also be important in HFmrEF and, although not assessed as part of the current study, may play a part in identifying subgroups of patients who may benefit from different treatment options.

6.4.2.2 Perfusion

There was no difference in stress MBF between HFmrEF and HFpEF or controls, but significantly higher values in HFmrEF compared to HFrEF. Overall, there was a moderate correlation between stress MBF and LVEF. These findings remained consistent when those with evidence of ischaemic heart disease were removed from the analysis.

An underlying hypothesis that microvascular dysfunction leads to chronic hypoperfusion, contributing to progressive deterioration in LV function, has been suggested(243). The correlation between EF and stress MBF that we have demonstrated supports these findings, although cannot provide evidence of a causal relationship between reduced MBF and LV function. Previous studies have shown decreased stress MBF in DCM(125,244) and severe systolic heart failure, which has been presumed secondary to microvascular disease. In addition, impaired stress MBF has been shown to be associated with poor prognosis in patients with LV dysfunction, independent of the level of impairment(244). This theory also presents an attractive potential target for medical therapy to slow progression.

Within HFmrEF, although no significant difference in seen in mean MBF, compared to controls, patients may be able to be identified for further investigation or targeted therapy.

MPR showed a significant correlation with EF, decreasing with worsening EF. Although MPR declines with EF, even in the HFrEF group it does not fall to levels that would be considered diagnostic of ischaemia in obstructive coronary disease in other studies using CMR quantitative perfusion(245), suggesting a different threshold may apply in microvascular disease.

We demonstrated no correlation between EF and resting MBF. A role for resting MBF in development of LV dysfunction has also been suggested with several PET and CMR studies suggesting that resting MBF is decreased in DCM(244,246), again leading to a state of chronic, low-grade ischaemia. However, these findings are less consistent between studies, with others showing different results. Other PET studies have shown no difference between controls and DCM groups(243), and one CMR study in DCM has shown a higher resting MBF in DCM compared to controls(125) suggesting possible higher metabolic requirement at rest in DCM. Lack of heterogeneity in results and relationships between resting

MBF and EF is likely to be associated with the numerous other factors that can influence rest MBF which are difficult to control for in these studies.

Reported outcomes within HFmrEF have varied between studies, and have been reported as similar to HFpEF(228,247), to HFrEF(248), intermediate(221) or independent of ejection fraction (222,227,249). From parametric markers measuring diffuse fibrosis and perfusion, HFmrEF resembles HFpEF while the HFrEF group demonstrates higher ECV and lower stress MBF (and MPR). These findings may represent part of the mechanism of deterioration in left ventricular function, and potentially could be markers of poor prognosis with in the HFmrEF group, leading to progression to HFrEF.

6.4.3 Nomenclature

Whilst HF with ejections fractions between 40 and 50% has been known to be a grey area before the publication of the ESC guidelines, the way in which it has been defined has varied. ESC guidelines classify this group as a separate entity, suggesting potential unique characteristics, while the AHA guidelines have broadly defined it as HFpEF(250). Within the AHA group, there are 2 categories, either HFpEF borderline (EF 40-49%) or HFpEF recovered (EF 40-49% with evidence of previous EF <40%). These two groups would differ significantly in treatment options, with one behaving similarly to HFpEF and unlikely to benefit prognostically from medical therapies, and the other requiring traditional HFrEF treatments to maintain this recovered EF. Evidence of how to distinguish between these groups, without prior imaging evidence is difficult, and it may be that tissue characteristics could differ between the two and help inform diagnosis. This distinction points to the potential importance of aetiology rather than EF as a marker for treatment, an area where CMR plays a key role, as demonstrated by the proportion of silent IHD identified in our cohort

With the development of more attention to this middle group of heart failure, a further hypothesis of heart failure as a continuous spectrum, rather than defined by EF has been proposed(251,252). This argues that heart failure is better described by phenotype and aetiology than by EF which is known to vary. In one prospective study of patients with a diagnosis of heart failure, 57% of those initially categorised as HFmrEF had changed their EF class within one year, with 24% reducing to HFrEF and 33% increasing in EF to the HFpEF category. Nevertheless, these patients' underlying disease aetiology will remain the same. Overall, our data

supports this continuous theory. We have shown linear relationships in EF with diffuse fibrosis and stress perfusion across the EF spectrum, and the lack of significant difference between HFmrEF and HFpEF in these parameters when considered as discrete groups may in part be due to the narrower range of EF in each compared to HFrEF (HFrEF 6.7-39.9%, HFmrEF 40-49.7% and HFpEF 50-72%).We have shown that CMR has an important role in the identification of different phenotypes of HFmrEF and advocate that it has an important role in risk stratification it such patients.

6.4.4 Limitations

Patients within this study were prospectively recruited from clinical care, where a CMR scan had been requested by the treating clinician. This may have introduced a degree of referral bias where patients not deemed to be suitable for CMR may have been excluded reducing the proportion of patients with AF, more frail or elderly patients, or those with more severe heart failure symptoms in our study compared with other cohorts. A large proportion of our patients had NYHA class I symptoms at the time of scanning, potentially because they had already been seen and started on appropriate medical therapy prior to their scan. Whilst patients were advised to avoid caffeine prior to their scan, this was not tested for, and previous studies have shown that some patients will still have detectable levels of caffeine at the time of their scan, which may influence the effects of adenosine.

6.4.5 Conclusion

We have demonstrated a trend in fibrosis and perfusion related to ejection fraction and supporting the consideration of heart failure as a spectrum rather than distinct entities divided by EF, and other factors such as aetiology may be important in determining best management.

This study provides a detailed description of the HFmrEF phenotype. Patients with HFmrEF have a high prevalence of occult ischaemic heart disease (similar to HFrEF) and without appropriate imaging may miss out on secondary prevention. Conversely patients with HFmrEF have less microvascular impairment and fibrosis (similar to HFpEF), further work is needed to confirm how the phenotype of HFmrEF responds to medical therapy.

Chapter 7

CMR phenotyping in heart failure and diabetes: the role of dysglycaemia and microvascular dysfunction

7.1 Background

Diabetes is an increasing challenge with current estimates of over 450 million people affected worldwide, and this figure projected to continue to rise(63). Heart failure and diabetes are associated, with heart failure as a common early manifestations of cardiovascular disease in diabetes(96). Diabetes has been identified as an independent risk factor for the development of heart failure(89,90) and prognosis in heart failure shown to be worse in those with diabetes (95).

Diabetes mellitus is associated with an increased risk of coronary artery disease and associated ischaemic cardiomyopathy. However, it is also associated with diabetic cardiomyopathy, with abnormal myocardial function in the absence of coronary disease. The exact mechanisms behind the development of this condition are unknown with a proposed mechanism of fibrosis and myocardial dysfunction progressing to systolic impairment(107). Tissue characterisation with cardiovascular magnetic resonance (CMR) has given insights into the early changes involved in this condition, demonstrating increased myocardial mass and interstitial fibrosis. The addition of quantitative myocardial perfusion will provide a new tool to assess microvascular abnormalities, another potential prognostic marker in this complex patient group.

We hypothesised that dysglycaemic patients presenting with a new diagnosis of heart failure have increased prevalence of occult ischaemic heart disease and microvascular dysfunction. We aimed to investigate if either of these factors is associated with major adverse cardiovascular events (MACE).

7.2 Methods

7.2.1 Study population

In this prospective clinical study, patients with newly diagnosed heart failure who had been referred for a CMR scan to investigate aetiology were recruited (n=365). Patients were excluded if they had a known history of coronary artery disease (stenosis >70% on angiography, myocardial infarction, previous percutaneous coronary intervention or coronary artery bypass grafting) or symptoms of angina. Other exclusion criteria included hypertrophic cardiomyopathy, amyloidosis, congenital heart disease, suspected acute pathology such as myocarditis, advanced renal failure, or any contraindication to CMR or gadolinium-based contrast agents.

The primary outcome was a MACE event, defined as the composite of cardiovascular death, non-fatal acute coronary syndrome, stroke and hospitalisation due to heart failure.

7.2.2 Patient characteristics

Patients underwent clinical assessment on the day of their CMR appointment, including medical history, New York Heart Association (NYHA) function class, risk factors and current medications. Haematocrit (Hct) and HbA1c were measured from a blood sample taken at the time of the CMR scan.

Patients were subsequently divided into normoglycaemic (HbA1c <42mmol/mol) and dysglycaemic (HbA1c>42 mmol/mol), with this group being further divided into pre-diabetes (HbA1c 42-47 mmol/mol) and diabetes (HbA1c>47mmol/mol)(68). All patients with a known diagnosis of diabetes were classified as dysglycaemic and diabetes, regardless of their HbA1c.

7.2.3 Study protocol

All CMR studies were undertaken on a 3T system (Siemens Magnetom Prisma, Erlangen, Germany). Participants were instructed to abstain from caffeine for 24 hours before the study. The protocol consisted of cine imaging, native and post contrast T1 mapping, stress and rest perfusion and late gadolinium enhancement imaging as

described in chapter 2. When it was unclear if enhancement seen on bright blood LGE was ischaemic a dark blood LGE stack was also acquired(225).

For perfusion imaging, adenosine was infused for a minimum of 3 minutes, at a rate of 140µg/kg/min and increased up to a maximum of 210µg/kg/min if there was insufficient haemodynamic response (heart rate increase less than 10bpm or systolic blood pressure change less than 10mmHg) or there was no symptomatic response. Images were acquired over 90 dynamics to allow for poor ventricular function. A minimum ten-minute interval was kept between perfusion acquisitions.

Blood pressure and heart rate were recorded during adenosine infusion. For perfusion imaging, an intravenous bolus of 0.05mmol/kg gadobutrol (Gadovist, Leverkusen, Germany) was administered at 5ml/s followed by a 20ml saline flush using an automated injection pump (Medrad MRXperion Injection System, Bayer). Perfusion mapping was performed using the Gadgetron streaming software image reconstruction framework(48).

7.2.4 Image analysis

Measurement of cardiac volume parameters and the presence of late gadolinium was performed using cvi42 software (Circle Cardiovascular Imaging, Calgary, Canada). LGE was reported if enhancement was identified on two orthogonal planes or, where available, on both bright and dark blood LGE images. Ischaemic LGE was defined as involving the subendocardium in a typical coronary distribution while non-ischaemic LGE did not involve the subendocardium. Inducible ischaemia was defined as a visual perfusion defect affecting >1 segment present at stress, but not at rest or matching infarct on LGE imaging, in a coronary distribution.

7.2.5 Quantitative analysis

T1 and perfusion maps, were analysed as described in chapter 2. In order to report global microvascular function (rather than the effects of occult coronary artery disease or replacement fibrosis) segments with ischaemia or late gadolinium enhancement were also excluded from analysis. T1 times and MBF values for all remaining segments were averaged to provide a global value.

Myocardial perfusion reserve (MPR) was calculated as stress/rest MBF. ECV was calculated using the formula “myocardial ECV = (1 − Hct) × (ΔR1myocardium/ΔR1blood), where R1 = 1/T1”. These were calculated for each segment and averaged to provide a global value.

7.2.6 Statistical analysis

Analysis was performed using SPSS 23 (IBM SPSS, Armonk, NY, USA). Normality of distribution was assessed using the Shapiro-Wilk test. Data is presented as mean (±standard deviation) or median and IQR for continuous data, and frequency(percentage) for categorical data.

Comparison between groups were performed using independent samples T-test or Mann-Whitney U test depending on normality and chi-square test or Fisher’s exact test for categorical data. Correlation was assessed using Pearson r correlation or Spearman’s rank correlation coefficient. Statistical tests were two-tailed and $p<0.05$ was considered significant.

Survival curves were constructed according to the Kaplan-Meier method and compared dichotomous groups using the presence of dysglycaemia and median values of MPR within the study population as cut-offs. Where more than one MACE occurred to a patient, the first event was taken as an endpoint.

To identify independent predictors of MACE, separate Cox proportional hazard regression analyses were performed with adjustment for covariates including age, left ventricular ejection fraction (LVEF), T1 values, stress MBF, HbA1c and MPR.

7.3 Results

A total of 351 patients were recruited to take part in the study, of these 8 were excluded from final analysis (3 due to amyloidosis, 4 with contra-indications to adenosine, and 1 where we were unable to accurately analyse due to arrhythmia).

Of the 343 patients included in final analysis, 176 were normoglycaemic (Mean HbA1c 37±3.3) and 167 dysglycaemic, further divided into 84 pre-diabetes (mean HbA1c 44±1.6) and 83 diabetes (mean HbA1c 57.2±18.3).

7.3.1 Patient characteristics

Patient characteristics are shown in Table 1. Patients with dysglycaemia were older than those with normoglycaemia and tended to have lower ejection fraction. This group also had a higher proportion of symptomatic patients, defined using NYHA class. Incidence of hypertension, hypercholesterolaemia and cerebrovascular disease were highest in the diabetes group.

Use of diuretics increased across the groups, lowest in normoglycaemic and highest in diabetes. The diabetes group were also more likely to be taking ACEi.

7.3.2 CMR assessment

CMR data can be seen in table 2. LVEF was lowest in the diabetes group, and LV mass was higher in dysglycaemia patients compared to normoglycaemia. No difference was seen in right ventricular parameters or left atrial size. The incidence of occult ischaemic heart disease was highest in the diabetes group with 27.7% having evidence of previous myocardial infarction.

Native T1 and ECV were higher in the dysglycaemic group. No significant difference was seen between pre-diabetes and diabetes groups. Stress MBF was lower in the dysglycaemic group, leading to lower MPR. No significant difference was seen in rest MBF (Table 3).

Table 7-1 - Patient characteristics

			Dysglycaemia		p values			
		Normoglycaemia (176)	Prediabetes (84)	Diabetes (83)	Normoglycaemia vs dysglycaemia	Normoglycaemia vs pre-diabetes	Pre-diabetes vs diabetes	Normoglycaemia vs diabetes
Age (yrs)		61 (53-70)	65 (54-72)	67 (55-73)	0.014	0.106	0.477	0.016
Male		111 (63.1)	56 (66.7)	53 (63.9)	0.671	0.571	0.703	0.902
BMI (kg/m^2)		28.1±4.7	28.2±5.3	28.3±5.7	0.967	0.994	0.907	0.940
NYHA	**I**	127 (72.2)	50 (59.5)	43 (51.8)	0.006	0.120	0.557	0.004
	II	44 (25.0)	30 (35.7)	34 (41.0)				
	III	5 (2.8)	4 (4.8)	6 (7.2)				
HbA1c		37±3.3	44.0±1.6	57.2±18.3	<0.001	<0.001	<0.001	<0.001
SOBOE		70 (39.8)	34 (40.5)	33 (39.8)	0.948	0.914	0.925	0.998
Orthopnoea		24 (13.6)	15 (17.9)	13 (15.7)	0.419	0.373	0.704	0.664
Oedema		26 (14.8)	10 (11.9)	14 (16.9)	0.916	0.531	0.361	0.663
Hypertension		72 (40.9)	35 (41.7)	48 (57.8)	0.102	0.908	0.037	0.011
Hypercholesterolaemia		35 (19.9)	19 (22.6)	31 (37.3)	0.031	0.611	0.038	0.003
Stroke		21 (11.9)	6 (7.1)	15 (18.1)	0.856	0.237	0.033	0.183
Atrial fibrillation		66 (37.5)	32 (38.1)	36 (43.4)	0.541	0.926	0.488	0.367
Antiplatelet		31 (17.6)	18 (21.4)	16 (19.3)	0.517	0.462	0.730	0.746

Betablocker	127 (72.2)	68 (81.0)	71 (85.5)	0.014	0.126	0.427	0.018
Statin	58 (33.0)	38 (45.2)	47 (56.6)	0.001	0.055	0.141	<0.001
ACEi/ARB	140 (79.5)	66 (78.6)	79 (95.2)	0.072	0.856	0.002	0.001
MRA	34 (19.3)	19 (22.6)	28 (33.7)	0.054	0.537	0.110	0.011
Diuretic	52 (28.5)	37 (44.0)	51 (61.4)	<0.001	0.021	0.024	<0.001
Anticoagulant	55 (31.3)	30 (35.7)	30 (36.1)	0.379	0.492	0.954	0.452

Those with dysglycaemia were older and had worse LVEF. The incidence of hypertension, hypercholesterolaemia and cerebrovascular disease were highest in the diabetes group. This group were more likely to be taking ACEi and diuretics and had higher symptom scores (NYHA).

NYHA – New York Heart association, ACEi - angiotensin converting enzyme inhibitor, ARB – angiotensin receptor blocker, MRA – mineralocorticoid receptor antagonist.

Table 7-2 - CMR Assessment

		Dysglycaemia		p values			
	Normoglycaemia 176	Pre-diabetes 84	Diabetes 83	Normoglycaemia vs dysglycaemia	Normoglycaemia vs pre-diabetes	Pre-diabetes vs diabetes	Normoglycaemia vs diabetes
Volumetrics							
LVEF (%)	41.9±11.9	39.6±13.4	35.6±12.5	0.001	0.125	0.053	<0.001
LVEDVi (ml/m^2)	107.7±33.1	112.7±39.4	112.8±37	0.251	0.425	0.833	0.285
LVMi (g/m^2)	65.1±18.4	71.3±18.7	69.4±19.5	0.004	0.004	0.305	0.068
RVEDVi (ml/m^2)	75.2±19.6	78.3±23.0	77.6±24.3	0.492	0.355	0.478	0.848
RVEF (%)	50.3±11.7	48.9±13.9	47.9±13.6	0.267	0.633	0.565	0.185
LA (ml/m^2)	78.4±35.4	84.6±42.8	77.5±36.8	0.819	0.514	0.411	0.779
Ischaemia and scar							
Ischaemia	10 (5.7)	4 (4.8)	9 (10.8)	0.436	0.759	0.142	0.137
Ischaemic LGE	29 (16.5)	15 (17.9)	23 (27.7)	0.143	0.781	0.129	0.035
Ischaemic heart disease	30 (17.0)	17 (20.2)	26 (31.3)	0.049	0.532	0.101	0.009
Non LGE	52 (29.5)	27 (32.1)	28 (33.7)	0.498	0.670	0.827	0.496

LVEF was lower in the dysglycaemic group while LVMi was higher. The incidence of ischaemic heart disease was highest in the diabetes group.

LVEF – left ventricular ejection fraction, LVEDVi – indexed left ventricular end diastolic volume, LVMi – indexed left ventricular mass, RVEDVi – indexed right ventricular end diastolic volume, RVEF – right ventricular ejection fraction, LA – left atrium. LGE – late gadolinium enhancement

Table 7-3 - CMR Parametric mapping

		Dysglycaemia		p values			
	Normoglycaemia 176	Pre-diabetes 84	Diabetes 83	Normoglycaemia vs dysglycaemia	Normoglycaemia vs pre-diabetes	Pre-diabetes vs diabetes	Normoglycaemia vs diabetes
T1 (ms)	1316.5±39.6	1331.0±39.9	1335.1±45.2	<0.001	0.006	0.532	0.001
ECV (%)	25.1±3.1	25.7±2.88	25.8±2.88	0.049	0.166	0.720	0.077
Stress MBF (ml/g/min)	1.93±0.62	1.59±0.54	1.53±0.52	0.000	0.003	0.466	<0.001
Rest MBF (ml/g/min)	0.73±0.20	0.69±0.19	0.70±0.26	0.130	0.130	0.771	0.312
MPR	2.61±0.90	2.41±0.88	2.37±0.85	0.020	0.089	0.758	0.040

Native T1 and ECV were higher in the dysglycaemic group. Stress MBF and MPR were lower in this group.

ECV – extracellular volume, MBF – myocardial blood flow, MPR – myocardial perfusion reserve.

7.3.3 Outcomes

MACE data were available over a median period of 623 days, (IQR 511-777). During this time, there were a total of 35 MACE occurring in 30 patients, including 23 hospitalisations due to HF (6.7%), 4 strokes (1.1%), 7 cardiovascular deaths (2.0%) and 1 (0.3%) coronary revascularisation in ACS. Median time to first MACE was 287 days (IQR 55-419). Individual events were similar in the normoglycaemia and dysglycaemia groups, the most common was hospital admission secondary to heart failure incidence (Table 4).

Table 7-4 - MACE events by glycaemic status

MACE Event	Normoglycaemia 176	Dysglycaemia 167	Pre-diabetes 84	Diabetes 83
HF admission	11 (6.3%)	12 (7.2%)	2 (2.4%)	10 (12%)
CV Death	1 (0.6%)	1 (0.6%)	1 (1.2%)	0
Stroke	2 (1.1%)	2 (1.2%)	1 (1.2%)	1 (1.2%)
ACS	1 (0.6%)	0	0	0

The main cause of MACE was hospital admission secondary to heart failure, with highest incidence seen in the diabetes group.

MACE – major adverse cardiovascular event, HF – heart failure, CV – cardiovascular, ACS – acute coronary syndrome

Patients with MACE were older (67.2 ±10.5yrs vs 61.8 ±12.4yrs, p=0.020) and had worse LVEF (33.0 ±15.1% vs 40.4 ±12.2%, p=0.014) than those without a MACE (Table 5). They had higher native T1 (1350 ±45ms vs 1322 ±41ms, p=0.001), ECV (27.0 ±3.2% vs 25.3 ±3.0%, p=0.004) and rest MBF (0.82 ±0.30ml/g/min vs 0.70 ±0.20ml/g/min, p=0.037) and lower MPR (2.08±0.76 vs 2.54±0.89, p=0.006).

Table 7-5 - Patient characteristics by presence of MACE

	No MACE 313	**MACE** 30	**p**
Age (yrs)	61.8±12.4	67.2±10.5	0.020
Male	203	17	0.372
BMI (kg/m^2)	28.2±5.1	27.6±5.3	0.518
HbA1c (mmol/mol)	43.1±10.6	49.6±23.6	0.150
Hypertension	139	16	0.348
Hypercholesterolaemia	78	7	0.848
Stroke	35	7	0.052
Atrial fibrillation	123	11	0.778
LVEF (%)	40.4±12.2	33.0±15.1	0.014
LVEDVi (ml/m^2)	109±34.6	122±44.3	0.059
LVMi (g/m^2)	67.3±18.7	71.1±20.4	0.295
RVEDVi (ml/m^2)	76.1±20.4	80.5±32.5	0.470
RVEF (%)	50.0±12.1	43.2±17.2	0.046
TI (ms)	1322±41	1350±45	0.001
ECV (%)	25.3±3.0	27.0±3.2	0.004
Stress MBF (ml/g/min)	1.71±0.59	1.59±0.55	0.280
Rest MBF (ml/g/min)	0.70±0.20	0.82±0.30	0.037
MPR	2.54±0.89	2.08±0.76	0.006

Those with MACE were older and had worse LVEF. The MACE group also had higher T1 and ECV and lower MPR.

The incidence of MACE did not differ significantly between the normoglycaemic groups and dysglycaemic groups when examined by presence of fibrosis, scar or ischaemia (Table 6). There was a higher incidence of MACE in the IHD group compared to those with non-ischaemic LGE or no LGE in both the normoglycaemic and dysglycaemic groups. This was only significant in the normoglycaemic group (IHD 20% vs NI LGE 8% vs other 5%, p=0.03).

Table 7-6 - MACE events and CMR findings

	Normoglycaemia (176)		**Dysglycaemia** (167)		**p**
	MACE (15)	**No MACE** (161)	**MACE** (15)	**No MACE** (152)	
Ischaemia	2 (20%)	8 (80%)	3 (23%)	10 (77%)	0.859
Ischaemic LGE	6 (21%)	23 (79%)	6 (16%)	32 (84%)	0.604
IHD	6 (20%)	24 (80%)	6 (14%)	37 (86%)	0.534
NI LGE	4 (8%)	48 (92%)	3 (5%)	52 (95%)	0.711
Other (no LGE or IHD)	5 (5%)	93 (95%)	6 (8%)	68 (92%)	0.533

No difference was seen between MACE in normoglycaemic and dysglycaemic groups divided by the presence of ischaemia, scar or fibrosis.

MACE – major adverse cardiovascular event, LGE – late gadolinium enhancement, IHD – ischaemic heart disease, NI – non-ischaemic.

Individual hazard ratios are shown in Figure 1. In both normoglycaemia and dysglycaemia, decreased LVEF and increased native T1 were both associated with increased risk of MACE. IHD was associated with an increased HR in normoglycaemia, decrease in MPR was associated with increased HR in dysglycaemia.

	Harard Ratio	Lower limit	Upper limit	p
Age (per 10 years)				
Normoglycaemia	1.599	1.002	2.553	0.049
Dysglycaemia	1.315	0.798	2.166	0.283
LVEF (per 10%)				
Normoglycaemia	0.627	0.421	0.932	0.021
Dysglycaemia	0.617	0.400	0.953	0.030
RVEF (per 10%)				
Normoglycaemia	0.590	0.378	0.923	0.021
Dysglycaemia	0.736	0.516	1.050	0.091
HbA1c				
Normoglycaemia	1.112	0.916	1.350	0.282
Dysglycaemia	1.027	1.009	1.045	0.003
T1 (per 10ms)				
Normoglycaemia	1.164	1.049	1.293	0.004
Dysglycaemia	1.126	1.005	1.261	0.041
ECV				
Normoglycaemia	1.177	1.031	1.344	0.016
Dysglycaemia	1.154	0.957	1.391	0.134
Stress MBF				
Normoglycaemia	0.912	0.400	2.079	0.827
Dysglycaemia	0.422	0.134	1.322	0.139
MPR				
Normoglycaemia	0.921	0.516	1.644	0.780
Dysglycaemia	0.188	0.077	0.457	<0.001
IHD				
Normoglycaemia	3.454	1.229	9.706	0.019
Dysglycaemia	1.939	0.690	5.452	0.209
NI LGE				
Normoglycaemia	1.171	0.373	3.678	0.787
Dysglycaemia	2.143	0.602	7.629	0.239

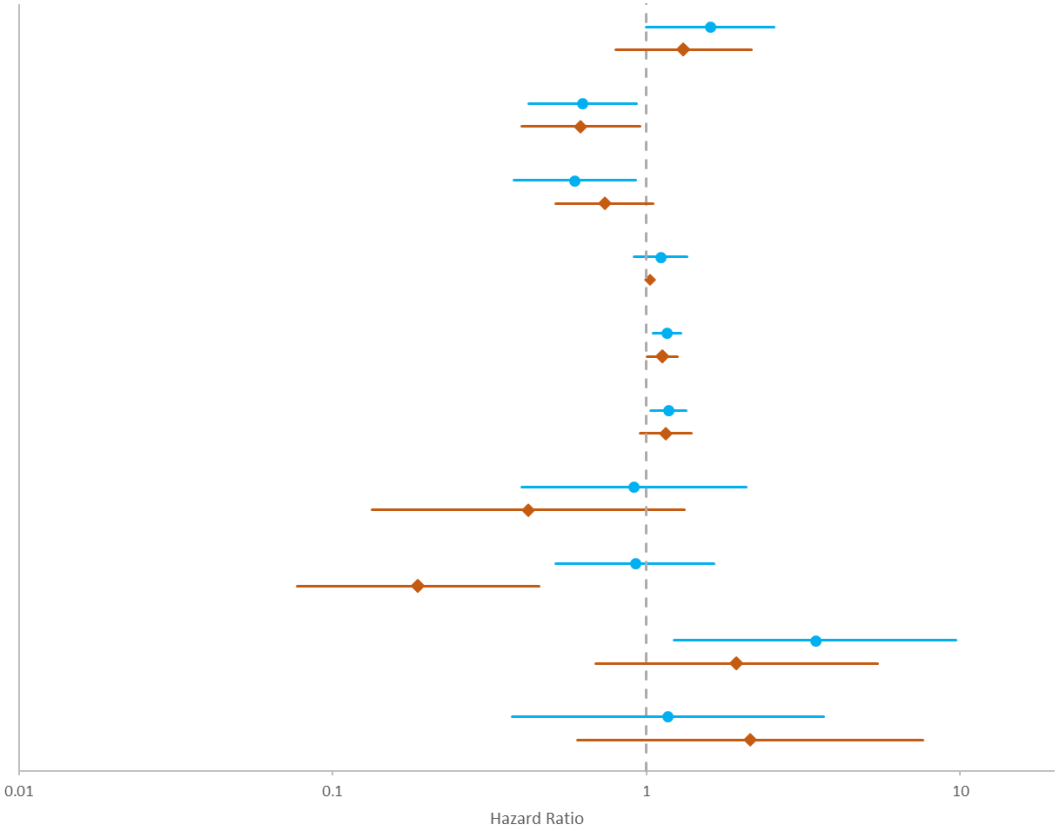


Figure 7-1 Univariate Cox regression analysis

Univariate Cox regression analysis of association with MACE. In both groups, worsening LVEF and increased native T1 were associated with increased risk. Worsening MPR and increased HbA1c were a predictor of poor outcomes in the dysglycaemic group

In multivariate Cox regression, corrected for age, LVEF, RVEF and HbA1c, MPR remained a predictor of MACE. (Table 7)

Table 7-7 - Multivariate Cox regression

Predictor	Hazard Ratio	95% CI	p
Corrected for age, LVEF, RVEF and HbA1c			
T1	1.008	0.999-1.017	0.080
ECV	1.114	0.970-1.280	0.127
MPR	0.553	0.318-0.962	0.036

Reduced MPR was associated with increased risk of MACE.

ECV – extracellular volume, MPR – myocardial perfusion reserve, CI confidence interval, LVEF – left ventricular ejection fraction, RVEF – right ventricular ejection fraction.

Kaplan Meier event free survival curves for the presence of IHD, and reduced MPR are shown in Figure 2 and Figure 3. In the dysglycaemic group lower MPR was associated with worse prognosis, p=0.005. In contrast, in the normoglycaemic group, the presence of IHD was associated with poorer outcomes, p=0.012, this effect was not seen in dysglycaemia.

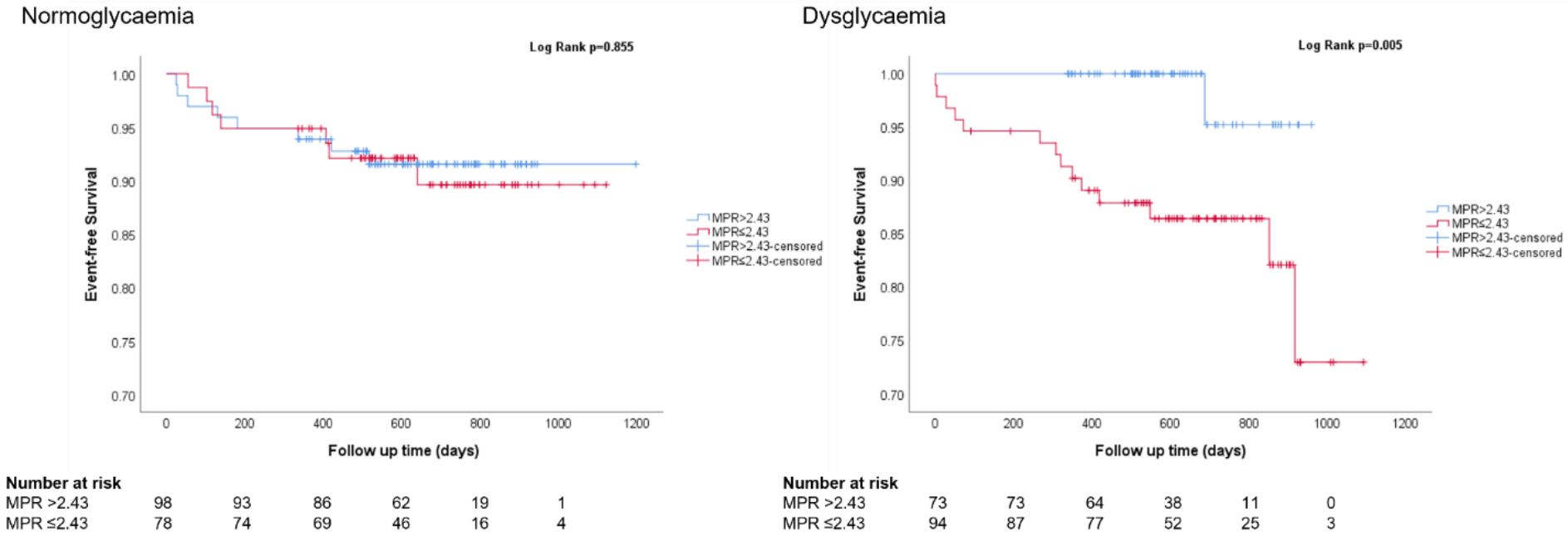


Figure 7-2 Kaplan Meier Survival curves by MPR

Lower MPR was associated with worse outcomes in dysglycaemia, with no significant difference seen in normoglycaemia.

MPR – myocardial perfusion reserve. MPR categories were divided by median MPR across the cohort (2.43).

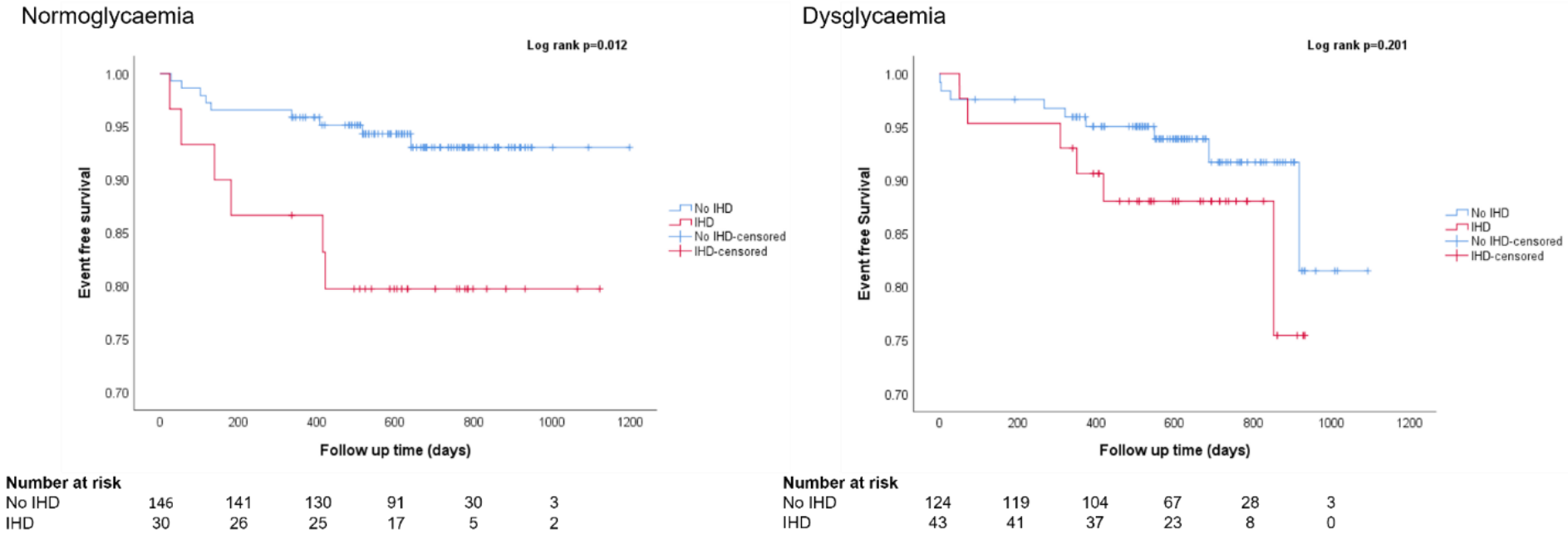


Figure 7-3 Kaplan Meier survival curves by IHD

The presence of IHD was associated with worse outcomes in normoglycaemia, with no significant difference seen in dysglycaemia.

IHD – ischaemic heart disease – defined as the presence of ischaemic scar or inducible ischaemia on CMR imaging.

7.4 Discussion

In this prospective study of 343 patients with newly diagnosed heart failure, we have reported that dysglycaemic patients have impaired myocardial microvascular function compared to normoglycaemic patients. Furthermore, impairment of microvascular function is associated with increased MACE only in dysglycaemic patients but not in those with normoglycaemia. The association between microvascular function and MACE is still seen after correction for baseline factors of age, LVEF and RVEF.

The prevalence of silent ischaemic heart disease was higher in dysglycaemic patients compared to normoglycaemic patients but was not associated with MACE.

7.4.1 Diabetes and Heart failure

Diabetic cardiomyopathy has been described for almost five decades, noting the occurrence of heart failure unrelated to coronary artery disease in patients with diabetes(88). Patients with diabetes have been demonstrated to have an increased incidence of heart failure(89–91), and to have worse outcomes from heart failure(92).

Recent data report the prevalence of diabetes as 7.1% in the general population in England (8.5% including those undiagnosed) and 17.5% in the over 65s(253). Within our population with a median age of 63, 83 (24%) had diabetes, with a further 84 (24%) having pre-diabetes.

Within our cohort, those with dysglycaemia were older with worse systolic impairment and were more symptomatic than the normoglycaemia group.

Those with diabetes had a higher prevalence of hypertension and hypercholesterolaemia than those in either the normoglycaemic group or the pre-diabetes group. They were more likely to be treated with statins than those with normoglycaemia. Studies in Denmark have shown that those with DM and no significant coronary artery disease were more likely to be taking preventative medication(73), potentially ameliorating the risk of developing CAD, they demonstrated no significant difference in risk of cardiovascular events between those with and without diabetes in this population. There was no difference in antiplatelet use between the groups, which may reflect the fact that aspirin is no longer recommended for primary prevention without a significant risk of

cardiovascular disease(254). The increased use of ACEi and diuretics across the groups matches the worsening LVEF and symptoms seen and is likely to be clinically guided.

7.4.2 Diffuse fibrosis

The exact mechanisms of diabetic cardiomyopathy are uncertain, but a progression from intracellular abnormalities followed by an asymptomatic increase in LV mass then myocardial fibrosis and subsequent diastolic impairment before the development of myocardial systolic dysfunction has been described(255). Imaging markers of strain and subsequent diastolic dysfunction have been used in echocardiography to detect these early changes(256,257). Increased mass and diastolic dysfunction has also been demonstrated in large CMR studies(113,115,116), within our cohort we similarly showed increased LV mass in the dysglycaemic group.

CMR studies have demonstrated higher T1 and ECV in diabetes(117–119), and shown association with poorer outcomes(120). Change in native T1 is another early marker of changes associated with diabetic heart disease, it has been seen to be raised in patients with diabetes and normal LVEF and has been shown to be associated with global longitudinal strain(117) and diastolic function(118).

Within our cohort we have seen increased native T1 values within the dysglycaemic group compared to normoglycemic, with no significant difference between pre-diabetes and diabetes. These findings fit with previous studies, but also indicate that changes within the myocardium may occur very early on in the disease process, within the pre-diabetic phase.

7.4.3 Ischaemic heart disease

There is an increased incidence of CAD in DM(71,72), including a known risk of silent ischaemia and infarction. Previous studies have shown incidences of silent MI between 17 and 28% in patients with diabetes and no known previous MI or CAD(81,85,86), while one study also examined patients with impaired fasting glucose and found an incidence of silent MI of 16%(86), suggesting that there is also risk in pre-diabetes. Within our cohort we found a similar incidence, with 23(27.7%) patients having evidence of silent myocardial infarction, compared to 29 (16.5%) in the normoglycaemic group. The higher prevalence in diabetes is in part due to diabetic neuropathy(258) although the presence in pre-diabetes, when

neuropathy is unlikely to be present, suggest additional mechanisms. The high incidence demonstrates the importance of diagnostic tests in this patient group to assess for CAD so that appropriate secondary prevention can be prescribed.

The presence of silent infarction has been shown to be equivalent in outcomes to known ischaemic heart disease(87). Within our study, although ischaemic heart disease was associated with increased chance of MACE in the normoglycaemic group, it was not significant in those with dysglycaemia. This may in part relate to the small numbers of ischaemic events seen during follow-up – with only 2 incidences of cardiovascular death and one of acute coronary syndrome, across the entire cohort.

In ICELAND MI, a study of 936 participants, worse outcomes were seen in those with MI, and no mortality difference between those with known MI compared to silent MI(87). Those with recognised MI were on more cardiac medications than those with unrecognised MI. As previously commented on, rates of statin and ACEi use were higher in the dysglycaemic group in our study, so may have been acting as secondary prevention in these patients, beyond the original reasons for treatment.

7.4.4 HbA1c

Within our cohort we saw no difference in incidence of MACE events between the normoglycaemic and dysglycaemic groups. Within the dysglycaemic group the main predictors of outcome were HbA1c and MPR.

In diabetes, higher HbA1c is associated with worse outcomes and higher co-morbidities, reflecting the poorer control of blood sugars and therefore increased risk of complications including microvascular dysfunction and cardiovascular disease(97,259–261). In diabetic cardiomyopathy, higher HbA1c has been shown to be associated with worse outcomes, although this has not been a linear pattern with poor outcomes in those with low HbA1c seen as well(262,263), other studies have suggested variability in HbA1c is a factor in predicting poor outcomes(264). While recent studies with newer diabetic medications such as SGLT2 inhibitors have shown promise in improving HF outcomes, the effects demonstrated have not been due to the improved glycaemic control(265) and other studies have shown no improvement in HF outcomes with better glycaemic control(100). Although small, the HR in our study, and changes seen early in the pre-diabetes group illustrate the potential importance of blood sugar control from initial

detection, in order to try and prevent complications due to poor control which may become irreversible.

7.4.5 Perfusion

MPR reflects microvascular dysfunction and is the strongest marker for cardiovascular events within our study. There is limited data available on the presence of impaired perfusion reserve in diabetic cardiomyopathy. Studies have shown reduced perfusion reserve in patients with diabetes and no known heart failure(134). Recent CMR studies have demonstrated an association between perfusion reserve and diastolic function and impaired strain rates in asymptomatic patients(132,133,135), but no association with systolic function in the same group(135) suggesting that this may play an early role in the development of cardiomyopathy.

Abnormal perfusion has been demonstrated in other non-ischaemic cardiomyopathies including dilated cardiomyopathy(125), valvular heart disease(124), amyloid(59) and Fabry's(56), usually associated with poorer outcomes . It has been suggested that microvascular dysfunction, resulting in low grade, chronic hypoperfusion plays a part in the development of DCM(125) and similar mechanisms may be present in diabetic heart disease.

We demonstrated reduced MPR in the dysglycaemic group compared to the normoglycaemic group with no difference was seen in either parameter between pre-diabetes and diabetes, supporting the early role of this change. In contrast to normoglycaemia, reduced MPR was associated with worse prognosis in dysglycaemia, suggesting microvascular dysfunction may be a key component of the worse prognosis in diabetic heart failure, and a potential target for modification.

7.4.6 Limitations

Patients recruited to this study were referred for CMR as part of routine clinical practice, therefore some elements of referral bias may have affected the make-up of our patient group, potentially excluding more frail patients who would not be felt able to undergo the test. The majority of our patients had few symptoms and were classed as NYHA I which may reflect the fact that they had already been started on treatment prior to their scan. Patients were asked to abstain from caffeine for 24hrs prior to the scan, but this was not tested, and previous studies have shown that a proportion may have residual caffeine, influencing adenosine affects.

7.4.7 Conclusions

In a prospective study of newly diagnosed patients with heart failure we have shown a high prevalence of diabetes and pre-diabetes. Myocardial tissue characteristics showed increased fibrosis and impaired perfusion compared to normoglycaemic patients, demonstrating changes early in the disease process, occurring before a clinical diagnosis of diabetes. Myocardial perfusion reserve was an independent marker of adverse cardiac events in these patients, with MPR in the presence of dysglycaemia being the strongest predictor of adverse outcomes and potentially providing an early mechanism in the development of myocardial dysfunction and a treatment target to improve outcomes.

Chapter 8
Final Discussion

CMR can provide accurate quantification of subclinical cardiac change in both health and disease and has been used to detect early fibrosis across a spectrum of cardiac disease. The detection of early change has potential for use as diagnostic and prognostic markers, initially in research, but ultimately in clinical practice. With quantitative perfusion CMR now able to be fully integrated into a standard scan, another important parameter is available to provide additional insights into disease pathways and prognosis.

In this thesis we have used quantitative myocardial perfusion to establish repeatability of this technique and normal values within the healthy population. We have applied these techniques to a range of subjects including patients with coronary artery disease, heart failure and diabetes.

8.1 Conclusions

The main findings were:

8.1.1 Repeatability of quantitative perfusion CMR

i. Inline quantitative perfusion CMR offers similar levels of repeatability to those seen in PET, the current reference method

ii. Repeatability was better in stress compared to rest, likely due to the presence of hyperaemia removing some physiological variation seen in rest MBF

iii. MPR had a worse repeatability than stress MBF, suggesting stress MBF may be the preferrable method for assessing ischaemia

8.1.2 Normal values in the healthy population

i. Normal values of MBF at stress and rest differed between sexes

ii. Females had higher MBF at stress and at rest, in part due to higher resting heart rates. No difference was seen in MPR between sexes

iii. Stress MBF and MPR declined with increasing age

8.1.3 Adenosine stress CMR

i. In healthy volunteers, no difference was seen in stress MBF when adenosine dose or length of administration were increased

ii. In patients with moderate to severe left ventricular dysfunction, stress MBF was significantly higher with higher dose adenosine

iii. Higher dose adenosine did not result in higher stress MBF in patients with mild left ventricular dysfunction or coronary artery disease

iv. Increased dose adenosine may be helpful in patients with heart failure, particularly when looking for underlying coronary artery disease

8.1.4 Heart failure defined by ejection fraction

i. Occult ischaemic heart disease was present in a high proportion of patients with significantly impaired systolic function

ii. The presence of non-ischaemic scar did not vary between groups, but markers of diffuse fibrosis and increased interstitial volume (T1 and ECV) were highest in severe systolic impairment and had a linear relationship to ejection fraction

iii. Stress MBF and MPR declined with ejection fraction, suggesting a microvascular component in more severe disease

iv. The association of worsening fibrosis and perfusion with decline in ejection fraction suggests potential targets for treatment, or markers for poor prognosis

8.1.5 Heart failure and dysglycaemia

i. The incidence of dysglycaemia was high within the heart failure population (49% in our cohort)

ii. In presumed non-ischaemic heart failure, there was still a large proportion of patients with silent ischaemic heart disease (21%)

iii. Patients with dysglycaemia had higher levels of diffuse fibrosis (T1) and lower stress MBF and MPR

iv. Within the dysglycaemic cohort, no difference was seen in tissue characteristics between those with diabetes and pre-diabetes indicating that these changes occur early in the disease process.

v. Reduced MPR was a prognostic marker in diabetes and heart failure, independent of age, ventricular function and HbA1c

vi. The worse prognosis in diabetic heart failure may be associated with microvascular dysfunction, providing a prognostic marker and potential treatment target

8.2 Future directions

We have demonstrated the utility of quantitative perfusion CMR in tissue characterisation in impaired ventricular function. Identifying early changes in heart failure and diabetes suggests that microvascular dysfunction plays a role in the development of diabetic cardiomyopathy and may account for some of the associated worse prognosis. Worse diabetic control (higher HbA1c) has been shown to be associated with increased microvascular complications in other organs(266), but has not reliably been seen to be associated with worse outcomes in diabetic cardiomyopathy(262,263). Similarly, better control of HbA1c has not resulted in better outcomes, and recent trials of SGLT2 inhibitors, the beneficial outcomes seen in heart failure have been independent of diabetes control(102). Quantitative perfusion provides the ability to quantify the extent of microvascular dysfunction and use this as a marker or endpoint in future studies, both to assess the effect of novel diabetes treatments on cardiac microvascular dysfunction, and then to evaluate whether modification may result in improved outcomes.

8.2.1 Further studies

Further studies within diabetes and heart failure are required to build on these results and explore the aetiologies and mechanism of diabetic heart disease and its prognosis. Future studies could assess the impact of medications known to improve outcomes in diabetes and heart failure, such SGLT2i (102,267,268) where assessment of subclinical changes in myocardium may inform on the, as yet unknown, mechanisms by which this effect is achieved.

In addition, given the influence of microvascular dysfunction on outcomes in these patients, targeted studies of the effect of agents such as ACE inhibitors or ARBs which have been shown to reverse some microvascular dysfunction in hypertension(269–272) may be helpful, with quantitative perfusion CMR able to measure the effect of treatment on microvascular dysfunction.

Within heart failure, our results showing improved stress perfusion with higher dose adenosine require further investigation for their potential impact on clinical practice. A larger study of patients with impaired LV function and coronary artery disease could show the impact of higher adenosine dosing on diagnosis of significant ischaemia, and whether an increased dose increased the positive diagnosis rate in this group and should be used in routine practice.

References

1. Timmis A, Townsend N, Gale CP, Torbica A, Lettino M, Petersen SE, et al. European society of cardiology: Cardiovascular disease statistics 2019. Eur Heart J. 2020 Jan 1;41(1):12–85.
2. World Health Organization. WHO - The top 10 causes of death [Internet]. 2018 [cited 2021 Jul 31]. p. 1–7. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death
3. Tonino PAL, Fearon WF, De Bruyne B, Oldroyd KG, Leesar MA, Ver Lee PN, et al. Angiographic Versus Functional Severity of Coronary Artery Stenoses in the FAME Study. Fractional Flow Reserve Versus Angiography in Multivessel Evaluation. J Am Coll Cardiol. 2010;55(25):2816–21.
4. Knuuti J, Wijns W, Achenbach S, Agewall S, Barbato E, Bax JJ, et al. 2019 ESC Guidelines for the diagnosis and management of chronic coronary syndromes. Eur Heart J. 2020 Jan 14;41(3):407–77.
5. Pijls NHJ, Fearon WF, Tonino PAL, Siebert U, Ikeno F, Bornschein B, et al. Fractional flow reserve versus angiography for guiding percutaneous coronary intervention in patients with multivessel coronary artery disease: 2-Year follow-up of the FAME (fractional flow reserve versus angiography for multivessel evaluation) study. J Am Coll Cardiol. 2010;56(3):177–84.
6. Patel MR, Peterson ED, Dai D, Brennan JM, Redberg RF, Anderson HV, et al. Low Diagnostic Yield of Elective Coronary Angiography. http://dx.doi.org/101056/NEJMoa0907272. 2010 Mar 17;362(10):886–95.
7. Overview | Recent-onset chest pain of suspected cardiac origin: assessment and diagnosis | Guidance | NICE.
8. Hou ZH, Lu B, Gao Y, Jiang SL, Wang Y, Li W, et al. Prognostic value of coronary CT angiography and calcium score for major adverse cardiac events in outpatients. JACC Cardiovasc Imaging. 2012;5(10):990–9.
9. Polonsky TS, McClelland RL, Jorgensen NW, Bild DE, Burke GL, Guerci AD, et al. Coronary artery calcium score and risk classification for coronary heart disease prediction. Vol. 303, JAMA - Journal of the American Medical

Association. 2010. p. 1610–6.

10. Knez A, Becker A, Leber A, White C, Becker CR, Reiser MF, et al. Relation of coronary calcium scores by electron beam tomography to obstructive disease in 2,115 symptomatic patients. Am J Cardiol. 2004;93(9):1150–2.

11. Villines TC, Hulten EA, Shaw LJ, Goyal M, Dunning A, Achenbach S, et al. Prevalence and severity of coronary artery disease and adverse events among symptomatic patients with coronary artery calcification scores of zero undergoing coronary computed tomography angiography: Results from the CONFIRM (Coronary CT Angiography Evalu. J Am Coll Cardiol. 2011;58(24):2533–40.

12. Meijboom WB, Meijs MFL, Schuijf JD, Cramer MJ, Mollet NR, van Mieghem CAG, et al. Diagnostic Accuracy of 64-Slice Computed Tomography Coronary Angiography. A Prospective, Multicenter, Multivendor Study. J Am Coll Cardiol. 2008;52(25):2135–44.

13. Meijboom WB, Van Mieghem CAG, van Pelt N, Weustink A, Pugliese F, Mollet NR, et al. Comprehensive Assessment of Coronary Artery Stenoses. Computed Tomography Coronary Angiography Versus Conventional Coronary Angiography and Correlation With Fractional Flow Reserve in Patients With Stable Angina. J Am Coll Cardiol. 2008;52(8):636–43.

14. Koo BK, Erglis A, Doh JH, Daniels D V., Jegere S, Kim HS, et al. Diagnosis of ischemia-causing coronary stenoses by noninvasive fractional flow reserve computed from coronary computed tomographic angiograms: Results from the prospective multicenter DISCOVER-FLOW (Diagnosis of Ischemia-Causing Stenoses Obtained Via Noni. J Am Coll Cardiol. 2011;58(19):1989–97.

15. Nørgaard BL, Leipsic J, Gaur S, Seneviratne S, Ko BS, Ito H, et al. Diagnostic performance of noninvasive fractional flow reserve derived from coronary computed tomography angiography in suspected coronary artery disease: The NXT trial (Analysis of Coronary Blood Flow Using CT Angiography: Next Steps). J Am Coll Cardiol. 2014;63(12):1145–55.

16. Lu MT, Ferencik M, Roberts RS, Lee KL, Ivanov A, Adami E, et al. Noninvasive FFR Derived From Coronary CT Angiography: Management and Outcomes in the PROMISE Trial. JACC Cardiovasc Imaging.

2017;10(11):1350–8.

17. Collet C, Onuma Y, Andreini D, Sonck J, Pompilio G, Mushtaq S, et al. Coronary computed tomography angiography for heart team decision-making in multivessel coronary artery disease. Eur Heart J. 2018 Nov 1;39(41):3689–98.

18. Coronary CT Angiography and 5-Year Risk of Myocardial Infarction. N Engl J Med. 2018 Sep 6;379(10):924–33.

19. Parkash R, DeKemp RA, Ruddy TD, Kitsikis A, Hart R, Beauschene L, et al. Potential utility of rubidium 82 PET quantification in patients with 3-vessel coronary artery disease. J Nucl Cardiol. 2004;11(4):440–9.

20. Kajander SA, Joutsiniemi E, Saraste M, Pietilä M, Ukkonen H, Saraste A, et al. Clinical Value of Absolute Quantification of Myocardial Perfusion With 15O-Water in Coronary Artery Disease. Circ Cardiovasc Imaging. 2011 Nov;4(6):678–84.

21. Herzog BA, Husmann L, Valenta I, Gaemperli O, Siegrist PT, Tay FM, et al. Long-Term Prognostic Value of 13N-Ammonia Myocardial Perfusion Positron Emission Tomography. Added Value of Coronary Flow Reserve. J Am Coll Cardiol. 2009;54(2):150–6.

22. Bruder O, Wagner A, Lombardi M, Schwitter J, Van Rossum A, Pilz G, et al. European cardiovascular magnetic resonance (EuroCMR) registry – multi national results from 57 centers in 15 countries.

23. Greenwood JP, Maredia N, Younger JF, Brown JM, Nixon J, Everett CC, et al. Cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC): a prospective trial. Lancet. 2012;379(379):453–60.

24. Jahnke C, Nagel E, Gebker R, Kokocinski T, Kelle S, Manka R, et al. Prognostic value of cardiac magnetic resonance stress tests: Adenosine stress perfusion and dobutamine stress wall motion imaging. Circulation. 2007;115(13):1769–76.

25. Cerqueira MD, Weissman NJ, Dilsizian V, Jacobs AK, Kaul S, Laskey WK, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. A statement for healthcare professionals from the Cardiac Imaging Committee of the Council on Clinical Cardiology of the American

Heart Association. Circulation. 2002;105(4):539–42.

26. Hussain ST, Paul M, Plein S, McCann GP, Shah AM, Marber MS, et al. Design and rationale of the MR-INFORM study: stress perfusion cardiovascular magnetic resonance imaging to guide the management of patients with stable coronary artery disease. J Cardiovasc Magn Reson 2012 141. 2012 Sep 19;14(1):1–10.

27. Shaw LJ, Berman DS, Picard MH, Friedrich MG, Kwong RY, Stone GW, et al. Comparative definitions for moderate-severe ischemia in stress nuclear, echocardiography, and magnetic resonance imaging. JACC Cardiovasc Imaging. 2014;7(6):593–604.

28. Vincenti G, Masci PG, Monney P, Rutz T, Hugelshofer S, Gaxherri M, et al. Stress Perfusion CMR in Patients With Known and Suspected CAD: Prognostic Value and Optimal Ischemic Threshold for Revascularization. JACC Cardiovasc Imaging. 2017;10(5):526–37.

29. Patel AR, Antkowiak PF, Nandalur KR, West AM, Salerno M, Arora V, et al. Assessment of advanced coronary artery disease: Advantages of quantitative cardiac magnetic resonance perfusion analysis. J Am Coll Cardiol. 2010;56(7):561–9.

30. Klocke FJ, Simonetti OP, Judd RM, Kim RJ, Harris KR, Hedjbeli S, et al. Limits of Detection of Regional Differences in Vasodilated Flow in Viable Myocardium by First-Pass Magnetic Resonance Perfusion Imaging. Circulation. 2001 Nov 13;104(20):2412–6.

31. Al-Saadi N, Nagel E, Gross M, Bornstedt A, Schnackenburg B, Klein C, et al. Noninvasive detection of myocardial ischemia from perfusion reserve based on cardiovascular magnetic resonance. Circulation. 2000;101(12):1379–83.

32. Schwitter J, Nanz D, Kneifel S, Bertschinger K, Büchi M, Knüsel PR, et al. Assessment of myocardial perfusion in coronary artery disease by magnetic resonance: A comparison with positron emission tomography and coronary angiography. Circulation. 2001;103(18):2230–5.

33. Christian TF, Rettmann DW, Aletras AH, Liao SL, Taylor JL, Balaban RS, et al. Absolute Myocardial Perfusion in Canines Measured by Using Dual-Bolus First-Pass MR Imaging. Radiology. 2004 Sep 1;232(3):677–84.

34. Utz W, Niendorf T, Wassmuth R, Messroghli D, Dietz R, Schulz-Menger J. Contrast-dose relation in first-pass myocardial MR perfusion imaging. J Magn Reson Imaging. 2007;25(6):1131–5.

35. Gatehouse PD, Elkington AG, Ablitt NA, Yang GZ, Pennell DJ, Firmin DN. Accurate assessment of the arterial input function during high-dose myocardial perfusion cardiovascular magnetic resonance. J Magn Reson Imaging. 2004;20(1):39–45.

36. Broadbent DA, Biglands JD, Larghat A, Sourbron SP, Radjenovic A, Greenwood JP, et al. Myocardial blood flow at rest and stress measured with dynamic contrast-enhanced MRI: Comparison of a distributed parameter model with a fermi function model. Magn Reson Med. 2013;70(6):1591–7.

37. Costa MA, Shoemaker S, Futamatsu H, Klassen C, Angiolillo DJ, Nguyen M, et al. Quantitative Magnetic Resonance Perfusion Imaging Detects Anatomic and Physiologic Coronary Artery Disease as Measured by Coronary Angiography and Fractional Flow Reserve. J Am Coll Cardiol. 2007;50(6):514–22.

38. Wilke N, Simm C, Zhang J, Ellermann J, Ya X, Merkle H, et al. Contrast-enhanced first pass myocardial perfusion imaging: Correlation between myocardial blood flow in dogs at rest and during hyperemia. Magn Reson Med. 1993 Apr 1;29(4):485–97.

39. Hsu LY, Groves DW, Aletras AH, Kellman P, Arai AE. A quantitative pixel-wise measurement of myocardial blood flow by contrast-enhanced first-pass CMR perfusion imaging: Microsphere validation in dogs and feasibility study in humans. JACC Cardiovasc Imaging. 2012;5(2):154–66.

40. Jerosch-Herold M, Wilke N, Stillman AE, Wilson RF. Magnetic resonance quantification of the myocardial perfusion reserve with a Fermi function model for constrained deconvolution. Med Phys. 1998 Jan 1;25(1):73–84.

41. Huber A, Sourbron S, Klauss V, Schaefer J, Bauner KU, Schweyer M, et al. Magnetic resonance perfusion of the myocardium: Semiquantitative and quantitative evaluation in comparison with coronary angiography and fractional flow reserve. Invest Radiol. 2012;47(6):332–8.

42. Morton G, Chiribiri A, Ishida M, Hussain ST, Schuster A, Indermuehle A, et al. Quantification of absolute myocardial perfusion in patients with coronary artery

disease: Comparison between cardiovascular magnetic resonance and positron emission tomography. J Am Coll Cardiol. 2012;60(16):1546–55.

43. Papanastasiou G, Williams MC, Dweck MR, Mirsadraee S, Weir N, Fletcher A, et al. Multimodality Quantitative Assessments of Myocardial Perfusion Using Dynamic Contrast Enhanced Magnetic Resonance and 15 O-Labeled Water Positron Emission Tomography Imaging. IEEE Trans Radiat Plasma Med Sci. 2018;2(3):259–71.

44. Mordini FE, Haddad T, Hsu LY, Kellman P, Lowrey TB, Aletras AH, et al. Diagnostic accuracy of stress perfusion CMR in comparison with quantitative coronary angiography: Fully quantitative, semiquantitative, and qualitative assessment. JACC Cardiovasc Imaging. 2014;7(1):14–22.

45. Biglands JD, Ibraheem M, Magee DR, Radjenovic A, Plein S, Greenwood JP. Quantitative Myocardial Perfusion Imaging Versus Visual Analysis in Diagnosing Myocardial Ischemia: A CE-MARC Substudy. JACC Cardiovasc Imaging. 2018;11(5):711–8.

46. Lockie T, Ishida M, Perera D, Chiribiri A, De Silva K, Kozerke S, et al. High-resolution magnetic resonance myocardial perfusion imaging at 3.0-tesla to detect hemodynamically significant coronary stenoses as determined by fractional flow reserve. J Am Coll Cardiol. 2010;57(1):70–5.

47. Villa ADM, Corsinovi L, Ntalas I, Milidonis X, Scannell C, Giovine G Di, et al. Importance of operator training and rest perfusion on the diagnostic accuracy of stress perfusion cardiovascular magnetic resonance. J Cardiovasc Magn Reson 2018 201. 2018 Nov 19;20(1):1–10.

48. Kellman P, Hansen MS, Nielles-Vallespin S, Nickander J, Themudo R, Ugander M, et al. Myocardial perfusion cardiovascular magnetic resonance: optimized dual sequence and reconstruction for quantification. J Cardiovasc Magn Reson. 2017;19(1):43.

49. Hsu LY, Jacobs M, Benovoy M, Ta AD, Conn HM, Winkler S, et al. Diagnostic Performance of Fully Automated Pixel-Wise Quantitative Myocardial Perfusion Imaging by Cardiovascular Magnetic Resonance. JACC Cardiovasc Imaging. 2018;11(5):697–707.

50. Hansen MS, Sørensen TS. Gadgetron: An open source framework for medical

image reconstruction. Magn Reson Med. 2013;69(6):1768–76.

51. Bassingthwaighte JB, Wang CY, Chan IS. Blood-tissue exchange via transport and transformation by capillary endothelial cells. Circ Res. 1989;65(4):997–1020.

52. Engblom H, Xue H, Akil S, Carlsson M, Hindorf C, Oddstig J, et al. Fully quantitative cardiovascular magnetic resonance myocardial perfusion ready for clinical use: a comparison between cardiovascular magnetic resonance imaging and positron emission tomography. J Cardiovasc Magn Reson. 2017;19(1):78.

53. Neglia D, Michelassi C, Giovanna Trivieri M, Sambuceti G, Giorgetti A, Pratali L, et al. Prognostic Role of Myocardial Blood Flow Impairment in Idiopathic Left Ventricular Dysfunction. Circulation. 2002 Jan 15;105(2):186–93.

54. Petersen SE, Jerosch-Herold M, Hudsmith LE, Robson MD, Francis JM, Doll HA, et al. Evidence for Microvascular Dysfunction in Hypertrophic Cardiomyopathy. Circulation. 2007 May 8;115(18):2418–25.

55. Cecchi F, Olivotto I, Gistri R, Lorenzoni R, Chiriatti G, Camici PG. Coronary Microvascular Dysfunction and Prognosis in Hypertrophic Cardiomyopathy. http://dx.doi.org/101056/NEJMoa025050. 2009 Oct 7;349(11):1027–35.

56. Tomberli B, Cecchi F, Sciagrà R, Berti V, Lisi F, Torricelli F, et al. Coronary microvascular dysfunction is an early feature of cardiac involvement in patients with Anderson–Fabry disease. Eur J Heart Fail. 2013 Dec 1;15(12):1363–73.

57. Elliott PM, Kindler H, Shah JS, Sachdev B, Rimoldi OE, Thaman R, et al. Coronary microvascular dysfunction in male patients with Anderson-Fabry disease and the effect of treatment with α galactosidase A. Heart. 2006 Mar 1;92(3):357–60.

58. Esposito R, Santoro C, Mandoli GE, Cuomo V, Sorrentino R, Mura L La, et al. Clinical Medicine Cardiac Imaging in Anderson-Fabry Disease: Past, Present and Future. J Clin Med. 2021;10.

59. Dorbala S, Vangala D, Bruyere J, Quarta C, Kruger J, Padera R, et al. Coronary Microvascular Dysfunction Is Related to Abnormalities in Myocardial Structure and Function in Cardiac Amyloidosis. JACC Hear Fail. 2014;2(4):358–67.

60. Graf S, Khorsand A, Gwechenberger M, Schütz M, Kletter K, Sochor H, et al.

Myocardial perfusion in patients with typical chest pain and normal angiogram. Eur J Clin Invest. 2006;36(5):326–32.

61. King H Herman W AR. Global Burden of Diabetes, 1995-2025 Prevalence,Estimates and Projections. Diabetes Care. 1998;21(9):1414–31.

62. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: Systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet. 2011;378(9785):31–40.

63. Cho NH, Shaw JE, Karuranga S, Huang Y, da Rocha Fernandes JD, Ohlrogge AW, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271–81.

64. IDF Diabetes Atlas 9th edition 2019 [Internet]. [cited 2021 Jul 11]. Available from: https://www.diabetesatlas.org/en/

65. Taylor KS, Heneghan CJ, Farmer AJ, Fuller AM, Adler AI, Aronson JK, et al. All-Cause and Cardiovascular Mortality in Middle-Aged People With Type 2 Diabetes Compared With People Without Diabetes in a Large U.K. Primary Care Database. Diabetes Care. 2013 Aug 1;36(8):2366–71.

66. Tancredi M, Rosengren A, Svensson A-M, Kosiborod M, Pivodic A, Gudbjörnsdottir S, et al. Excess Mortality among Persons with Type 2 Diabetes. N Engl J Med. 2015;373(18):1720–32.

67. Morrish NJ, Wang S-L, Stevens LK, Fuller JH, Keen H. Mortality and causes of death in the WHO multinational study of vascular disease in diabetes. Diabetologia. 2001;44(2):14–21.

68. Diabetes UK. Prediabetes or Borderline Diabetes. Diabetes UK. 2017;1–5.

69. Gu K, Catherine C, Maureen I. Mortality in adults with and without diabetes in a national cohort of the U.S. population, 1971-1993. Diabetes Care. 1998;21(7):1138–45.

70. Eberly L, Cohen J, Prineas R, Yang L. Impact of Incident Diabetes and Incident 18-Year Mortality The Multiple Risk Factor Intervention Trial experience. Diabetes Care. 2003;26(3):848–54.

71. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from Coronary Heart Disease in Subject with Type 2 Diabetes and in Nondiabetic Subjects with and without Prior Myocardial Infarction. N Engl J Med. 1998;339(4):229–34.

72. Bulugahapitiya U, Siyambalapitiya S, Sithole J, Idris I. Is diabetes a coronary risk equivalent? Systematic review and meta-analysis. Diabet Med. 2009 Feb 1;26(2):142–8.

73. Olesen KKW, Madsen M, Egholm G, Thim T, Jensen LO, Raungaard B, et al. Patients With Diabetes Without Significant Angiographic Coronary Artery Disease Have the Same Risk of Myocardial Infarction as Patients Without Diabetes in a Real-World Population Receiving Appropriate Prophylactic Treatment. Diabetes Care. 2017 Aug 1;40(8):1103–10.

74. Giusca S, Kelle S, Nagel E, Buss SJ, Voss A, Puntmann V, et al. Differences in the prognostic relevance of myocardial ischaemia and scar by cardiac magnetic resonance in patients with and without diabetes mellitus. Eur Heart J Cardiovasc Imaging. 2016;17(7):812–20.

75. Zellweger MJ, Hachamovitch R, Kang X, Hayes SW, Friedman JD, Germano G, et al. Prognostic relevance of symptoms versus objective evidence of coronary artery disease in diabetic patients. Eur Heart J. 2004;25(7):543–50.

76. Johansson I, Dahlström U, Edner M, Näsman P, Rydén L, Norhammar A. Prognostic Implications of Type 2 Diabetes Mellitus in Ischemic and Nonischemic Heart Failure. J Am Coll Cardiol. 2016;68(13):1404–16.

77. Nandalur KR, Dwamena BA, Choudhri AF, Nandalur MR, Carlos RC. Diagnostic Performance of Stress Cardiac Magnetic Resonance Imaging in the Detection of Coronary Artery Disease. A Meta-Analysis. J Am Coll Cardiol. 2007;50(14):1343–53.

78. Schwitter J, Wacker CM, Van Rossum AC, Lombardi M, Al-Saadi N, Ahlstrom H, et al. MR-IMPACT: comparison of perfusion-cardiac magnetic resonance with single-photon emission computed tomography for the detection of coronary artery disease in a multicentre, multivendor, randomized trial. Eur Heart J. 2008;29:480–9.

79. Klein L, Gheorghiade M. Management of the patient with diabetes mellitus and

myocardial infarction: Clinical trials update. Am J Med. 2004;116(5 SUPPL. 1):47–63.

80. Weiner DA, Ryan TJ, Parsons L, Fisher LD, Chaitman BR, Sheffield LT, et al. Significance of silent myocardial ischemia during exercise testing in patients with diabetes mellitus: A report from the coronary artery surgery study (CASS) registry. Am J Cardiol. 1991;68(8):729–34.

81. Kwong RY, Sattar H, Wu H, Vorobiof G, Gandla V, Steel K, et al. Incidence and prognostic implication of unrecognized myocardial scar characterized by cardiac magnetic resonance in diabetic patients without clinical evidence of myocardial infarction. Circulation. 2008;118(10):1011–20.

82. Muhlestein JB, Lappé DL, Lima JAC, Rosen BD, May HT, Knight S, et al. Effect of Screening for Coronary Artery Disease Using CT Angiography on Mortality and Cardiac Events in High-Risk Patients With Diabetes. JAMA. 2014;312(21):2234.

83. Young LH, Wackers FJT, Chyun DA, Davey JA, Barrett EJ, Taillefer R, et al. Cardiac Outcomes After Screening for Asymptomatic Coronary Artery Disease in Patients With Type 2 Diabetes. JAMA. 2009;301(15):1547.

84. Kwong RY, Chan AK, Brown KA, Chan CW, Reynolds HG, Tsang S, et al. Impact of unrecognized myocardial scar detected by cardiac magnetic resonance imaging on event-free survival in patients presenting with signs or symptoms of coronary artery disease. Circulation. 2006;113(23):2733–43.

85. Swoboda PP, McDiarmid AK, Erhayiem B, Haaf P, Kidambi A, Fent GJ, et al. A Novel and Practical Screening Tool for the Detection of Silent Myocardial Infarction in Patients With Type 2 Diabetes. J Clin Endocrinol Metab. 2016 Sep 1;101(9):3316–23.

86. Yoon YE, Kitagawa K, Kato S, Nakajima H, Kurita T, Ito M, et al. Prognostic significance of unrecognized myocardial infarction detected with MR imaging in patients with impaired fasting glucose compared with those with diabetes. Radiology. 2012;262(3):807–15.

87. Schelbert EB, Cao JJ, Sigurdsson S, Aspelund T, Kellman P, Aletras AH, et al. Prevalence and prognosis of unrecognized myocardial infarction determined by cardiac magnetic resonance in older adults. JAMA - J Am Med Assoc. 2012 Aug

29;308(9):890–7.

88. Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A. New type of cardiomyopathy associated with diabetic glomerulosclerosis. Am J Cardiol. 1972;30(6):595–602.

89. Gottdiener JS, Arnold AM, Aurigemma GP, Polak JF, Tracy RP, Kitzman DW, et al. Predictors of congestive heart failure in the elderly: The cardiovascular health study. J Am Coll Cardiol. 2000;35(6):1628–37.

90. Nichols GA, Gullion CM, Koro CE, Ephross SA, Brown JB. The Incidence of Congestive Heart Failure in Type 2 Diabetes. Diabetes Care. 2004 Aug 1;27(8):1879–84.

91. Nichols GA, Hillier TA, Erbey JR, Brown JB. Congestive Heart Failure in Type 2 Diabetes. Diabetes Care. 2001;24(9):1614 LP-1619.

92. MacDonald MR, Petrie MC, Varyani F, Ostergren J, Michelson EL, Young JB, et al. Impact of diabetes on outcomes in patients with low and preserved ejection fraction heart failure: An analysis of the Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity (CHARM) programme. Eur Heart J. 2008;29(11):1377–85.

93. Bertoni AG, Hundley WG, Massing MW, Bonds DE, Burke GL, Goff DC. Heart Failure Prevalence, Incidence, and Mortality in the Elderly with Diabetes. Diabetes Care. 2004;27(3):699–703.

94. Vaur L, Gueret P, Lievre M, Chabaud S, Passa P. Development of Congestive Heart Failure in Type 2 Diabetic Patients With Microalbuminuria or Proteinuria. Congest Hear Fail. 2003;26(3).

95. Cubbon RM, Adams B, Rajwani A, Mercer BN, Patel PA, Gherardi G, et al. Diabetes mellitus is associated with adverse prognosis in chronic heart failure of ischaemic and non-ischaemic aetiology. Diabetes Vasc Dis Res. 2013;10(4):330–6.

96. Shah AD, Langenberg C, Rapsomaniki E, Denaxas S, Pujades-Rodriguez M, Gale CP, et al. Type 2 diabetes and incidence of cardiovascular diseases: a cohort study in 1·9 million people. Lancet Diabetes Endocrinol. 2015 Feb 1;3(2):105–13.

97. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000 Aug 12;321(7258):405–12.

98. Filho AP, Kottgen A, Bertoni AG, Russell SD, Selvin E, Rosamond WD, et al. HbA 1c as a risk factor for heart failure in persons with diabetes: the Atherosclerosis Risk in Communities (ARIC) study. Diabetologia. 2008;51(12):2197–204.

99. Matsushita K, Blecker S, Pazin-Filho A, Bertoni A, Chang PP, Coresh J, et al. The Association of Hemoglobin A1c With Incident Heart Failure Among People Without Diabetes: The Atherosclerosis Risk in Communities Study. Diabetes. 2010 Aug;59(8):2020.

100. Castagno D, Baird-Gunning J, Jhund PS, Biondi-Zoccai G, MacDonald MR, Petrie MC, et al. Intensive glycemic control has no impact on the risk of heart failure in type 2 diabetic patients: Evidence from a 37,229 patient meta-analysis. Am Heart J. 2011;162(5).

101. Iribarren C, Karter AJ, Go AS, Ferrara A, Liu JY, Sidney S, et al. Glycemic control and heart failure among adult patients with diabetes. Circulation. 2001;103:2668–73.

102. Zinman B, Wanner C, Lachin JM, Fitchett D, Bluhmki E, Hantel S, et al. Empagliflozin, Cardiovascular Outcomes, and Mortality in Type 2 Diabetes. N Engl J Med. 2015;373(22):2117–28.

103. Scirica BM, Bhatt DL, Braunwald E, Steg PG, Davidson J, Hirshberg B, et al. Saxagliptin and Cardiovascular Outcomes in Patients with Type 2 Diabetes Mellitus. N Engl J Med. 2013;369:1317–26.

104. Marso SP, Daniels GH, Brown-Frandsen K, Kristensen P, Mann JFE, Nauck MA, et al. Liraglutide and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med. 2016;375(4):311–22.

105. Boudina S, Abel ED. Diabetic cardiomyopathy revisited. Vol. 115, Circulation. 2007. p. 3213–23.

106. Van Heerebeek L, Hamdani N, Handoko ML, Falcao-Pires I, Musters RJ,

Kupreishvili K, et al. Diastolic stiffness of the failing diabetic heart: Importance of fibrosis, advanced glycation end products, and myocyte resting tension. Circulation. 2008;117(1):43–51.

107. Seferović PM, Paulus WJ. Clinical diabetic cardiomyopathy: A two-faced disease with restrictive and dilated phenotypes. European Heart Journal 2015 p. 1718–27.

108. Rotkvić PG, Planinić Z, Pršo A-ML, Šikić J, Galić E, Rotkvić L, et al. The mystery of diabetic cardiomyopathy: From early concepts and underlying mechanisms to novel therapeutic possibilities. Int J Mol Sci. 2021 Jun 1;22(11):5973.

109. Das AK, Das JP, Chandrasekar S. Specific heart muscle disease in diabetes mellitus — a functional structural correlation. Int J Cardiol. 1987;17(3):299–302.

110. Fang ZY, Prins JB, Marwick TH. Diabetic Cardiomyopathy: Evidence, Mechanisms, and Therapeutic Implications. Endocr Rev. 2004;25(4):543–67.

111. Murarka S, Reza Movahed M. Diabetic Cardiomyopathy. J Card Fail. 2010;16:971–9.

112. Galderisi M, Anderson KM, Wilson PWF, Levy D. Echocardiographic evidence for the existence of a distinct diabetic cardiomyopathy (The Framingham Heart Study). Am J Cardiol. 1991;68(1):85–9.

113. Heckbert SR, Post W, Pearson GDN, Arnett DK, Gomes AS, Jerosch-Herold M, et al. Traditional Cardiovascular Risk Factors in Relation to Left Ventricular Mass, Volume, and Systolic Function by Cardiac Magnetic Resonance Imaging. The Multiethnic Study of Atherosclerosis. J Am Coll Cardiol. 2006;48(11):2285–92.

114. Devereux RB, Roman MJ, Paranicas M, O'Grady MJ, Lee ET, Welty TK, et al. Impact of Diabetes on Cardiac Structure and Function : The Strong Heart Study Impact of Diabetes on Cardiac Structure and Function The Strong Heart Study. Circulation. 2000;101:2271–6.

115. Poirier P, Bogaty P, Garneau C, Marois L, Dumesnil JG. Diastolic dysfunction in normotensive men with well-controlled type 2 diabetes: Importance of

maneuvers in echocardiographic screening for preclinical diabetic cardiomyopathy. Diabetes Care. 2001;24(1):5–10.

116. Boyer JK, Thanigaraj S, Schechtman KB, Pérez JE. Prevalence of ventricular diastolic dysfunction in asymptomatic, normotensive patients with diabetes mellitus. Am J Cardiol. 2004;93(7):870–5.

117. Ng ACT, Auger D, Delgado V, van Elderen SGC, Bertini M, Siebelink H-M, et al. Association Between Diffuse Myocardial Fibrosis by Cardiac Magnetic Resonance Contrast-Enhanced T1 Mapping and Subclinical Myocardial Dysfunction in Diabetic Patients: A Pilot Study. Circ Cardiovasc Imaging. 2012;

118. Jellis C, Wright J, Kennedy D, Sacre J, Jenkins C, Haluska B, et al. Association of imaging markers of myocardial fibrosis with metabolic and functional disturbances in early diabetic cardiomyopathy. Circ Cardiovasc Imaging. 2011;

119. Swoboda PP, McDiarmid AK, Erhayiem B, Ripley DP, Dobson LE, Garg P, et al. Diabetes mellitus, microalbuminuria, and subclinical cardiac disease: Identification and monitoring of individuals at risk of heart failure. J Am Heart Assoc. 2017 Jul 1;6(7).

120. Wong TC, Piehler K, Meier CG, Testa SM, Klock AM, Aneizi AA, et al. Association Between Extracellular Matrix Expansion Quantified by Cardiovascular Magnetic Resonance and Short-Term Mortality. Circulation. 2012 Sep 4;126(10):1206–16.

121. Jellis CL, Sacre JW, Wright J, Jenkins C, Haluska B, Jeffriess L, et al. Biomarker and imaging responses to spironolactone in subclinical diabetic cardiomyopathy. Eur Heart J Cardiovasc Imaging. 2014;15(7):776–86.

122. Gao Y, Yang Z gang, Ren Y, Liu X, Jiang L, Xie L jun, et al. Evaluation myocardial fibrosis in diabetes with cardiac magnetic resonance T1-mapping: Correlation with the high-level hemoglobin A1c. Diabetes Res Clin Pract. 2019;150:72–80.

123. Cao Y, Zeng W, Cui Y, Kong X, Wang M, Yu J, et al. Increased myocardial extracellular volume assessed by cardiovascular magnetic resonance T1 mapping and its determinants in type 2 diabetes mellitus patients with normal myocardial systolic strain Open Access. Cardiovasc Diabetol. 2018;17:7.

124. Steadman CD, Jerosch-Herold M, Grundy B, Rafelt S, Ng LL, Squire IB, et al. Determinants and functional significance of myocardial perfusion reserve in severe aortic stenosis. JACC Cardiovasc Imaging. 2012;5(2):182–9.

125. Gulati A, Ismail TF, Ali A, Hsu L-Y, Gonçalves C, Ismail NA, et al. Microvascular Dysfunction in Dilated Cardiomyopathy. JACC Cardiovasc Imaging. 2019 Aug;12(8):1699–708.

126. Tio RA, Slart RHJA, de Boer RA, van der Vleuten PA, de Jong RM, van Wijk LM, et al. Reduced regional myocardial perfusion reserve is associated with impaired contractile performance in idiopathic dilated cardiomyopathy. Neth Heart J. 2009 Dec;17(12):470–4.

127. Eguchi KK, Boden-Albala B, Jin Z, Rundek T, Sacco RL, Homma S, et al. Association Between Diabetes Mellitus and Left Ventricular Hypertrophy in a Multiethnic Population. Am J Cardiol. 2008;101(12):1787–91.

128. Asbun J, Villarreal FJ. The pathogenesis of myocardial fibrosis in the setting of diabetic cardiomyopathy. J Am Coll Cardiol. 2006;47(4):693–700.

129. Taskiran M, Fritz-Hansen T, Rasmussen V, Larsson HBW, Hilsted J. Decreased myocardial perfusion reserve in diabetic autonomic neuropathy. Diabetes. 2002;51(11):3306–10.

130. Rubinshtein R, Yang EH, Rihal CS, Prasad A, Lennon RJ, Best PJ, et al. Coronary microcirculatory vasodilator function in relation to risk factors among patients without obstructive coronary disease and low to intermediate Framingham score. Eur Heart J. 2010;31(8):936–42.

131. Lanza GA, Crea F. Primary coronary microvascular dysfunction: Clinical presentation, pathophysiology, and management. Circulation. 2010;121(21):2317–25.

132. Sørensen MH, Bojer AS, Broadbent DA, Plein S, Madsen PL, Gæde P. Cardiac perfusion, structure, and function in type 2 diabetes mellitus with and without diabetic complications. Eur Hear J - Cardiovasc Imaging. 2020 Aug 1;21(8):887–95.

133. Liu X, Yang Z, Gao Y, Xie L, Jiang L, Hu B, et al. Left ventricular subclinical myocardial dysfunction in uncomplicated type 2 diabetes mellitus is associated

with impaired myocardial perfusion: a contrast-enhanced cardiovascular magnetic resonance study. Cardiovasc Diabetol. 2018;

134. Jiang L, Shi K, Guo Y, Ren Y, Li Z, Xia C, et al. The additive effects of obesity on myocardial microcirculation in diabetic individuals: a cardiac magnetic resonance first-pass perfusion study. Cardiovasc Diabetol 2020 191. 2020 May 6;19(1):1–13.

135. Halabi A, Nolan M, Potter E, Wright L, Asham A, Marwick TH. Role of microvascular dysfunction in left ventricular dysfunction in type 2 diabetes mellitus. J Diabetes Complications. 2021 May 1;35(5):107907.

136. Messroghli DR, Bainbridge GJ, Alfakih K, Jones TR, Plein S, Ridgway JP, et al. Assessment of Regional Left Ventricular Function: Accuracy and Reproducibility of Positioning Standard Short-Axis Sections in Cardiac MR Imaging1. https://doi.org/101148/radiol2351040249. 2005 Apr 1;235(1):229–36.

137. Fischer JJ, Samady H, McPherson JA, Sarembock IJ, Powers ER, Gimple LW, et al. Comparison between visual assessment and quantitative angiography versus fractional flow reserve for native coronary narrowings of moderate severity. Am J Cardiol. 2002;90(3):210–5.

138. Hamilos M, Muller O, Cuisset T, Ntalianis A, Chlouverakis G, Sarno G, et al. Long-term clinical outcome after fractional flow reserve-guided treatment in patients with angiographically equivocal left main coronary artery stenosis. Circulation. 2009;120:1505–12.

139. Wilke N, Jerosch-Herold M, Wang Y, Huang Y, Christensen B V, Stillman AE, et al. Myocardial perfusion reserve: assessment with multisection, quantitative, first-pass MR imaging. Radiology. 1997 Aug 1;204(2):373–84.

140. Jerosch-Herold M, Swingen C, Seethamraju RT. Myocardial blood flow quantification with MRI by model-independent deconvolution. Med Phys. 2002;29:886–97.

141. Larghat AM, Maredia N, Biglands J, Greenwood JP, Ball SG, Jerosch-Herold M, et al. Reproducibility of first-pass cardiovascular magnetic resonance myocardial perfusion. J Magn Reson Imaging. 2013;37:865–74.

142. Kramer CM, Barkhausen J, Bucciarelli-Ducci C, Flamm SD, Kim RJ, Nagel E.

Standardized cardiovascular magnetic resonance imaging (CMR) protocols: 2020 update. J Cardiovasc Magn Reson. 2020 Feb 24;22(1):17.

143. Coefficient of variation from duplicate measurements [Internet]. [cited 2018 Mar 14]. Available from: https://www.medcalc.org/manual/cvfromduplicates.php

144. Ng MKC, Yeung AC, Fearon WF. Invasive assessment of the coronary microcirculation: Superior reproducibility and less hemodynamic dependence of index of microcirculatory resistance compared with coronary flow reserve. Circulation. 2006;113:2054–61.

145. Kaufmann PA, Gnecchi-Ruscone T, Yap JT, Rimoldi O, Camici PG. Assessment of the reproducibility of baseline and hyperemic myocardial blood flow measurements with 15O-labeled water and PET. J Nucl Med. 1999;40(11):1848–56.

146. Manabe O, Yoshinaga K, Katoh C, Naya M, Dekemp RA, Tamaki N. Repeatability of Rest and Hyperemic Myocardial Blood Flow Measurements with 82 Rb Dynamic PET. J Nucl Med. 2009;50(1):68–71.

147. Nagamachi S, Czernin J, Kim A, Sun K, Bottcher M, Phelps ME, et al. Reproducibility of Measurements of Regional Resting and Hyperemic Myocardial Blood Flow Assessed with PET. J Nucl Med. 1996;37:1626–31.

148. Sdringola S, Johnson NP, Kirkeeide RL, Cid E, Gould KL. Impact of unexpected factors on quantitative myocardial perfusion and coronary flow reserve in young, asymptomatic volunteers. JACC Cardiovasc Imaging. 2011;4(4):402–12.

149. Ocneanu AF, DeKemp RA, Renaud JM, Adler A, Beanlands RSB, Klein R. Optimally Repeatable Kinetic Model Variant for Myocardial Blood Flow Measurements with 82Rb PET. Comput Math Methods Med. 2017;

150. Kitkungvan D, Johnson NP, Roby AE, Patel MB, Kirkeeide R, Gould KL. Routine Clinical Quantitative Rest Stress Myocardial Perfusion for Managing Coronary Artery Disease: Clinical Relevance of Test-Retest Variability. JACC Cardiovasc Imaging. 2017;10:565–77.

151. Jerosch-Herold M, Vazquez G, Wang L, Jacobs DRJ, Folsom AR. Variability of myocardial blood flow measurements by magnetic resonance imaging in the multi-ethnic study of atherosclerosis. Invest Radiol. 2008;43(3):155–61.

152. Likhite D, Suksaranjit P, Adluru G, Hu N, Weng C, Kholmovski E, et al. Interstudy repeatability of self-gated quantitative myocardial perfusion MRI. J Magn Reson Imaging. 2016;43(6):1369–78.

153. Nitzsche EU, Choi Y, Czernin J, Hoh CK, Huang S, Schelbert HR. Noninvasive Quantification of Myocardial Blood Flow in Humans A Direct Comparison of the [N]Ammonia and the [O]Water Techniques. Circ Cardiovasc Imaging. 1996;93:2000–6.

154. Wyss CA, Koepfli P, Mikolajczyk K, Burger C, Von Schulthess GK, Kaufmann PA. Bicycle Exercise Stress in PET for Assessment of Coronary Flow Reserve: Repeatability and Comparison with Adenosine Stress. J Nucl Med. 2003;44:146–54.

155. Schindler TH, Zhang XL, Prior JO, Cadenas J, Dahlbom M, Sayre J, et al. Assessment of intra- and interobserver reproducibility of rest and cold pressor test-stimulated myocardial blood flow with 13N-ammonia and PET. Eur J Nucl Med Mol Imaging. 2007;

156. Keith GA, Rodgers CT, Chappell MA, Robson MD. A Look-Locker Acquisition Scheme for Quantitative Myocardial Perfusion Imaging with FAIR Arterial Spin Labeling in Humans at 3 Tesla. Magn Reson Med. 2017;78(2):541–9.

157. Johnson NP, Gould KL. Regadenoson versus dipyridamole hyperemia for cardiac PET imaging. JACC Cardiovasc Imaging. 2015;8(4):438–47.

158. Kitkungvan D, Lai D, Zhu H, Roby AE, Patel MB, Kirkeeide R, et al. Optimal Adenosine Stress for Maximum Stress Perfusion, Coronary Flow Reserve, and Pixel Distribution of Coronary Flow Capacity by Kolmogorov–Smirnov Analysis. Circ Cardiovasc Imaging. 2017;

159. Elkington AG, Gatehouse PD, Ablitt NA, Yang G-Z, Firmin DN, Pennell DJ. Interstudy reproducibility of quantitative perfusion cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2005;7(5):815–22.

160. Uren NG, Melin JA, de Bruyne B, Wijns W, Baudhuin T, Camici PG. Relation Between Myocardial Blood Flow and the Severity of Coronary Artery Stenosis. N Engl J Med. 1994;330:1782–8.

161. Dakak N, Quyyumi AA, Eisenhofer G, Goldstein DS, Iii RC. Sympathetically

Mediated Effects of Mental Stress on the Cardiac Microcirculation of Patients With Coranary Artery Disease. Am J Cardiol. 1995;76:125–30.

162. Bottcher M, Czernin J, Sun K, Phelps ME, Schelbert HR. Effect of Caffeine on Myocardial Blood Flow at Rest and During Pharmacological Vasodilation. J Nucl Med. 1995;36:2016–21.

163. Oudkerk M, Kuijpers D, Prakken NH, Vliegenthart R, M van Dijkman pvdijkman PR, Pim van der Harst pvanderharst bronovonl, et al. Caffeine intake inverts the effect of adenosine on myocardial perfusion during stress as measured by T1 mapping. Int J Cardiovasc Imaging. 2016;32:1545–53.

164. Banko LT, Haq SA, Rainaldi DA, Klem I, Siegler J, Fogel J, et al. Incidence of Caffeine in Serum of Patients Undergoing Dipyridamole Myocardial Perfusion Stress Test by an Intensive Versus Routine Caffeine History Screening. AJC. 2010;105:1474–9.

165. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J. 2019;40(2):87–165.

166. De Bruyne B, Baudhuin T, Melin JA, Pijls NH, Sys SU, Bol A, et al. Coronary flow reserve calculated from pressure measurements in humans. Validation with positron emission tomography. Circulation. 1994;89(3):1013–22.

167. Brown LAEE, Onciul SC, Broadbent DA, Johnson K, Fent GJ, Foley JRJJ, et al. Fully automated, inline quantification of myocardial blood flow with cardiovascular magnetic resonance: Repeatability of measurements in healthy subjects. J Cardiovasc Magn Reson. 2018 Dec 9;20(1):48.

168. Xue H, Brown LAE, Nielles-Vallespin S, Plein S, Kellman P. Automatic in-line quantitative myocardial perfusion mapping: Processing algorithm and implementation. Magn Reson Med. 2020 Feb 23;83(2):712–30.

169. Xue H, Tseng E, Knott KD, Kotecha T, Brown L, Plein S, et al. Automated detection of left ventricle in arterial input function images for inline perfusion mapping using deep learning: A study of 15,000 patients. Magn Reson Med. 2020 Nov 7;84(5):2788–800.

170. Czernin J, Muller P, Chan S, Brunken RC, Porenta G, Krivokapich J, et al.

Influence of Age and Hemodynamics on Myocardial Blood Flow and Flow Reserve. Circulation. 1993;88:62–9.

171. Higgins DM, Keeble C, Juli C, Dawson DK, Waterton JC. Reference range determination for imaging biomarkers: Myocardial T1. J Magn Reson Imaging. 2019 Sep 1;50(3):771–8.

172. Vasu S, Bandettini WP, Hsu L-Y, Kellman P, Leung S, Mancini C, et al. Regadenoson and adenosine are equivalent vasodilators and are superior than dipyridamole- a study of first pass quantitative perfusion cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2013;15:1.

173. Niedermayer S, Sourbron S, Prompona M, Cyran C, Reiser M, Huber A. Dose response of the intravascular contrast agent gadofosveset trisodium in MR perfusion imaging of the myocardium using a quantitative evaluation. Int J Cardiovasc Imaging. 2013;29(7):1527–36.

174. Fairbairn TA, Motwani M, Mather AN, Biglands JD, Larghat AM, Radjenovic A, et al. Cardiac MR imaging to measure myocardial blood flow response to the cold pressor test in healthy smokers and nonsmokers. Radiology. 2014;270(1):82–90.

175. Tomiyama Y, Manabe O, Oyama-Manabe N, Naya M, Sugimori H, Hirata K, et al. Quantification of myocardial blood flow with dynamic perfusion 3.0 Tesla MRI: Validation with (15) o-water PET. J Magn Reson Imaging. 2015;42(3):754–62.

176. Motwani M, Kidambi A, Uddin A, Sourbron S, Greenwood JP, Plein S. Quantification of myocardial blood flow with cardiovascular magnetic resonance throughout the cardiac cycle. J Cardiovasc Magn Reson. 2015;17(1):4.

177. El Fakhri G, Kardan A, Sitek A, Dorbala S, Abi-Hatem N, Lahoud Y, et al. Reproducibility and accuracy of quantitative myocardial blood flow assessment with (82)Rb PET: comparison with (13)N-ammonia PET. J Nucl Med. 2009;50(7):1062–71.

178. Efseaff M, Klein R, Ziadi MC, Beanlands RS, Dekemp RA. Short-term repeatability of resting myocardial blood flow measurements using rubidium-82 PET imaging. J Nucl Cardiol. 2012;19(5):997–1006.

179. Moody JB, Murthy VL, Lee BC, Corbett JR, Ficaro EP. Variance Estimation for Myocardial Blood Flow by Dynamic PET. IEEE Trans Med Imaging. 2015;34(11):2343–53.

180. Nickander J, Themudo R, Sigfridsson A, Xue H, Kellman P, Ugander M. Females have higher myocardial perfusion, blood volume and extracellular volume compared to males – an adenosine stress cardiovascular magnetic resonance study. Sci Rep. 2020 Dec 1;10(1):1–9.

181. Nagamachi S, Czernin J, Kim a S, Sun KT, Böttcher M, Phelps ME, et al. Reproducibility of measurements of regional resting and hyperemic myocardial blood flow assessed with PET. J Nucl Med. 1996;37(10):1626–31.

182. Chan SY, Brunken RC, Czernin J, Porenta G, Kuhle W, Krivokapich J, et al. Comparison of maximal myocardial blood flow during adenosine infusion with that of intravenous dipyridamole in normal men. J Am Coll Cardiol. 1992;20(4):979–85.

183. Muzik O, Duvernoy C, Beanlands RSB, Sawada S, Dayanikli F, Wolfe ER, et al. Assessment of diagnostic performance of quantitative flow measurements in normal subjects and patients with angiographically documented coronary artery disease by means of nitrogen-13 ammonia and positron emission tomography. J Am Coll Cardiol. 1998;31(3):534–40.

184. Chareonthaitawee P, Kaufmann PA, Rimoldi O, Camici PG. Heterogeneity of resting and hyperemic myocardial blood flow in healthy humans. Cardiovasc Res. 2001;50(1):151–61.

185. Tomiyama T, Kumita S ichiro, Ishihara K, Suda M, Sakurai M, Hakozaki K, et al. Patients with reduced heart rate response to adenosine infusion have low myocardial flow reserve in 13N-ammonia PET studies. Int J Cardiovasc Imaging. 2015;31(5):1089–95.

186. Knott KD, Camaioni C, Ramasamy A, Augusto JA, Bhuva AN, Xue H, et al. Quantitative myocardial perfusion in coronary artery disease: A perfusion mapping study. J Magn Reson Imaging. 2019 Jan 25;

187. Kaufmann PA, Gnecchi-Ruscone T, Schäfers KP, Lüscher TF, Camici PG. Low density lipoprotein cholesterol and coronary microvascular dysfunction in hypercholesterolemia. J Am Coll Cardiol. 2000;36(1):103–9.

188. Hajjiri MM, Leavitt MB, Zheng H, Spooner AE, Fischman AJ, Gewirtz H. Comparison of Positron Emission Tomography Measurement of Adenosine-Stimulated Absolute Myocardial Blood Flow Versus Relative Myocardial Tracer Content for Physiological Assessment of Coronary Artery Stenosis Severity and Location. JACC Cardiovasc Imaging. 2009;2(6):751–8.

189. Biglands JD, Magee DR, Sourbron SP, Plein S, Greenwood JP, Radjenovic A. Comparison of the Diagnostic Performance of Four Quantitative Myocardial Perfusion Estimation Methods Used in Cardiac MR Imaging: CE-MARC Substudy. Radiology. 2015;275(2):393–402.

190. Dayanikli F, Grambow D, Muzik O, Mosca L, Rubenfire M, Schwaiger M. Early Detection of Abnormal Coronary Flow Reserve in Asymptomatic Men at High Risk for Coronary Artery Disease Using Positron Emission Tomography. Circulation. 1994;90:808–17.

191. Wieneke H. Determinants of coronary blood flow in humans: quantification by intracoronary Doppler and ultrasound. J Appl Physiol. 2004;98(3):1076–82.

192. Yamamoto Y, de Silva R, Rhodes CG, Araujo LI, Iida H, Rechavia E, et al. A new strategy for the assessment of viable myocardium and regional myocardial blood flow using 15O-water and dynamic positron emission tomography. Circulation. 1992;86(1):167–78.

193. Wang L, Jerosch-Herold M, Jacobs DR, Shahar E, Folsom AR. Coronary Risk Factors and Myocardial Perfusion in Asymptomatic Adults The Multi-Ethnic Study of Atherosclerosis (MESA). J Am Coll Cardiol. 2006;47(3):565–72.

194. Duvernoy C, Vanadinseifert-Klauss, Firatdayanikli, Corneliahöss, Hennergraeff, Markusschwaiger. Gender Differences in Myocardial Blood Flow Dynamics Lipid Profile and Hemodynamic Effects. JACC. 1999;33:463–70.

195. Uren NG, Camici PG, Melin JA, Bol A, de Bruyne B, Radvan J, et al. Effect of Aging on Myocardial Perfusion Reserve. J Nucl Med. 1995;36:2032–6.

196. Yokoyama I. Reduced Coronary Flow Reserve in Familial Hypercholesterolemia. J Nucl Med. 1996;37:1937–42.

197. Collins P, Rosano GM, Sarrel PM, Ulrich L, Adamopoulos S, Beale CM, et al. 17 beta-Estradiol attenuates acetylcholine-induced coronary arterial constriction

in women but not men with coronary heart disease. Circulation. 1995 Jul 1;92(1):24–30.

198. Miller VM. Gender and vascular reactivity. Lupus. 1999;8:409–15.

199. Senneff MJ, Geltman EM, Bergmann SR, Hartman J. Noninvasive Delineation of the Effects of Moderate Aging on Myocardial Perfusion. J Nucl Med. 1991;32:2037–42.

200. Greenwood JP, Ripley DP, Berry C, McCann GP, Plein S, Bucciarelli-Ducci C, et al. Effect of care guided by cardiovascular magnetic resonance, myocardial perfusion scintigraphy, or NICE guidelines on subsequent unnecessary angiography rates :The CE-MARC 2 randomized clinical trial. JAMA - J Am Med Assoc. 2016 Sep 13;316(10):1051–60.

201. Wilson RF, Wyche K, Christensen B V, Zimmer S, Laxson DD. Clinical Investigation Effects of Adenosine on Human Coronary Arterial Circulation. Circulation. 1990;82(5):1595–606.

202. Mishra RK, Dorbala S, Logsetty G, Hassan A, Heinonen T, Schelbert HR, et al. Quantitative Relation Between Hemodynamic Changes During Intravenous Adenosine Infusion and the Magnitude of Coronary Hyperemia Implications for Myocardial Perfusion Imaging. J Am Coll Cardiol. 2005;45:553–8.

203. Modi BN, Rahman H, Sherif SA, Ellis · Howard, Kseniia Eruslanova ·, Chiribiri A, et al. Is heart rate response a reliable marker of adenosine-induced coronary hyperemia? Int J Cardiovasc Imaging. 2018;34:1117–25.

204. Gulsin GS, Abdelaty AMSEK, Shetye A, Lai FY, Bajaj A, Das I, et al. Haemodynamic effects of pharmacologic stress with adenosine in patients with left ventricular systolic dysfunction. Int J Cardiol. 2019 Mar;278:157–61.

205. Bravo PE, Hage FG, Woodham RM, Heo J, Iskandrian AE. Heart Rate Response to Adenosine in Patients With Diabetes Mellitus and Normal Myocardial Perfusion Imaging. Am J Cardiol. 2008;102:1103–6.

206. Hage FG, Heo J, Franks B, Belardinelli L, Blackburn B, Wang W, et al. Differences in heart rate response to adenosine and regadenoson in patients with and without diabetes mellitus. Am Heart J. 2009;157(4):771–6.

207. Manisty C, Ripley DP, Herrey AS, Captur G, Wong TC, Petersen SE, et al.

Splenic switch-off: A tool to assess stress adequacy in adenosine perfusion cardiac MR imaging. Radiology. 2015;276(3):732–40.

208. Lymburner P, Webber ;, Neill ;, Strugnell W, Hamilton-Craig C. P443 Simplified Splenic Switch Off-an easy method for determining adequacy of vasodila-tion during adenosine stress CMR.

209. Treuth M. Tolerance and diagnostic accuracy of an abbreviated adenosine infusion for myocardial scintigraphy: A randomized, prospective study. J Nucl Cardiol. 2001 Sep;8(5):548–54.

210. Verdoia M, Erbetta R, Sagazio E, Barbieri L, Negro F, Suryapranata H, et al. Impact of increasing dose of intracoronary adenosine on peak hyperemia duration during fractional flow reserve assessment. International J Cardiol. 2019;184:16–21.

211. Röther J, Achenbach S, Tröbs M, Blachutzik F, Nef H, Marwan M, et al. Comparison of standard- and high-dose intracoronary adenosine for the measurement of coronary fractional flow reserve (FFR). Clin Res Cardiol. 2016;105(12):1003–10.

212. Sparv D, Götberg M, Harnek J, Persson T, Hardig BM, Erlinge D. Assessment of increasing intravenous adenosine dose in fractional flow reserve. BMC Cardiovasc Disord. 2017;17:60.

213. Karamitsos TD, Ntusi NA, Francis JM, Holloway CJ, Myerson SG, Neubauer S. Feasibility and safety of high-dose adenosine perfusion cardiovascular magnetic resonance. J Cardiovasc Magn Reson. 2010;12(1).

214. Asakura M, Asanuma H, Kim J, Liao Y, Nakamaru K, Fujita M, et al. Impact of Adenosine Receptor Signaling and Metabolism on Pathophysiology in Patients with Chronic Heart Failure. Hypertens Res. 2007;30(9):781–7.

215. Headrick JP, Peart JN, Reichelt ME, Haseler LJ. Adenosine and its receptors in the heart: Regulation, retaliation and adaptation ☆. BBA - Biomembr. 2011;1808:1413–28.

216. Johnston DL, Hodge DO, Hopfenspirger MR, Gibbons RJ. Clinical Determinants of Hemodynamic and Symptomatic Responses in 2,000 Patients During Adenosine Scintigraphy. Mayo Clin Proc. 1998 Apr 1;73(4):314–20.

217. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JGF, Coats AJS, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Vol. 37, European Heart Journal. Oxford University Press; 2016. p. 2129–2200m.

218. Lam CSP, Gamble GD, Ling LH, Sim D, Leong KTG, Yeo PSD, et al. Mortality associated with heart failure with preserved vs. reduced ejection fraction in a prospective international multi-ethnic cohort study. Eur Heart J. 2018 May 21;39(20):1770–80.

219. Bhatia RS, Tu J V, Lee DS, Austin PC, Fang J, Haouzi A, et al. Outcome of Heart Failure with Preserved Ejection Fraction in a Population-Based Study A bs tr ac t. 2006.

220. Fonarow GC, Stough WG, Abraham WT, Albert NM, Gheorghiade M, Greenberg BH, et al. Heart Failure Characteristics, Treatments, and Outcomes of Patients With Preserved Systolic Function Hospitalized for Heart Failure A Report From the OPTIMIZE-HF Registry. 2007;

221. Chioncel O, Lainscak M, Seferovic PM, Anker SD, Crespo-Leiro MG, Harjola VP, et al. Epidemiology and one-year outcomes in patients with chronic heart failure and preserved, mid-range and reduced ejection fraction: an analysis of the ESC Heart Failure Long-Term Registry. Eur J Heart Fail. 2017 Dec 1;19(12):1574–85.

222. Fröhlich H, Rosenfeld N, Täger T, Goode K, Kazmi S, Hole T, et al. Epidemiology and long-term outcome in outpatients with chronic heart failure in Northwestern Europe. Heart. 2019 Feb 21;

223. Koh AS, Tay WT, Teng THK, Vedin O, Benson L, Dahlstrom U, et al. A comprehensive population-based characterization of heart failure with mid-range ejection fraction. Eur J Heart Fail. 2017 Dec 1;19(12):1624–34.

224. Reddy YNV V, Carter RE, Obokata M, Redfield MM, Borlaug BA. A simple, evidence-based approach to help guide diagnosis of heart failure with preserved ejection fraction. Circulation. 2018;138(9):861–70.

225. Francis R, Kellman P, Kotecha T, Baggiano A, Norrington K, Martinez-Naharro A, et al. Prospective comparison of novel dark blood late gadolinium enhancement with conventional bright blood imaging for the detection of scar. J

Cardiovasc Magn Reson. 2017 Nov 21;19(1):91.

226. Vedin O, Lam CSP, Koh AS, Benson L, Teng THK, Tay WT, et al. Significance of Ischemic Heart Disease in Patients with Heart Failure and Preserved, Midrange, and Reduced Ejection Fraction: A Nationwide Cohort Study. Circ Hear Fail. 2017 Jun 1;10(6).

227. Farré N, Lupon J, Roig E, Gonzalez-Costello J, Vila J, Perez S, et al. Clinical characteristics, one-year change in ejection fraction and long-term outcomes in patients with heart failure with mid-range ejection fraction: a multicentre prospective observational study in Catalonia (Spain). BMJ Open. 2017;7.

228. Pascual-Figal DA, Ferrero-Gregori A, Gomez-Otero I, Vazquez R, Delgado-Jimenez J, Alvarez-Garcia J, et al. Mid-range left ventricular ejection fraction: Clinical profile and cause of death in ambulatory patients with chronic heart failure. Int J Cardiol. 2017 Aug;240:265–70.

229. Webb J, Draper J, Fovargue L, Sieniewicz B, Gould J, Claridge S, et al. Is heart failure with mid range ejection fraction (HFmrEF) a distinct clinical entity or an overlap group? IJC Hear Vasc. 2018;21:1–6.

230. Acharya T, Aspelund T, Jonasson TF, Schelbert EB, Cao JJ, Sathya B, et al. Association of Unrecognized Myocardial Infarction with Long-term Outcomes in Community-Dwelling Older Adults: The ICELAND MI Study. JAMA Cardiol. 2018 Nov 1;3(11):1101–6.

231. Kannel WB, Abbott RD. Incidence and Prognosis of Unrecognized Myocardial Infarction. N Engl J Med. 1984 Nov;311(18):1144–7.

232. Iles L, Pfluger H, Phrommintikul A, Cherayath J, Aksit P, Gupta SN, et al. Evaluation of Diffuse Myocardial Fibrosis in Heart Failure With Cardiac Magnetic Resonance Contrast-Enhanced T1 Mapping. J Am Coll Cardiol. 2008;52(19):1574–80.

233. Nakamori S, Dohi K, Ishida M, Goto Y, Imanaka-Yoshida K, Omori T, et al. Native T1 Mapping and Extracellular Volume Mapping for the Assessment of Diffuse Myocardial Fibrosis in Dilated Cardiomyopathy. JACC Cardiovasc Imaging. 2018 Jan 1;11(1):48–59.

234. Doeblin P, Hashemi D, Tanacli R, Lapinskas T, Gebker R, Stehning C, et al.

CMR Tissue Characterization in Patients with HFmrEF. J Clin Med. 2019 Nov 5;8(11):1877.

235. Rommel KP, Von Roeder M, Latuscynski K, Oberueck C, Blazek S, Fengler K, et al. Extracellular Volume Fraction for Characterization of Patients with Heart Failure and Preserved Ejection Fraction. J Am Coll Cardiol. 2016 Apr 19;67(15):1815–25.

236. Schelbert EB, Fridman Y, Wong TC, Abu Daya H, Piehler KM, Kadakkal A, et al. Temporal relation between myocardial fibrosis and heart failure with preserved ejection fraction: Association with baseline disease severity and subsequent outcome. JAMA Cardiol. 2017 Sep 1;2(9):995–1006.

237. Kanagala P, Cheng ASH, Singh A, Khan JN, Gulsin GS, Patel P, et al. Relationship Between Focal and Diffuse Fibrosis Assessed by CMR and Clinical Outcomes in Heart Failure With Preserved Ejection Fraction. JACC Cardiovasc Imaging. 2019 Feb;

238. Puntmann VO, Voigt T, Chen Z, Mayr M, Karim R, Rhode K, et al. Native T1 mapping in differentiation of normal myocardium from diffuse disease in hypertrophic and dilated cardiomyopathy. JACC Cardiovasc Imaging. 2013 Apr 1;6(4):475–84.

239. Dass S, Suttie JJ, Piechnik SK, Ferreira VM, Holloway CJ, Banerjee R, et al. Myocardial tissue characterization using magnetic resonance noncontrast T1 mapping in hypertrophic and dilated cardiomyopathy. Circ Cardiovasc Imaging. 2012;5(6):726–33.

240. Treibel TA, Fridman Y, Bering P, Sayeed A, Maanja M, Frojdh F, et al. Extracellular Volume Associates With Outcomes More Strongly Than Native or Post-Contrast Myocardial T1. JACC Cardiovasc Imaging. 2020 May;13(1):44–54.

241. Schelbert EB, Piehler KM, Zareba KM, Moon JC, Ugander M, Messroghli DR, et al. Myocardial Fibrosis Quantified by Extracellular Volume Is Associated With Subsequent Hospitalization for Heart Failure, Death, or Both Across the Spectrum of Ejection Fraction and Heart Failure Stage. J Am Heart Assoc. 2015 Dec 18;4(12).

242. Inui K, Asai K, Tachi M, Yoshinaga A, Izumi Y, Kubota Y, et al. Extracellular

volume fraction assessed using cardiovascular magnetic resonance can predict improvement in left ventricular ejection fraction in patients with dilated cardiomyopathy. Heart Vessels. 2018 Oct 20;33(10):1195–203.

243. Van Den Heuvel AFM, Van Veldhuisen DJ, Van Der Wall EE, Blanksma PK, Siebelink HMJ, Vaalburg WM, et al. Regional myocardial blood flow reserve impairment and metabolic changes suggesting myocardial ischemia in patients with idiopathic dilated cardiomyopathy. J Am Coll Cardiol. 2000 Jan;35(1):19–28.

244. Neglia D, Michelassi C, Giovanna Trivieri M, Sambuceti G, Giorgetti A, Pratali L, et al. Prognostic Role of Myocardial Blood Flow Impairment in Idiopathic Left Ventricular Dysfunction.

245. Kotecha T, Martinez-Naharro A, Boldrini M, Knight D, Hawkins P, Kalra S, et al. Automated Pixel-Wise Quantitative Myocardial Perfusion Mapping by CMR to Detect Obstructive Coronary Artery Disease and Coronary Microvascular Dysfunction. JACC Cardiovasc Imaging. 2019 Oct;12(10):1958–69.

246. Jerosch-Herold M, Sheridan DC, Kushner JD, Nauman D, Burgess D, Dutton D, et al. Cardiac magnetic resonance imaging of myocardial contrast uptake and blood flow in patients affected with idiopathic or familial dilated cardiomyopathy. Am J Physiol Hear Circ Physiol. 2008;295:1234–42.

247. Lund LH. Heart Failure with Mid-range Ejection Fraction: Lessons from CHARM. Card Fail Rev. 2018;4(2):70–2.

248. Bhambhani V, Kizer JR, Lima JAC, Van Der Harst P, Bahrami H, Nayor M, et al. Predictors and Outcomes of Heart Failure with Mid-Range Ejection Fraction HHS Public Access. Eur J Hear Fail. 2018;20(4):651–9.

249. Miró Ò, Javaloyes P, Gil V, Martín-Sánchez FJ, Jacob J, Herrero P, et al. Comparative Analysis of Short-Term Outcomes of Patients With Heart Failure With a Mid-Range Ejection Fraction After Acute Decompensation. Am J Cardiol. 2019 Jan;123(1):84–92.

250. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE, Drazner MH, et al. 2013 ACCF/AHA guideline for the management of heart failure: A report of the American college of cardiology foundation/american heart association task force on practice guidelines. J Am Coll Cardiol. 2013;

251. Triposkiadis F, Butler J, Abboud FM, Armstrong PW, Adamopoulos S, Atherton JJ, et al. The continuous heart failure spectrum: moving beyond an ejection fraction classification. Eur Heart J. 2019 Apr 8;

252. Lam CSP, Voors AA, Piotr P, McMurray JJV, Solomon SD. Time to rename the middle child of heart failure: heart failure with mildly reduced ejection fraction. Eur Heart J. 2020 Jul 1;41(25):2353–5.

253. Diabetes - Data - PHE [Internet]. [cited 2021 Jul 21]. Available from: https://fingertips.phe.org.uk/profile/diabetes-ft/data

254. Do not offer aspirin for the primary prevention of cardiovascular disease to adults with type 1 diabetes. | NICE [Internet]. [cited 2021 Jul 21]. Available from: https://www.nice.org.uk/donotdo/do-not-offer-aspirin-for-the-primary-prevention-of-cardiovascular-disease-to-adults-with-type-1-diabetes

255. Rajbhandari J, James Fernandez C, Agarwal M, Xin Yi Yeap B, Pappachan JM. Diabetic heart disease: A clinical update. World J Diabetes. 2021;12(4):383–406.

256. Ernande L, Bergerot C, Rietzschel ER, De Buyzere ML, Thibault H, Pignonblanc PG, et al. Diastolic dysfunction in patients with type 2 diabetes mellitus: Is it really the first marker of diabetic cardiomyopathy? J Am Soc Echocardiogr. 2011;24(11):1268–1275.e1.

257. Ng ACT, Delgado V, Bertini M, van der Meer RW, Rijzewijk LJ, Shanks M, et al. Findings from Left Ventricular Strain and Strain Rate Imaging in Asymptomatic Patients With Type 2 Diabetes Mellitus. Am J Cardiol. 2009 Nov 15;104(10):1398–401.

258. Serhiyenko VA, Serhiyenko AA. Cardiac autonomic neuropathy: Risk factors, diagnosis and treatment. World J Diabetes. 2018;9(1):1.

259. Selvin E, Marinopoulos S, Berkenblit G, Rami T, Brancati FL, Powe NR, et al. Meta-analysis: Glycosylated hemoglobin and cardiovascular disease in diabetes mellitus. Ann Intern Med. 2004 Sep 21;141(6).

260. Khaw KT, Wareham N, Bingham S, Luben R, Welch A, Day N. Association of hemoglobin A1c with cardiovascular disease and mortality in adults: The European prospective investigation into cancer in Norfolk. Ann Intern Med.

2004 Sep 21;141(6).

261. Coutinho M, Gerstein HC, Wang Y, Yusuf S. The relationship between glucose and incident cardiovascular events: A metaregression analysis of published data from 20 studies of 95,783 individuals followed for 12.4 years. Diabetes Care. 1999 Feb;22(2):233–40.

262. Lawson CA, Jones PW, Teece L, Dunbar SB, Seferovic PM, Khunti K, et al. Association Between Type 2 Diabetes and All-Cause Hospitalization and Mortality in the UK General Heart Failure Population: Stratification by Diabetic Glycemic Control and Medication Intensification. JACC Hear Fail. 2018 Jan 1;6(1):18–26.

263. Aguilar D, Bozkurt B, Ramasubbu K, Deswal A. Relationship of Hemoglobin A1C and Mortality in Heart Failure Patients with Diabetes. J Am Coll Cardiol. 2009 Jul 28;54(5):422.

264. Gu J, Pan J-A, Fan Y-Q, Zhang H-L, Zhang J-F, Wang C-Q. Prognostic impact of HbA1c variability on long-term outcomes in patients with heart failure and type 2 diabetes mellitus. Cardiovasc Diabetol. 2018;17:96.

265. Zinman B. Empagliflozin, cardiovascular outcomes, and mortality in type 2 diabetes. Vol. 373, NEJM. 2016. p. 2117–28.

266. Stratton IM, Adler AI, Neil HAW, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): Prospective observational study. Br Med J. 2000 Aug 12;321(7258):405–12.

267. Neal B, Perkovic V, Mahaffey KW, de Zeeuw D, Fulcher G, Erondu N, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med. 2017;377(7):644–57.

268. Kato ET, Silverman MG, Mosenzon O, Zelniker TA, Cahn A, Furtado RHM, et al. Effect of Dapagliflozin on Heart Failure and Mortality in Type 2 Diabetes Mellitus. Circulation. 2019;139(22):2528–36.

269. Schiffrin EL. Correction of remodeling and function of small arteries in human hypertension by cilazapril, an angiotensin I-converting enzyme inhibitor. J Cardiovasc Pharmacol. 1996;27(SUPPL. 2):13–8.

270. Schiffrin EL. Vascular remodeling and endothelial function in hypertensive patients: Effects of antihypertensive therapy. Scand Cardiovasc Journal, Suppl. 1998;32(47):15–21.

271. Smith RD, Yokoyama H, Averill DB, Cooke L, Brosnihan KB, Schiffrin EL, et al. The protective effects of angiotensin II blockade with olmesartan medoxomil on resistance vessel remodeling (The VIOS study): Rationale and baseline characteristics. Am J Cardiovasc Drugs. 2006;6(5):335–42.

272. Neglia D, Fommei E, Varela-Carver A, Mancini M, Ghione S, Lombardi M, et al. Perindopril and indapamide reverse coronary microvascular remodelling and improve flow in arterial hypertension. J Hypertens. 2011;29(2):364–72.

Appendices

Appendix 1 – Ethical approval for chapters 3,4 and 5

NHS
Health Research Authority

Yorkshire & The Humber - Leeds West Research Ethics Committee
Jarrow Business Centre
Rolling Mill Road
Jarrow
NE32 3DT

Tel: 0207 104 8117

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

24 October 2017

Dr John Greenwood
University of Leeds
Academic Unit of Cardiovascular Medicine
G floor, Jubilee Wing
Leeds General Infirmary
Leeds
LS1 3EX

Dear Dr Greenwood

Study title:	**CE-MARC 2: Optimization of Image Acquisition and Analysis Methods**
REC reference:	**12/YH/0551**
Amendment number:	**SA5**
Amendment date:	**05 October 2017**
IRAS project ID:	**116093**

The above amendment was reviewed at the meeting of the Sub-Committee held in correspondence.

Summary of amendment

This substantial amendment was submitted to add an extra possible imaging sequence called Diffusion Weighted Imaging, due to this change the protocol was updated.

Ethical opinion

The Sub-Committee did not raise any ethical issues.

The members of the Committee taking part in the review gave a **favourable ethical opinion** of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP) [Substantial amendment form]	SA5	05 October 2017
Research protocol or project proposal [Protocol - tracked]	1.4	05 October 2017

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our Research Ethics Committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

12/YH/0551: Please quote this number on all correspondence

Yours sincerely
pp

Dr Vera Neumann
Vice Chair

E-mail: nrescommittee.yorkandhumber-leedswest@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Anne Gowing*

Appendix 2 – Ethical approval for chapter 4

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Prof Sven Plein
LICAMM
University of Leeds
Leeds
LS2 9JT

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

08 June 2018

Dear Prof Plein

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Ethnic Variation in Diabetes Mellitus**
IRAS project ID:	**239982**
REC reference:	**18/YH/0125**
Sponsor	**University of Leeds**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

IRAS project ID	239982

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: Ms Clare Skinner
Email: governance-ethics@leeds.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **239982**. Please quote this on all correspondence.

Yours sincerely

Thomas Fairman
HRA Assessor

IRAS project ID	239982

Email: hra.approval@nhs.net

Copy to: *NHS Research Ethics Office, Leeds University, (Sponsor)*
Ms Anne Gowing, Leeds Teaching Hospitals NHS Trust,
(Lead NHS R&D Contact)

Appendix 3 – Ethical approval for chapter 4

NHS
Health Research Authority

East Midlands - Nottingham 1 Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

28 November 2019

Dr D Adlam
Associate Professor and Honorary Consultant
University of Leicester
Department of Cardiovascular Sciences
Glenfield Hospital
Groby Road, Leicester
LE3 9QP

Dear Dr Adlam,

Study title:	**Spontaneous coronary artery dissection (SCAD): vascular pathophysiology, epidemiology and genetics**
REC reference:	**14/EM/0056**
Amendment number:	**SA08**
Amendment date:	**05 September 2019**
IRAS project ID:	**141202**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	SA08	05 September 2019
Research protocol or project proposal	8.0	15 September 2019

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

14/EM/0056:	**Please quote this number on all correspondence**

Yours sincerely,

P.P.

Professor Cris Constantinescu
Chair

E-mail: NRESCommittee.EastMidlands-Nottingham1@nhs.net

Enclosures: *List of names and professions of members who took part in the review*

Copy to: *Mrs Wendy Gamble*

Appendix 4 – Ethical approval for chapter 4

Health Research Authority

West Midlands - Solihull Research Ethics Committee

The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS
Tel: 0207 1048310

Please note: This is the favourable opinion of the REC only and does not allow the amendment to be implemented at NHS sites in England until the outcome of the HRA assessment has been confirmed.

02 March 2021

Dr Gaurav Singh Gulsin
Department of Cardiovascular Sciences
Cardiovascular Research Centre, Glenfield General Hospital
Groby Road, Leicester
LE39QP

Dear Dr Gulsin

Study title:	**Prevalence and Determinants of Subclinical Cardiovascular Dysfunction in Adults with Type 2 Diabetes**
REC reference:	**17/WM/0192**
Amendment number:	**0580_7_SA05**
Amendment date:	**05 February 2021**
IRAS project ID:	**226498**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Completed Amendment Tool [Amendment Toolkit]	N/A	05 February 2021
Participant consent form [	3.0	17 February 2021

PREDICT_MEMRI_PIL_v3.0_17.02.2021_MARKED]		
Participant consent form [PREDICT_MEMRI_consent_v3.0_17.02.2021_MARKED]	3.0	17 February 2021
Participant information sheet (PIS) [PREDICT_MEMRI_consent_v3.0_17.02.2021_CLEAN]	3.0	17 February 2021
Participant information sheet (PIS) [PREDICT_MEMRI_PIL_v3.0_17.02.2021_CLEAN]	3.0	17 February 2021
Research protocol or project proposal [PREDICT_protocol_UoL_v6.0_17.02.2021_CLEAN]	6.0	17 February 2021
Research protocol or project proposal [PREDICT_protocol_UoL_v6.0_17.02.2021_MARKED]	6.0	17 February 2021

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

Working with NHS Care Organisations

Sponsors should ensure that they notify the R&D office for the relevant NHS care organisation of this amendment in line with the terms detailed in the categorisation email issued by the lead nation for the study.

Amendments related to COVID-19

We will update your research summary for the above study on the research summaries section of our website. During this public health emergency, it is vital that everyone can promptly identify all relevant research related to COVID-19 that is taking place globally. If you have not already done so, please register your study on a public registry as soon as possible and provide the HRA with the registration detail, which will be posted alongside other information relating to your project.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

HRA Learning

We are pleased to welcome researchers and research staff to our HRA Learning Events and online learning opportunities– see details at: https://www.hra.nhs.uk/planning-and-improving-research/learning/

IRAS Project ID - 226498:	**Please quote this number on all correspondence**

Yours sincerely

Dr Rex J Polson
Chair
E-mail: solihull.rec@hra.nhs.uk

Enclosures: *List of names and professions of members who took part in the review*

Appendix 5 – Ethical approval for chapters 4 and 5

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Professor Sven Plein
BHF Professor of Cardiology and Honorary Consultant Cardiologist
University of Leeds
LICAMM
LIGHT building
University of Leeds
LS2 9JT

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

15 June 2018

Dear Professor Plein

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Advanced Magnetic Resonance Imaging: Optimization of Image Acquisition and Analysis Methods (AMaRI)**
IRAS project ID:	**245109**
REC reference:	**18/YH/0168**
Sponsor	**University of Leeds**

I am pleased to confirm that HRA and Health and Care Research Wales (HCRW) Approval has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

How should I continue to work with participating NHS organisations in England and Wales?
You should now provide a copy of this letter to all participating NHS organisations in England and Wales, as well as any documentation that has been updated as a result of the assessment.

Following the arranging of capacity and capability, participating NHS organisations should **formally confirm** their capacity and capability to undertake the study. How this will be confirmed is detailed in the "*summary of assessment*" section towards the end of this letter.

You should provide, if you have not already done so, detailed instructions to each organisation as to how you will notify them that research activities may commence at site following their confirmation of capacity and capability (e.g. provision by you of a 'green light' email, formal notification following a site initiation visit, activities may commence immediately following confirmation by participating organisation, etc.).

IRAS project ID	245109

It is important that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details of the research management function for each organisation can be accessed here.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within the devolved administrations of Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) has been sent to the coordinating centre of each participating nation. You should work with the relevant national coordinating functions to ensure any nation specific checks are complete, and with each site so that they are able to give management permission for the study to begin.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?
The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

I am a participating NHS organisation in England or Wales. What should I do once I receive this letter?
You should work with the applicant and sponsor to complete any outstanding arrangements so you are able to confirm capacity and capability in line with the information provided in this letter.

The sponsor contact for this application is as follows:

Name: NHS Research Ethics Officer
Email: governance-ethics@leeds.ac.uk

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **245109**. Please quote this on all correspondence.

IRAS project ID	245109

Yours sincerely

Thomas Fairman
HRA Assessor

Email: hra.approval@nhs.net

Copy to: *NHS Research Ethics Office, Leeds University, (Sponsor Contact)*
Ms Anne Gowing, Leeds Teaching Hospitals NHS Trust, (Lead NHS R&D Contact)

Appendix 6 – Ethical approval for chapters 5, 6 and 7

NHS
Health Research Authority

Dr PS Swoboda
Lecturer in Cardiology
University of Leeds
University of Leeds
Leeds
LS2 9JT

Email: hra.approval@nhs.net

23 October 2017

Dear Dr Swoboda

Letter of HRA Approval

Study title:	**Myocardial tissue characteristics in patients with heart failure according to glycaemic status.**
IRAS project ID:	**228222**
REC reference:	**17/YH/0300**
Sponsor	**University of Leeds**

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read *Appendix B* carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

IRAS project ID	228222

It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators",* issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application

IRAS project ID	228222

procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **228222**. Please quote this on all correspondence.

Yours sincerely

Juliana Araujo
Assessor
Email: hra.approval@nhs.net

Copy to: *Sponsor Representative: Ms Clare Skinner, University of Leeds*
Lead NHS R&D Office Representative: Ms Anne Gowin, Leeds Teaching Hospitals NHS Trust