

**Tissue Characterisation in Acquired Heart Disease with
Cardiovascular Magnetic Resonance Imaging**

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Chapter Two

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Abstract

Objectives: To apply and refine conventional and developing cardiac magnetic resonance (CMR) imaging tissue characterisation techniques, with the aim of applying them to better understand cardiac remodelling in health, disease and as an outcome surrogate in clinical trials.

Background: Cardiac structure, function and perfusion are routinely assessed with a range of imaging modalities in both research and clinical practice. Cardiac magnetic resonance (CMR) imaging is emerging as the gold standard tool for many of these assessments. The use of gadolinium containing contrast agents in CMR protocols allows the detection of myocardial scar and focal fibrosis and provides important prognostic information. The developing field of T1 mapping allows measurement of the extracellular volume, a surrogate for fibrosis that offers further insights into diffuse myocardial change not previously possible.

Methods: CMR tissue characterisation techniques were applied in sequential studies of: ischaemic cardiomyopathy, health, athletic cardiac adaptation and in a randomised controlled study examining the effects of spironolactone in heart failure with preserved ejection fraction.

Results & Conclusions: Late gadolinium enhancement (LGE) imaging is of limited application in predicting functional recovery of dysfunctional segments in ischaemic cardiomyopathy (Chapter 3). Modified Look-Locker Inversion recovery (MOLLI) T1 and extracellular mapping techniques can be applied reproducibly in health and following either bolus or split dose gadolinium administration (Chapters 4 & 5). T1 mapping provides important insights into athletic cardiac remodelling that may allow its application in distinguishing between athletic and myopathic change (Chapter 6). Individuals with heart failure with preserved ejection fraction (HF-PEF) have diffuse myocardial fibrosis as measured with T1 mapping. Early results of a randomised controlled study suggest that the beneficial effects of spironolactone in HF-PEF may in part be due to regression of diffuse myocardial fibrosis (Chapter 7).

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1. Chapter 1

General Introduction

1.1 Introduction

Cardiovascular magnetic resonance (CMR) imaging is well established in clinical practice for the assessment of cardiac structure, function and perfusion[1]. The excellent spatial resolution and reproducibility of CMR[2] mean that it is ideal for accurate quantification of cardiac function, whilst first pass adenosine perfusion imaging is becoming established as a key clinical modality for ischaemia detection and quantification[3, 4].

For both clinical and research use the ability to accurately characterise myocardium is of increasing importance, not only for accurate diagnosis but also to track disease progression and the efficacy of interventions. This is a developing field and whilst some characterisation techniques are firmly established others remain predominantly research tools.

Tissue characterisation techniques are made possible by interrogating the T1 and T2 properties of tissue, as well as the effects of gadolinium based contrast agents (GBCA) upon these. This thesis focuses on T1 based techniques, though both T1 and T2 based techniques are of clinical importance.

1.2 T1 Based Techniques

T1 is the longitudinal relaxation time of tissue, and differs according to tissue composition, the presence of contrast and field strength[5]. T1 is measured following a 180° inversion, or 90° saturation, pulse along the z-axis in the magnetic field, and represents approximately 63% of the time for spin to return to equilibrium along the z-axis. Energy, detected as signal, is released by hydrogen nuclei as their spin returns to equilibrium following the inversion or saturation pulse. The signal released is related to size of the molecule with which a hydrogen nucleus is associated: large molecules return to equilibrium slowly with a longer T1 (e.g. fibrotic tissue), whilst smaller molecules (e.g. fat) return to equilibrium more quickly with shorter T1[5].

A crude assessment of tissue characteristics may be made on T1 weighted survey images though beyond use for planning further acquisitions, or detection of incidental masses, there is limited application in either research or clinical practice.

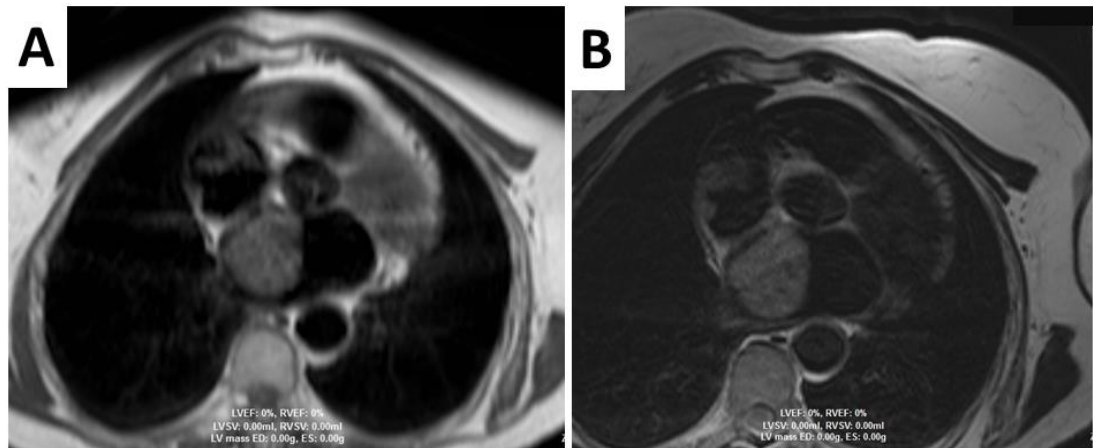


Figure 1.1 Incidental finding in CMR

A) T1 and B) T2 weighted survey images demonstrate a left atrial mass, later found to be a left atrial myxoma

Both subjective and quantitative methods of T1 based tissue characterisation are in routine practice. These include visual assessment of focal scar and fibrosis with late gadolinium enhancement (LGE) imaging and quantification of tissue T1 with the use of 'mapping' methods. T1 mapping techniques remain under development and the optimal methodology has not yet been agreed[6].

1.2.1 Late Gadolinium Enhancement Imaging

The addition of GBCA to scan protocols greatly increases the potential of tissue characterisation with CMR. Due to their large molecular size GBCAs are excluded from the intracellular space, consequently following intravenous injection GBCAs remain in the circulation and extracellular space before being renally excreted. In the presence of fibrosis or acute injury normal GBCA kinetics are altered, the clearance of GBCAs is impaired and contrast persists in the tissue thus shortening T1.

Late gadolinium enhancement images are acquired a minimum of 5 minutes after GBCA administration with a T1 weighted image using an inversion time (TI) such that the signal from normal myocardium is nulled, but in tissue where gadolinium has persisted high signal is evident[7].

LGE imaging allows the detection of focal myocardial processes, and both its presence and extent have prognostic importance[8-10]. LGE imaging is now also recommended for determining the aetiology of heart failure[11], made possible by characteristic enhancement patterns displayed in a range of conditions[12, 13].

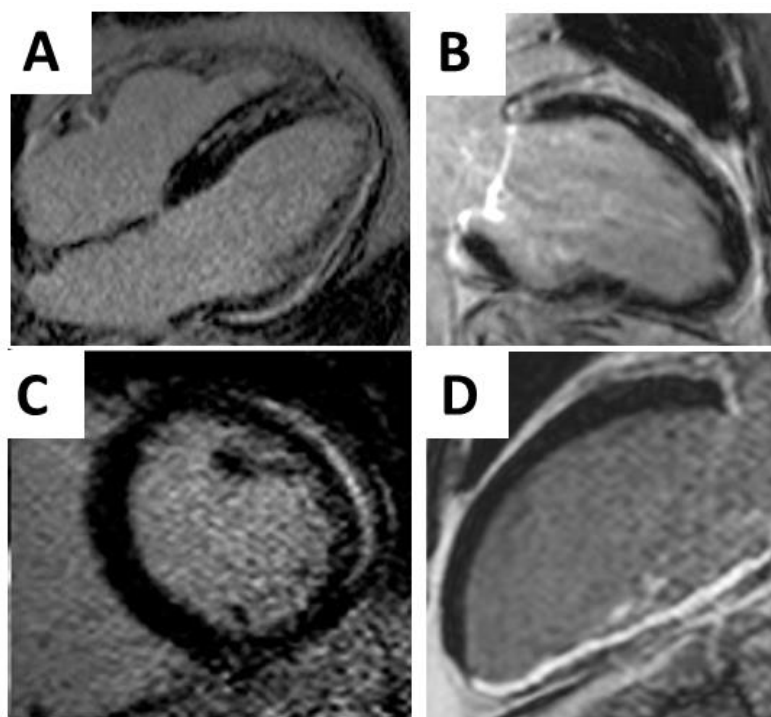


Figure 1.2 Characteristic LGE patterns

A & B) Septal, anterior and lateral mid-wall enhancement in dilated cardiomyopathy. C) Epicardial lateral wall enhancement in myocarditis. D) Full thickness inferior enhancement and thinning in chronic myocardial infarction.

However despite its utility, there are limitations of this technique including:

- 1) the need for normal tissue as 'contrast' for diagnosis
- 2) the binary nature of abnormality detection.

As a contrast between normal and abnormal tissue is required diffuse abnormalities are poorly appreciated on LGE imaging. This is a particular problem in conditions characterised by diffuse fibrosis where, as an inherent part of the LGE approach, global nulling of the myocardium may occur. Furthermore, though the extent of LGE may be quantified, this may vary greatly by quantification technique employed[14]. As well as difficulty quantifying the extent of abnormalities, the underlying pathology responsible may only be termed as present or absent, rather than defined by severity of tissue change. The inability to characterise the underlying tissue change fully means surrogates are sometimes employed such as 'greyness' or 'haziness'. Use of terms such as these introduces subjectivity and decreases reproducibility in CMR interpretation. Furthermore grey areas on LGE imaging may reflect partial volume phenomenon or patchy change[15]. In addition,

without the ability to reliably quantify abnormalities tracking change in tissue composition, either in disease progression or the effect of an intervention, is not possible. These limitations may be overcome by the quantitative technique, T1 mapping[16].

1.2.2 T1 Mapping & Extracellular Volume Estimation

T1 mapping allows the T1 of any given pixel to be defined and displayed as either a grayscale or colour image (once colour scaling has been performed)[17, 18]. T1 mapping techniques are often vendor specific, though some may be used across platforms, and include MOLLI, ShMOLLI, SASHA and SAPHIRE. T1 is calculated by sampling signal at various time points along the tissue recovery curve following an inversion or saturation pulse. The signal intensity readout is then fitted to a model that enables tissue T1 to be estimated[19]. T1 measurement methods have differing degrees of accuracy and precision[20], and consensus on the optimal methodology has not yet been reached[6].

1.2.2.1 Sequences & T1 Sampling Methodology

1.2.2.1.1 Modified Look-Locker Inversion Recovery (MOLLI)

There are multiple alternative methods to measure myocardial T1, as mentioned above, but they share some common principles.

The modified Look-Locker Inversion recovery (MOLLI) method was first published in 2004[19]. The nomenclature used to describe MOLLI schemes describes the number of samples made following each inversion pulse, and in parentheses the minimum pause (recovery period defined in either seconds (s) or R-R interval (b)) following the prior sample e.g. a 4(3s)3(3s)2 scheme would comprise: three inversion pulses with 4 samples after the first inversion pulse, three after the second and two after the final pulse, and a minimum 3 second pause from the last sample of the prior set before the next inversion pulse.

The initially proposed MOLLI scheme employed three inversion pulses with eleven subsequent samples in three sets with variable trigger delays set to obtain a representative sample of the inversion recovery curve. Multiple sample points allow better fitting of the recovery curve, however sampling itself affects recovery as in the sampling process signal is 'lost'. A schematic of a 5(3s)3 MOLLI acquisition is shown in figure 1.3.

Potential limitations: A balanced steady-state free precession (bSSFP), rather than spoiled gradient echo, readout is used in MOLLI due to improved signal-to-noise

ratio[19]. However the use of a bSSFP readout speeds tissue recovery, as a result it is necessary to apply a correction factor, which in turn affects accuracy.

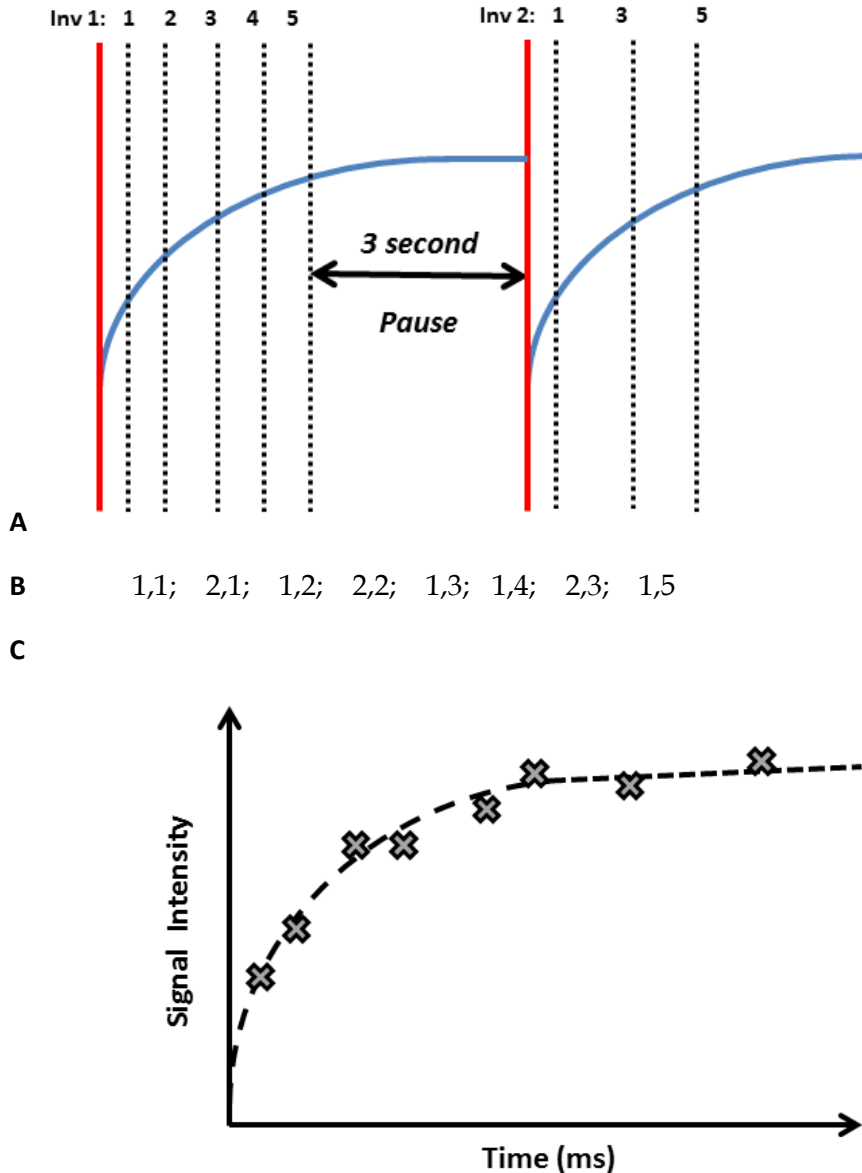


Figure 1.3 Explanatory diagram of a 5b(3s)3b MOLLI Scheme.

A) After each 180° inversion pulse (red lines) magnetisation recovers (blue line), as magnetisation recovers signal is sampled with differing trigger delays (dashed lines). Subsequent inversion pulses are delivered to the tissue after a minimum specified delay. B) Re-ordering of acquisitions by length of trigger delay. C) A T1 fitting curve is generated allowing estimation of tissue T1.

Following an inversion pulse, tissue magnetisation recovers to normal over time. After the final sample, it is necessary to allow magnetisation to fully recover prior

to a further inversion pulse. Incomplete recovery leads to fitting errors as recovery occurs from differing baseline level of tissue magnetisation, with subsequent knock on effect on T1 accuracy, as recovery does not occur along the same recovery curve.

Multiple alternatives to the initial 3(3b)3(3b)5 MOLLI scheme have been suggested. The aim of these alternatives include improved accuracy and precision of T1 and shortened breath-hold. Many sequences now employ only two inversion pulses to minimise the effects of prior magnetisation on subsequent readouts, whilst it is now also common for differing pre and post contrast T1 mapping strategies to be used, due to differing relaxation properties following contrast administration. For example a three second pause to allow complete recovery is unnecessary when T1 is shortened, whilst standard pre-contrast, 'native T1' sequences may sample insufficiently early on the recovery curve when recovery is swift to allow for accurate fitting. In an attempt to counteract the effects of heart rate on T1 estimation modifications to the original MOLLI sequence have been suggested. The use of fixed pause duration, measured in seconds, ensures that the interval between inversions is constant, and cannot become as squeezed, and the use of front weighted MOLLI schemes e.g. 5b(3s)3b both mean that the effects of prior magnetisation are lessened [17].

1.2.2.1.2 Shortened Modified Look-Locker Inversion Recovery (ShMOLLI)

The ShMOLLI technique shares much with the MOLLI method, but was developed to mitigate the systematic error encountered at higher heart rates on T1 with MOLLI. Underestimation of longer T1 with MOLLI at higher heart rates is well recognised [19, 21], and is predominantly attributable to an insufficient recovery interval between inversions: In early MOLLI schemes pause duration was measured in beats (b), and as a result the interval between inversions using a 3(3b)3(3b)5 scheme with a heart rate of 60 and 100min⁻¹ are 6000 milliseconds (ms) and 3600ms respectively. Therefore when longer T1 times are encountered recovery between inversions may be incomplete. The ShMOLLI method[22] (5(1b)1(1b)1) acquires 5 samples without the influence of prior tissue inversions, and only utilises the final two when recovery (which is estimated from the prior 5) is estimated to have been complete. This adaption decreases the influence of heart rate on T1 estimation, but also means that when T1 is long the later acquisitions are discarded with subsequent effect on accuracy, due to fewer points with which to fit a curve.

1.2.2.1.3 Saturation Recovery Single Shot Acquisition (SASHA)

Rather than using a 180° inversion pulse as in MOLLI/ShMOLLI both SASHA and SAPHIRE mapping methods employ a saturation pulse. A saturation pulse at 90° to the z axis nulls the innate magnetism of tissue, from which it then recovers to normal[23]. With the SASHA technique a saturation pulse every R waves nulls the

signal, which is then sampled at a variety of intervals as it recovers to allow the recovery curve to be fitted.

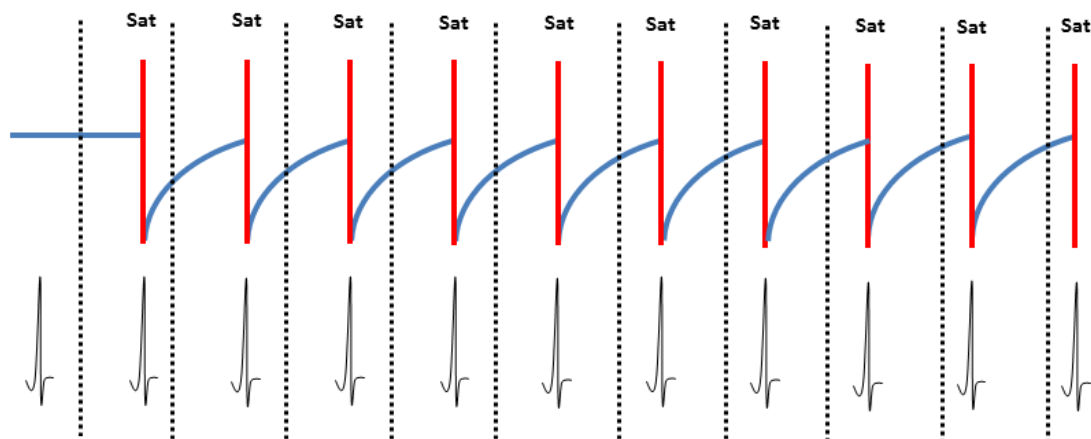


Figure 1.4 Explanatory diagram of a SASHA scheme

An initial sample (dashed line) is performed prior to saturation pulse deliver to determine fully recovered tissue T1. Following that 90° saturation pulses (red line) are delivered with every R wave, and signal sampled with different trigger delay to allow plotting of a T1 recovery curve as it recovers (blue line).

Unlike MOLLI, where recovery is affected by the bSSFP readout, saturation recovery is unaffected and as a result it is not necessary to apply a correction algorithm. This, and the absence of effect from prior inversions, mean that SASHA has better accuracy compared to MOLLI[20]. When T1 is long, a recovery period longer than one R-R interval may be used. However as a first read out is performed prior to delivery of any saturation pulses a good estimate of fully recovered T1 is obtained with this technique.

Tissue T1 derived with the SASHA method is likely more accurate than MOLLI and ShMOLLI however measurements have lower precision and reproducibility. Furthermore if the subject has a low heart rate the acquisition time may well be longer than both ShMOLLI and more recently suggested MOLLI schemes[20], as SASHA acquisitions are typically made over ten heart beats.

1.2.2.1.4 Saturation Pulse Prepared Heart Rate Independent Inversion Recovery (SAPPHIRE)

SAPPHIRE is a hybrid scheme in which a saturation pulse is first applied prior to subsequent inversion pulse and subsequent read out. This method has been shown to perform similarly to SASHA.

1.2.2.1.5 Summary

The MOLLI method is the most established of the T1 mapping techniques in common use. However it has limitations, including inaccuracy at high heart rates and the necessity of a correction algorithm, that have led to alternative schemes being developed. The ShMOLLI method is less sensitive to heart rate than MOLLI but may result in a loss of accuracy as when T1 is long, as some points are discarded. Indeed with newer MOLLI schemes with 'front-loading' of sampling (e.g 5b(3s)3b) and fixed minimum pause duration the advantages of the ShMOLLI technique are limited. Though less commonly employed than MOLLI based techniques the saturation pulse based methods e.g. SASHA have superior accuracy[20].

1.2.2.2 Quantitative Tissue Characterisation

Native T1 reflects both cellular and intracellular characteristics, and is affected by both field strength and tissue water content[5]. Prolonged native T1 is seen in a range of conditions including myocarditis, acute myocardial infarction and hypertrophic cardiomyopathy[24], whilst decreased T1 may be seen in Anderson-Fabry disease and iron deposition[25]. Further tissue characterisation may be performed by combining native and post contrast myocardial and blood pool T1 with haematocrit to determine the extracellular volume (ECV):

$$ECV = (1 - Hct) \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)}$$

**Where $R1=1/T1$ and Hct is Haematocrit.*

The 'wash in/wash out' kinetics of gadolinium mean that the distribution, and persistence, of gadolinium in the extra-cellular space allows the relative shortening of T1 following GBCA to be used to approximate the extracellular volume[17]. However for this method to be applied it is important that post-contrast T1 is sampled at a point when gadolinium has reached an equilibrium[16], or steady-state, between the intravascular and interstitial space i.e. the components of the extracellular volume. Failure to do so will result in over-estimation of ECV, as T1 is inappropriately shortened when compared to steady state. Reliable measurement of ECV has previously been shown to be possible following both bolus and steady state infusion methods of GBCA administration [16].

As well as determining ECV numerically, swift interpretation of ECV maps may be facilitated with use of a colour image. Colour ECV maps display identical

information to grayscale images but pixels are coloured according to a predefined scale.

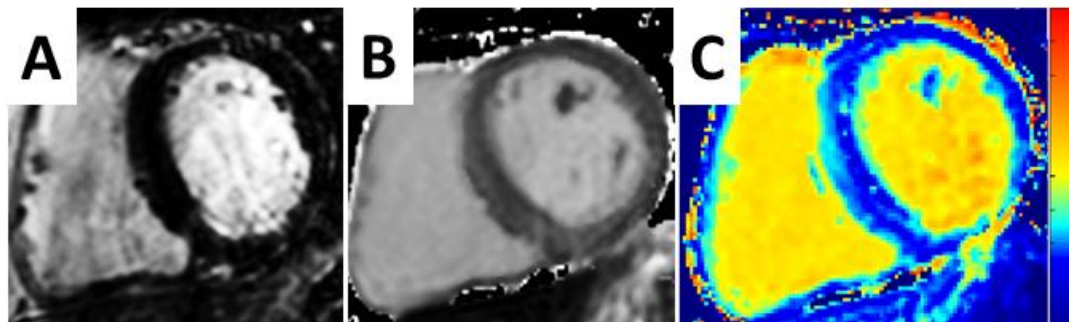


Figure 1.5 T1 Based techniques in chronic MI

Sub-endocardial inferior myocardial infarction. A) LGE image demonstrating sub-endocardially based partial thickness myocardial infarction. B) Grayscale native T1 map and C) ECV colour map of the same slice demonstrating elevated ECV adjacent to the area displaying late enhancement.

ECV has been demonstrated to correlate well with histological measurement of tissue fibrosis in a range of conditions[16]. ECV is often used as a surrogate for fibrosis or infiltration in both research and clinical arenas, though this may be an oversimplification as this assumption does not account for the effects of capillary dilation. The ability to measure diffuse fibrosis objectively enables disturbance of the extracellular space to be tracked. This new method will potentially provide new opportunities for monitoring the tissue effects of therapeutic intervention.

There are limitations of both native T1 and extracellular volume calculation. T1 is sensitive to both field strength, field inhomogeneity and sequence, as a consequence absolute cut-offs for native T1 are not yet applicable across multiple sites. Multiple acquisitions are necessary for T1 mapping techniques; as a result T1 acquisition can be long with introduction of respiratory motion artefact[17]. Furthermore as both pre and post T1 are necessary for ECV calculation reproducible image planning is mandatory to sample the same myocardium on both native and post contrast T1 measurement.

1.3 T2 Based Techniques

T2 is the time constant of transverse relaxation and related to random spin-spin interactions of molecules within, tissue and is more affected by water than T1[5]. If free water is present, these molecules are further apart, interactions less frequent, and relaxation slower. Whereas if water is bound to large molecules it moves more slowly, they are more liable to interact and relaxation occurs more quickly.

1.3.1 T2 Weighted Imaging

T2 weighted image characterisation is applied in clinical protocols for subjective detection of oedema and inflammation[26], research use includes quantification of myocardial salvage and area at risk[27]. T2 weighted images are generated with spin echo sequences, particularly turbo spin-echo and short-TI triple inversion recovery prepared fast spin echo (STIR)[28]. The presence of oedema demonstrated on T2 weighted images has been shown to agree with histological specimens[29, 30].

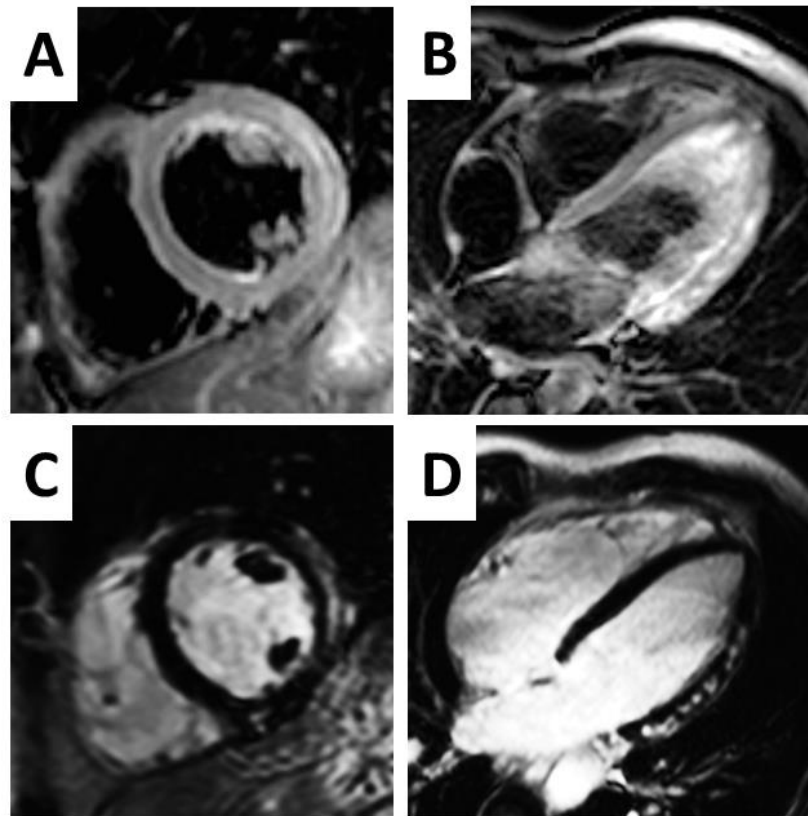


Figure 1.6 Acute Myocarditis

A & B) hyperintense signal in the LV lateral wall. C & D) demonstrate LGE in the same area in an epicardial distribution

Oedema is demonstrated as a hyperintense area of signal, and allows distinction between acute and chronic presentations of cardiac disease[31]. Semi-quantitative assessment is possible by measuring the signal ratio of myocardium and skeletal muscle. However despite this T2 image interpretation is limited by:

- 1) Lack of uniformity of signal, and signal loss in tissue distant from the coil, making nearer myocardium appear relatively hyper intense
- 2) Artefact from slow flowing blood adjacent to myocardium often resulting in a bright rim, adjacent to the endocardium.

1.3.2 T2 Mapping

T2 mapping may help overcome some of the above limitations, whilst at the same time providing quantitative information regarding the severity of a given abnormality[32]. As with T1 mapping, sequence development is ongoing and is an active area of CMR research. Similarly to T1 mapping multiple acquisitions are performed to generate a final 'map' with pixel wise T2 values[33]. The quantification of myocardial water content and oedema in health, aging and chronic disease may offer new opportunities to study sub-clinical manifestation of disease[34, 35]. Artefact, particularly susceptibility artefact infero-laterally, and relatively low signal when compared to T1 based techniques limits its adoption in routine clinical practice.

1.3.3 T2* Mapping

Of all quantitative techniques T2* mapping is the most established, and also the technique most used for clinical decision making[36, 37]. T2* is shorter than T2 due to interaction between the T2 properties and local magnetic field inhomogeneities due to the presence of iron containing compounds[5].

In clinical practice T2* maps are predominantly used for the quantification of cardiac iron deposition, and T2* has been demonstrated to agree well with biopsy specimens. The ability to identify and track iron deposition is used to guide iron chelation therapy in both haemoglobinopathies and transfusion dependent patients. There is also growing research interest in T2* mapping based techniques to identify intramyocardial haemorrhage in acute MI[38], a factor that has been associated with adverse prognosis.

1.4 Conclusion

Myocardial tissue characterisation is a developing field of CMR. Increasingly the research focus is moving away from subjective to quantitative techniques. Both T1 and T2 quantification techniques are applied in clinical practice and are providing important insights to disease in cardiovascular research.

This thesis focuses on T1 weighted based techniques: Initially describing the use of LGE imaging in chronic severe ischaemic cardiomyopathy and later T1 mapping sequence development and its application in health and disease.

Chapter 2

Taxonomy in Clinical Practice for Myocardial Segments in Left Ventricular Systolic Dysfunction

2.1. Abstract

2.1.1. Objectives

To describe differing states of myocardial health and disease in systolic dysfunction, in an effort to establish standard nomenclature for future clinical research studies.

2.1.2. Background

Multiple terms are applied to describe myocardium in clinical studies, particularly with reference to chronic ischaemia. In addition studies employ a range of imaging modalities to assess myocardial health. The lack of consistency in description of myocardium where terms are often used interchangeably, across a range of imaging modalities hinders the interpretation and application of study findings in clinical practice.

2.1.3. Conclusions

We have described differing myocardial states in left ventricular systolic dysfunction with reference to histology and comprehensive imaging assessment with the aim of establishing consistent nomenclature in future clinical studies.

2.2 Introduction

The leading cause of left ventricular systolic dysfunction (LVSD) is ischaemic heart disease (IHD), but other aetiologies may also lead to LVSD. The pharmacological and device therapy for the treatment of LVSD are well established and continues to improve. Multiple studies have demonstrated morbidity and mortality benefit for β -blockade[39] [40] [41], Angiotensin converting enzyme (ACE) inhibitors[42] [43] [44], aldosterone antagonists[45] [46] and device therapy[47] [48].

In contrast to medical therapy and device treatment, the role of revascularisation in LVSD of ischaemic origin is less certain. Whilst there are clear theoretical benefits in improving blood supply to large areas of dysfunctional 'viable' myocardium, improved outcomes following revascularisation have not been demonstrated in recent large clinical trials: STICH[49], HEART[50] and PARR-2[51]. The, largely unexpected, results of these trials have led to renewed discussion of the merits of viability assessment in LVSD.

An important confounder in this debate is the inconsistent and often imprecise terminology employed to describe myocardial states. In particular the terms 'viable' and 'hibernating' are often used interchangeably and sometimes incorrectly. 'Viable' is a summative term used to describe a range of myocardial states including: normal, thinned dyskinetic myocardium, myocardium retaining metabolically active respiring cells and dysfunctional non-transmurally scarred tissue. 'Hibernating' myocardium on the other hand is very narrowly defined as chronically dysfunctional, ischaemic myocardium that recovers contractile function following improvement in the myocardial perfusion supply/demand ratio. It follows from these definitions that an assessment of 'viability' can be made prior to revascularisation, but that an assessment of myocardium as 'hibernating' can only be retrospective. Furthermore, identifying myocardium as 'viable' does not necessarily imply functional recovery following revascularisation or 'hibernation', as is often wrongly assumed.

A second pertinent issue in this context is that myocardial viability and the likelihood of contractile recovery of viable, dysfunctional myocardium can be assessed with a range of non-invasive imaging techniques. These techniques differ fundamentally in their methodological approach, the properties of the acquired images and the tests' limitations, so that discrepancies between the available tests are inevitable. This makes consistency in the definition of myocardial states across imaging fields challenging. Understanding these differences is imperative, especially in the design and interpretation of studies designed to determine the role of revascularisation in patients with chronic LVSD.

This article aims to define the different states of myocardium that may exist and coexist in ischaemic heart disease and cardiomyopathy with reference to histology and non-invasive imaging techniques, without reference to heart failure with preserved ejection fraction (HF-PEF).

2.3 Available Imaging Techniques

2.3.1 Echocardiography

Echocardiography enables real-time assessment of cardiac structure and function using a range of techniques. 2D echocardiography allows comprehensive assessment of chamber size, wall thickness and contractile function. Doppler imaging enables assessment of blood flow through the heart, allowing quantification of valvular heart disease, and myocardial motion in systole and diastole. There are many echo parameters that provide functional assessment of left ventricular function including biplane or 3D ejection fraction (LVEF). Global longitudinal strain (GLS) is now an established technique that detects subtle systolic and diastolic abnormalities, as well as having superior prognostic value when compared to LVEF[52] [53]. The addition of pharmacological (dobutamine) or physiological (treadmill/static bike) stress enables the detection of ischaemia and viable, dysfunctional myocardium[54] [55] through demonstration of contractile reserve. Perfusion may be quantified using myocardial contrast echocardiography (MCE)[56] however this remains primarily a research tool, rather than commonplace in clinical practice.

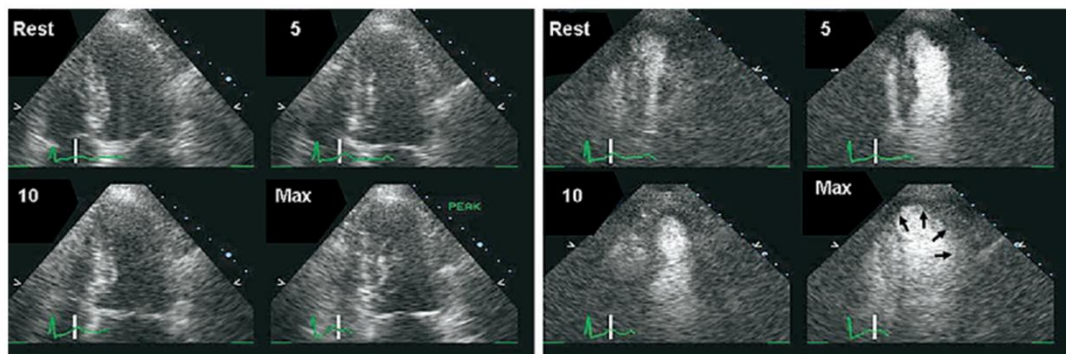


Figure 2.1 Intra-venous contrast agent use in echocardiography.

Left, rest images have poorly defined endocardial border. Right, following administration of intra-venous contrast agent the apical wall motion defect is evident. Taken from Plana *et al*[57].

An important limitation of echocardiography is inconsistent image quality and inter-observer variability[58]. Poor image quality can often be overcome with the addition of intra-venous contrast agents[59]. However, these are not licensed following recent myocardial infarction and image quality can remain limited in some due to chest wall abnormality or chronic respiratory disease.

2.3.2 Cardiovascular Magnetic Resonance

Cardiovascular magnetic resonance (CMR) produces high resolution images of the heart in unrestricted imaging planes. Cine imaging with whole heart coverage enables measurement of chamber size and systolic function similar to 2D echocardiography, but with better accuracy and reproducibility [60, 61]. CMR with pharmacological stress agents is used for ischaemia detection: vasodilator induced hyperaemic first pass perfusion CMR delineates myocardium with reduced perfusion reserve[62] while dobutamine stress allows identification of inducible regional wall motion abnormalities to detect ischaemia and hibernation in a similar manner to stress echocardiography[63]. An additional strength of CMR is its ability to characterise specific tissue properties. Different tissues and tissue states have characteristic signal patterns on T1 and T2 weighted imaging, allowing for delineation of the extracellular volume, fat and oedema. The addition of intra-venous extra-cellular contrast agents allows detection of intra-cardiac thrombus, micro-vascular obstruction (MO), scar and fibrosis[64]. Late gadolinium enhancement (LGE) images are acquired 10 to 20 minutes after contrast administration, when reduced clearance of contrast agent leads to relative signal enhancement in tissues with an expanded extracellular space, such as infarct scar and replacement fibrosis. LGE thus enables an anatomical delineation of infarct extent and shows distinctive patterns of fibrosis in myocarditis, myo-pericarditis and cardiomyopathy [65].

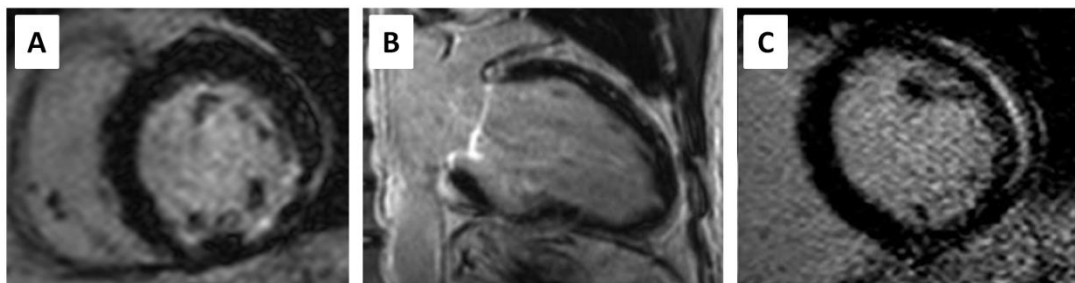


Figure 2.2 Patterns of LGE seen in different pathologies

A. Sub-endocardial infero-lateral enhancement in myocardial infarction. **B.** Anterior mid wall enhancement in dilated cardiomyopathy. **C.** Lateral epicardial and pericardial enhancement in myo-pericarditis.

Quantitative analysis of relaxation times with mapping methods is becoming more widely used; T1 mapping both before and after contrast administration allows estimation of the myocardial extracellular volume (ECV) fraction, which is increased in infiltrative and fibrotic processes[66, 67].

Magnetic Resonance Spectroscopy (MRS) allows insight into cardiac metabolism without invasive measurement or ionising radiation. However this remains a research tool and is not used in clinical care or decision making.

Limitations of CMR include availability, cost and presence of contraindications such as implantable devices.

2.3.3 Single Photon Emission Computed Tomography

Single Photon Emission Computed Tomography (SPECT) allows assessment of perfusion, metabolism and, with the addition of image gating, contractile function.

Thallium SPECT imaging allows identification of myocytes that retain a functional Na⁺/K⁺ ATPase and are metabolically active. The Na⁺/K⁺ ATPase maintains the physiological environment within the cell. Thallium readily crosses this channel and is taken up by myocytes in direct proportion to myocardial blood flow. Cellular thallium uptake is inhibited by concomitant digoxin administration, but does not preclude study interpretation. On stress thallium SPECT, diminished tracer uptake therefore reflects regional perfusion abnormalities as a surrogate of myocardial ischaemia[68]. Importantly, Thallium persists in the circulation following injection due to its long half-life, and delayed imaging allows the identification of hypo-perfused but respiring tissue as a marker of *viable myocardium*.

Technetium is now the most commonly used tracer in SPECT, primarily due to superior imaging characteristics, as well as lower whole body radiation exposure[69]. However due to its high binding affinity with myocyte mitochondria and shorter half-life (6hrs vs 73hrs) it does not persist in the circulation and redistribute in the same manner as Thallium[69]. Techniques have been developed to increase initial uptake of technetium into metabolically active hypo-perfused myocytes, and diagnostic accuracy for viability is now similar to thallium SPECT[70].

When compared to other imaging modalities SPECT may miss, or overestimate the extent, of scar[71]. In addition SPECT reliability may be limited by artefact due to gastro-intestinal or breast attenuation which can affect the detection of ischaemia[72]. As perfusion is assessed relative to remote normal myocardium,

SPECT in common with first pass perfusion CMR, is dependent upon a reference area. As a result accuracy may also be limited in balanced ischaemia and left main stem disease[73] however it remains a widely used and available technique.

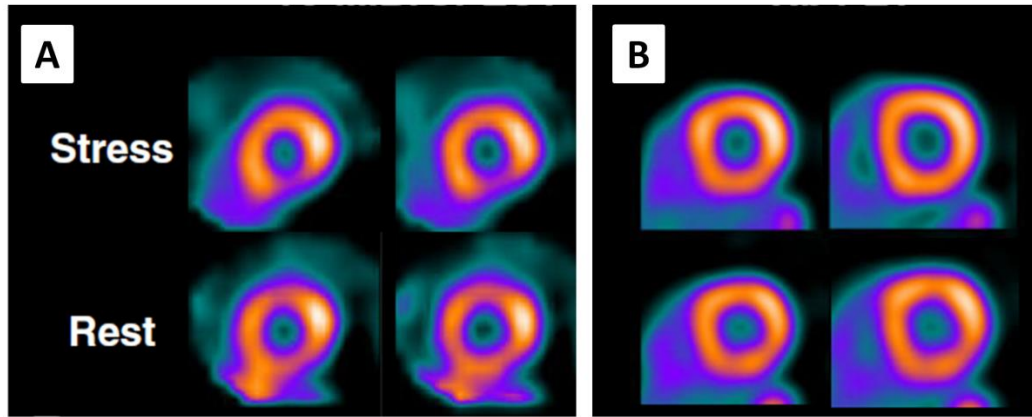


Figure 2.3 Inferior wall attenuation artefact on SPECT

The inferior defect seen on stress SPECT in A) is absent in B), PET imaging, suggesting SPECT attenuation artefact. Adapted from Bengel *et al*, Cardiac Positron Emission Tomography[74].

2.3.4 Positron Emission Tomography

Positron Emission Tomography (PET) imaging, whilst less available and accessible than SPECT imaging, offers better diagnostic accuracy and higher spatial resolution than SPECT. The typical resolution of PET images is between 4 – 7mm, whereas SPECT typically has a resolution around 10mm[75]. In addition to myocardial perfusion imaging and quantification, PET imaging allows assessment of myocardial glucose uptake with the tracer F^{18} -2-deoxy-2-fluoro-D-glucose (FDG).

FDG is taken into myocytes via the same sarcolemmal channel as glucose and accumulates intracellularly[75][93]. Tracer signal obtained is therefore proportional to metabolically active myocardium. The combination of perfusion and metabolism imaging with PET enables high resolution perfusion/metabolism mismatch images to be obtained, thus accurately identifying hypo-perfused metabolically active myocardium[76].

Table 2.1 Assessment of myocardial function, and relative strengths of non-invasive imaging techniques

	Morphology	Function	Perfusion	Metabolic	Scar
Echo	++	++	-	-	+
CMR	+++	+++	++	-	+++
SPECT	+	+	++	+	++
PET	+	+	+++	+++	++

Legend: +++ excellent assessment, ++ good assessment (with some weaknesses), + not best assessed with this modality, - not assessed with this modality

2.3.5 Summary

In summary, assessment of myocardial viability by echocardiography interrogates the functional, contractile reserve of an entire myocardial region, typically reported on a segmental basis; CMR allows a similar functional segmental assessment and in addition a morphological assessment of non-viable tissue with high spatial detail; and with SPECT or PET, assessment of viability is based on metabolic changes of non-viable tissue but limited to the entire myocardial extent or at best the endo- and epicardial layers.

2.4 Taxonomy

Myocardium can be divided broadly into different states of health and perfusion:

Normal

Ischaemic

Acute ischaemia

Chronic ischaemia

Stunned

Hibernating

Myocardial Infarction

Myopathic

2.4.1 Normal

2.4.1.1 Definition

Normal myocardium is metabolically active with normal contractile function at rest and exhibits contractile reserve in response to increased oxygen demand, and is by definition viable.

2.4.1.2 Metabolism

Normal cardiac function, including contraction and ionic regulation, is dependent upon ATP metabolism. The majority of ATP consumption occurs in myofibrils throughout the cardiac cycle. Further ATP is used to regulate sarcolemmal calcium and, at the membrane, Na^+/K^+ ATPase transporter[77, 78]. The heart consumes ATP rapidly, and is dependent upon constant renewal of ATP, which in turn is dependent upon creatine phosphate levels. Were ATP production to cease and consumption continue unchecked, cardiac stores would be depleted in approximately 10 to 15 seconds[77]. There are three main pathways by which ATP is synthesised: fatty acid oxidation, ketone body and carbohydrate metabolism. Fatty acid oxidation yields the most ATP, however all three pathways share a common endpoint of ATP synthesis at the electron transport[79]. In situations of increased metabolic demands, the proportion of ATP derived from carbohydrate metabolism increases[78].

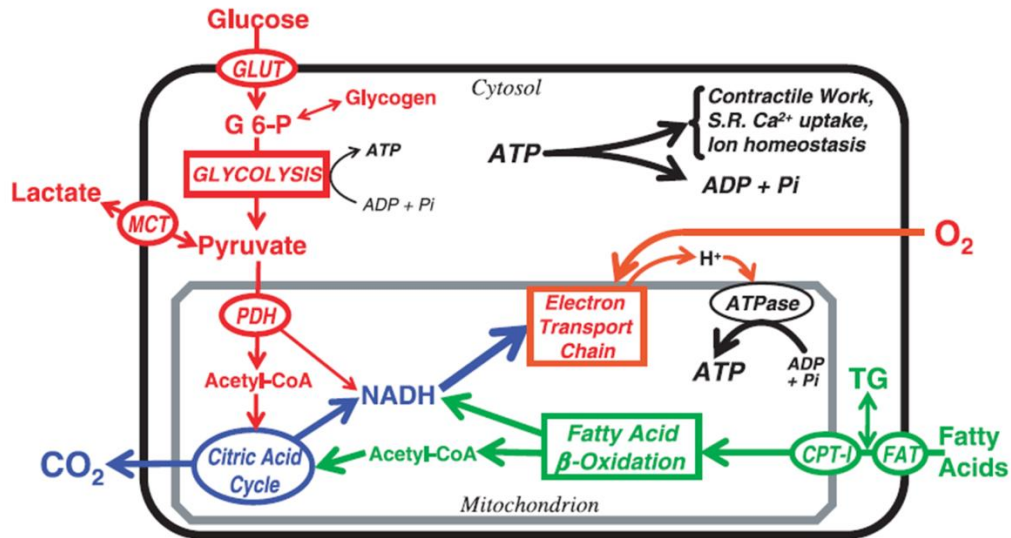


Figure 2.4 Normal myocyte metabolism

Taken from Myocardial Substrate Metabolism in the Normal and Failing Heart[79].

2.4.1.3 Histology

Healthy myocardial tissue is composed of myocytes, conducting tissue, vasculature and extra-cellular matrix. Approximately 75% of LV myocardial tissue is cardiac myocytes, the remaining 25% is the extracellular matrix(ECM)[80]. The ECM is predominantly composed of collagen types I & III. ECM composition is regulated by a number of factors, including circulating neuro-hormones and mechanical strain[81, 82].

2.4.1.4 Imaging, Morphology and Function

The left ventricle (LV) is a conical structure that tapers from base to apex. The normal LV wall has a thickness of between 7-11mm in diastole and thickens uniformly by at least 50% in systole. In response to increasing systemic oxygen demand, heart rate increases and myocardial contraction becomes increasingly dynamic. Systolic thickening is quantified on echocardiography using a standard 16 or 17 segment model with a visual scale[83] (1-7, 1=normal, 2= hypokinetic, 3= severely hypokinetic, 4= kinetic, 5= dyskinetic, 6= dyskinetic with scar, 7= aneurysmal) or by percentage wall thickening.

As well as assessing function, it is also possible to assess the constituents of the myocardium and the homogeneity of the tissue. By CMR, signal in healthy myocardium is uniform on T1 and T2 weighted and contrast enhanced images.

Semi-quantitative assessment of the ratio of T2 signal in healthy heart to skeletal muscle is <1.9 [84]. Quantitative measurement of the T1 signal, as measured by T1 mapping, shows normal values in narrowly defined ranges[85], but these depend on the field strength at which the scanner operates and the pulse-sequence used[85]. The ECV, calculated using pre and post contrast T1 mapping, is less method dependent and is $26 \pm 3\%$ in healthy myocardium[66].

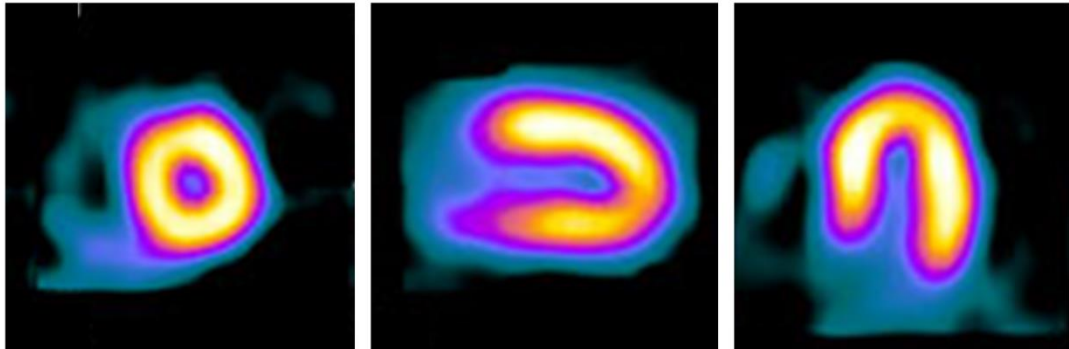


Figure 2.5 Normal SPECT examination

Left ventricle seen in short axis, vertical long axis and horizontal long axis.
Adapted from Hasegawa *et al* [86].

In healthy myocardium, nuclear techniques of perfusion and metabolism display uniform signal throughout the myocardium. Myocardial perfusion may be quantified both at rest and hyperaemia, thus allowing calculation of myocardial perfusion reserve. By PET normal resting myocardial blood flow is $0.7\text{ml}/\text{min}/\text{g}$, increasing to $2.75\text{ml}/\text{min}/\text{g}$ on stress, with a flow reserve of 4.17 [87]. Though absolute values of myocardial blood flow by PET and CMR correlate poorly, derived coronary flow reserve correlates well[88].

2.4.2 Reversible Ischaemia

2.4.2.1 Definition

Myocardial ischaemia is a mismatch of oxygen supply and demand that precipitates a change from aerobic to anaerobic respiration.

2.4.2.2 Metabolism

Ischaemia may either be complete, due to an occlusion of a coronary artery, or limited due to epicardial coronary artery stenosis. The degree of ischaemia is also

determined by the presence and extent of a collateral circulation that can develop in humans with established coronary artery disease.

Changes in cell metabolism begin within one minute of onset of severe ischaemia. Sub-endocardial tissue becomes ischaemic first followed by sub-epicardial tissue[89]. Shortly after the onset of severe ischaemia, oxygen present in myocardium is consumed and normal oxidative metabolism ceases. At the same time, electron transport across cell membranes decreases and myocyte contraction becomes markedly impaired. During this initial phase, anaerobic respiration replaces aerobic respiration as the dominant source of ATP, and glycogen replaces fatty acids and glucose as the substrate for energy production[77]. Anaerobic respiration in this setting provides approximately a quarter of the amount of ATP as aerobic metabolism. However due to adverse intra-cellular conditions, including falling pH, ATP production at this rate is only sustained for approximately one minute before continuing at a much lower rate for up to one hour[77].

In non-severe ischaemia a degree of aerobic respiration continues, and as a result more ATP is produced when compared with anaerobic glycolysis. Furthermore, hydrogen ions and lactate that accumulate in severe ischaemia are produced less quickly, and 'washed out' of still perfused tissue, allowing preservation of a more physiological environment.

Once ischaemia has resolved, recovery of normal function is variable. Regional abnormalities of systolic function may persist for up to several days, and myocardium that fails to recover normal systolic function immediately is said to be 'stunned'[90].

2.4.2.3 Histology

Abnormal function of cell membrane channels leads to myocyte oedema shortly after onset of ischaemia. In addition to oedema, following short duration of severe ischaemia, depletion of glycogen stores and the presence of 'I-bands' in myo-fibrils are seen on electron microscopy[91]

2.4.2.4 Imaging, Morphology and Function

2.4.2.4.1 Acute Ischaemia

Shortly following the onset of ischaemia, regional systolic function becomes impaired and may remain so for days after the ischaemic insult[90]. Transthoracic echocardiography is most commonly used to identify the wall motion abnormalities associated with acute ischaemia. SPECT and PET are rarely used clinically in the setting of acute ischaemia. On CMR, wall motion and thickness can

be assessed in a similar fashion to echocardiography and in addition, oedema is readily detectable and quantifiable as areas of high signal on T2 weighted images[13]. The oedematous zone may be quantified to determine the degree of benefit following intervention and delineate the 'area at risk'.

2.4.2.4.2 Chronic Ischaemia

Exercise or dobutamine stress echocardiography allow for detection of ischaemia as well as determining its location and extent[92]. Ischaemic myocardium shows reduced contractile reserve with regional wall motion abnormalities developing at increasing levels of stress.

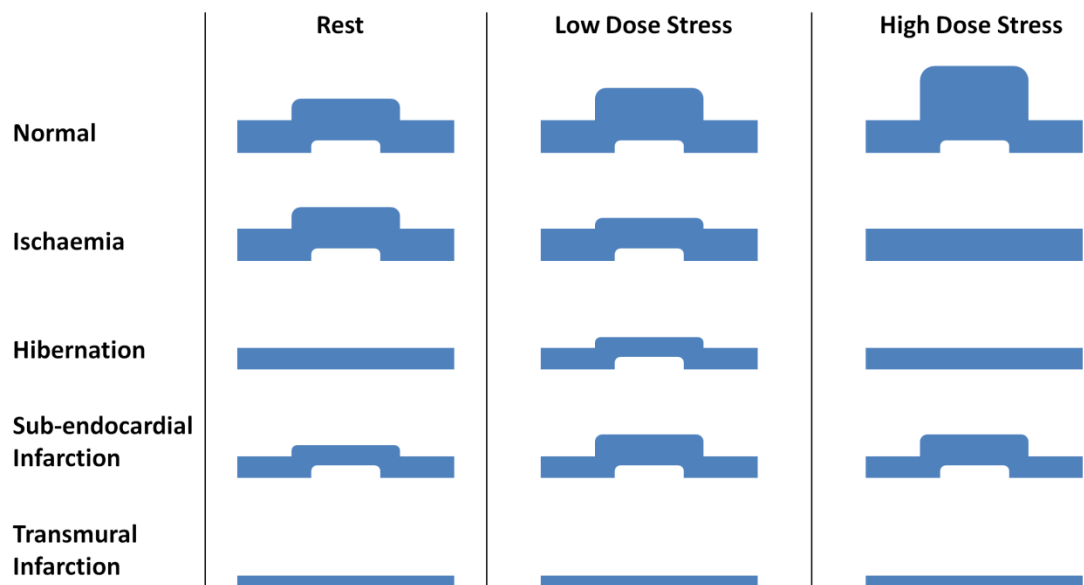


Figure 2.6 Schematic of wall motion abnormalities in ischaemia, hibernation, sub-endocardial ischaemia and infarction.

CMR allows detection of ischaemia through assessment of regional systolic function and perfusion. Tissue perfusion is assessed using first pass adenosine stress CMR as above. Quantitative assessment of perfusion with CMR has a good correlation with PET imaging[93] but is less commonly used in clinical practice than qualitative assessment.

SPECT is commonly used in the investigation of chronic ischaemia, and whilst image quality on PET is superior to SPECT, low availability limits utility. The sensitivity and specificity of SPECT compare well with other non-invasive imaging techniques[94], and the whole heart acquisition allows accurate quantification of the extent of ischaemia, a factor that may have important prognostic value[95]. Image interpretation may be limited by attenuation artefact in the inferior wall and

anterior wall in females[96]. In addition to perfusion imaging and LV function, transient LV dilation (Transient ischaemic dilation -TID) may be appreciated on stress SPECT imaging. TID may either represent true dilation as a result of severe coronary disease and stunning, or rather may reflect sub-endocardial defects not appreciated on perfusion imaging[97]. In individuals with normal perfusion the presence of severe TID marks adverse prognosis [98].

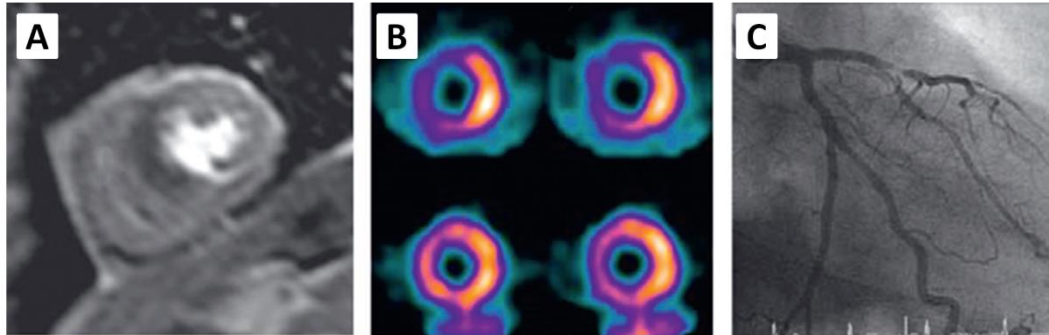


Figure 2.7 Imaging in chronic ischaemia

A) CMR first pass perfusion demonstrating mid-ventricular anterior and septal abnormality. B) SPECT demonstrating the anterior and septal stress (top row) abnormality that resolves at rest. C) Angiography in the same patient showing severe LAD stenosis. Adapted from Greenwood *et al* Cardiovascular magnetic resonance and single-photon emission computed tomography for diagnosis of coronary heart disease (CE-MARC): a prospective trial[3].

PET perfusion imaging allows detection of ischaemia. There are several possible tracers for use in PET imaging, however the most commonly used in clinical practice is Rubidium-82 due to its relative availability and practicality. In head to head studies the theoretical advantages of PET over SPECT have been demonstrated[74, 99], and PET perfusion imaging is established as the gold standard for the detection of myocardial ischaemia. Further to ischaemia detection PET may also measure myocardial blood flow[100], enabling qualitative assessment of myocardial blood flow reserve, which correlates strongly with coronary artery stenosis severity[101].

2.4.3 Stunning

2.4.3.1 Definition

Myocardium is 'stunned' when contractile function is depressed following transient ischaemia, prior to a full recovery, and having sustained no irreversible myocyte damage. The mechanism of sustained systolic dysfunction in stunning is incompletely understood. However it is believed that oxygen free radical formation and elevated myocardial calcium levels may lead to damage of myocardial proteins or the sarcoplasmic reticulum[102].

2.4.3.2 Metabolism

Sub-epicardial and sub-endocardial blood flow normalises quickly following restoration of normal coronary flow, however normal myocardial metabolism takes time to recover. Metabolic changes seen in transient ischaemia, including fall in myocyte ATP, phosphocreatine and pH, take several hours to reverse in stunned myocardium[103]. Post ischaemic myocardial oxidative and glucose metabolism remain depressed by approximately 20% of normal levels for several hours after an ischaemic insult, and take up to one week to recover to near normal levels[104].

2.4.3.3 Histology

Histological changes seen in stunned myocardium reflect ischaemia sustained. In common with metabolic change; resolving myocardial oedema, myocardial glycogen and ATP depletion[90] may be detected several days later.

2.4.3.4 Imaging, Morphology and Function

Systolic function of the affected segments is impaired in stunning. The speed of recovery of systolic function is variable and may be related to the duration and severity of the ischaemic insult[90, 105, 106]. Abnormalities of diastolic function, whether assessed by CMR or tissue Doppler persist beyond systolic abnormalities[107] [108]. It is likely that stunning is an underappreciated phenomena, abnormalities associated with acute ischaemia may have recovered when imaging is performed. Furthermore, in clinical practice the label attached to the dysfunctional myocardium will depend upon the time point at which the myocardium is assessed, as serial imaging is unlikely to be performed.

CMR examination demonstrates changes in keeping with ischaemia including high signal on T2 weighted images indicative of oedema, as well as regional systolic wall motion abnormalities.

PET FDG metabolism assessment may demonstrate depressed levels of glucose metabolism, but no significant metabolism perfusion mismatching as in hibernation are seen[58].

2.4.4 Myocardial Infarction

2.4.4.1 Definition

Myocardial infarction follows sustained ischaemia leading to myocyte necrosis and later, subsequent remodelling and fibrosis.

2.4.4.2 Metabolism

Necrosis occurs when sustained severe ischaemia leads to irreversible structural changes within the myocyte, including mitochondrial swelling and disruption of the sarcolemma[91]. In the course of ischaemic injury, necrosis begins in the sub-endocardium where tissue perfusion is lowest and energy consumption is highest, leading to ATP supply exhaustion and accumulation of the by-products of glycolysis to accumulate there first. Sub-endocardial infarction commences approximately 20 minutes after the onset of ischaemia. Prolonged ischaemia leads to increasingly transmural necrosis, which moves as a 'wave front' from the endocardium to the epicardium[89].

2.4.4.3 Histology

Characteristic histological changes occur during myocardial infarction, and evolve until the infarcted region has undergone scar replacement. On macroscopic examination there are few detectable changes over the first four hours, but from four to twelve hours the myocardium becomes mottled. Over the next week the infarct centre becomes pale and yellows whilst developing red margins. After this, the infarcted myocardium becomes greyer as fibrous scar tissue replaces the necrotic infarct[109, 110].

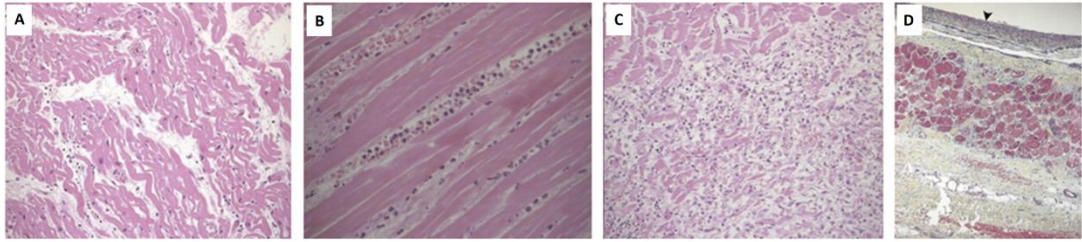


Figure 2.8 Histological change following myocardial infarction

Pathology of Myocardial Infarction, Diagnostic Histopathology 2013. **A.** Light microscopy of wavy mitochondrial fibres and interstitial oedema 4 hours postmyocardial infarction. **B.** Myocardial fibres 24 hours post myocardial infarction, myocardial thinning and interstitial infiltration by polymorphonuclear leukocytes. **C.** Granulation tissue 7-10 days post infarction. Near complete removal of myocytes. **D.** Light microscopy 2-3 weeks after infarction. Subendocardial fibrosis marked by arrowhead, with a small area of myocardial fibre preservation between layers of collagen deposition. Adapted from Chang *et al*[111].

Microscopically, the myocardium undergoes a series of changes responsible for the macroscopically appreciable abnormalities. During the initial phase, glycogen depletion and oedema, in keeping with severe ischaemia is seen on electron microscopy[112]. Between four and twelve hours, oedema, necrosis and intra-myocardial haemorrhage are seen. From twelve to twenty four hours hypereosinophilia, neutrophil infiltration and ongoing necrosis develop. Acute injury in myocardial infarction is followed within 24 to 48 hours by the disappearance of nuclei and striations, and macrophages remove dead cells at the infarct border. Following removal of dead myocytes, granulation tissue and collagen deposition begins, eventually leading to the formation of collagen scar[113] [114].

2.4.4.4 Imaging, Morphology and Function

On echocardiography, wall motion abnormalities develop before ECG changes or symptoms following coronary occlusion[115]. As well as regional wall motion abnormalities, tissue Doppler systolic velocities decrease rapidly following onset of ischaemia[116]. Following transmural infarction, the myocardium is akinetic and as time progresses, thins. On stress or exercise there is no improvement in wall thickening and there is an absence of contractile reserve[117]. Sub-endocardial infarction results in wall motion abnormalities of varying severities, and may be differentiated from transmural infarction using peak systolic circumferential strain and strain rate on stress echocardiography[118].

Similar to echo, CMR demonstrates gross anatomical changes associated with infarction including remodelling, wall thinning and regional wall motion abnormalities[13]. In addition, CMR imaging can be used to delineate infarct extent, indicate the area at risk and detect microvascular and reperfusion injury[119]. T2 weighted CMR is sensitive to free water content of tissue and in acute MI can be used to delineate the myocardial area at risk of ischaemic injury[120]. LGE CMR after acute MI displays high signal within the infarcted area due to persistence of gadolinium within areas of increased extra-cellular volume and abnormal washout kinetics within the infarcted tissue[13]. In animal models, infarct enhancement with LGE CMR has been observed within 90 minutes of the onset of ischaemia[121]. Over time, infarct expansion is reflected by an expanding area of enhanced myocardium. In the acute phase, infarct extent may be overestimated due to increased signal and contrast in the oedematous peri-infarct zone[122]. In acute myocardial infarction, LGE CMR may show an area of low signal within the otherwise high signal infarct zone. This reflects loss of normal capillary function due to microvascular obstruction (MO) leading to an absence of gadolinium delivery to the centre of the infarct. Extensive MO is associated with no-flow on invasive coronary angiography and adverse LV remodelling[123]. Within an area of MO, myocardial haemorrhage may occur, which causes low signal on T2 and T2* weighted CMR[124]. Haemorrhage represents more severe structural change in the setting of acute MI and is also associated with adverse LV remodelling following infarction[125]. The combination of the information gained from T2 weighted, early gadolinium and late gadolinium images allows measurement of the area at risk (T2 weighted CMR), infarct size (LGE) and myocardial salvage (difference between area at risk and infarct size)[120].

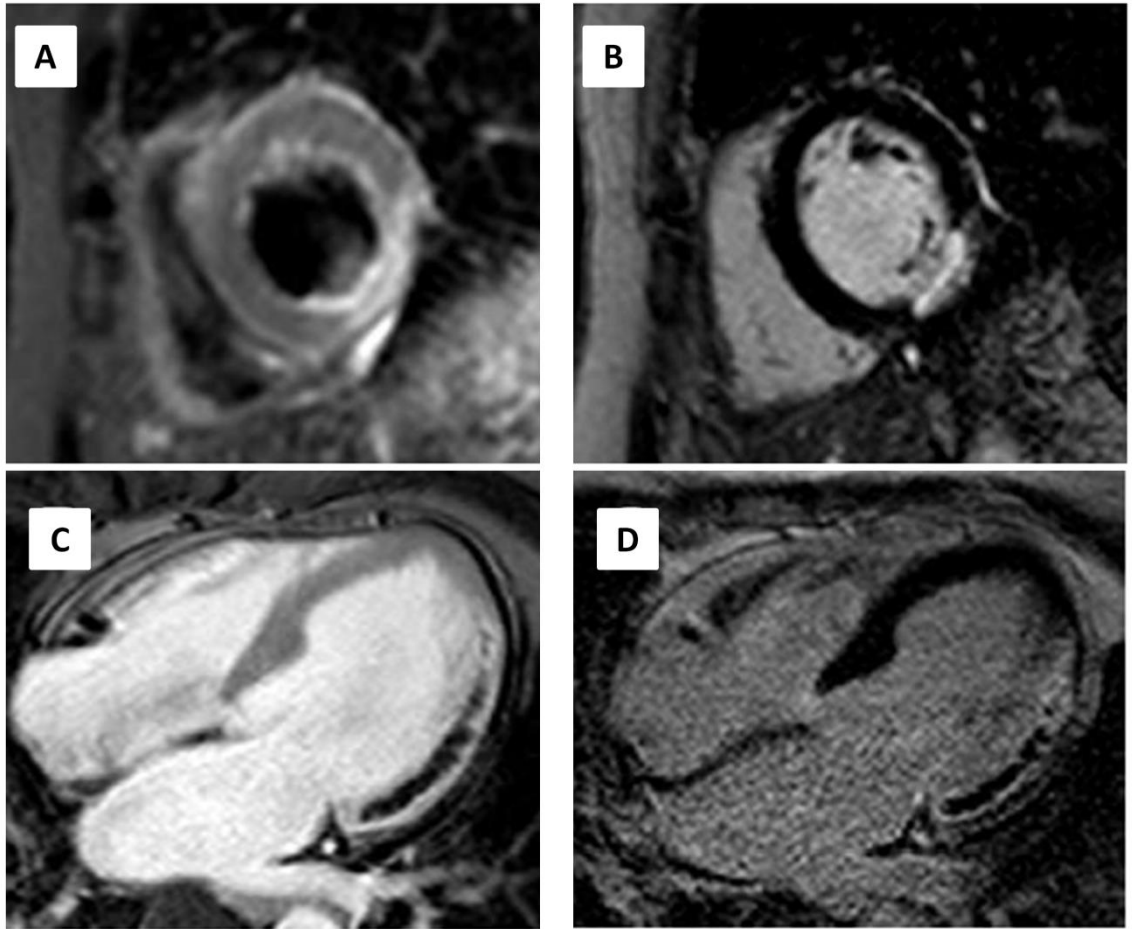


Figure 2.9 CMR study in acute myocardial infarction

Top row: A) High signal on T2 weighted image demonstrating Infero-lateral oedema and 'area at risk'. B) Sub-endocardial infarction in the same patient on LGE. **Bottom Row:** C) EGE image in a second patient with extensive lateral wall hypoenhancement D) LGE confirms infarction and microvascular obstruction.

At the time of acute infarction SPECT imaging is not usually performed. However in research settings, infarct area as delineated by tracer uptake on SPECT correlates well with infarct size on pathological specimens[126]. In chronic infarction, metabolically active myocytes are replaced by scar and the absence of an intact Na^+/K^+ ATPase leads to lack of uptake of the SPECT tracer within the region of infarction. The limited spatial resolution of SPECT can lead to an under-appreciation of the extent of infarction because small subendocardial infarction may not be detected[127].

In acute myocardial infarction, FDG PET allows detection of infarction and the presence of viable tissue in or adjacent to the infarcted territory. The absence of detectable glucose metabolism is associated with irreversible myocardial

injury[128]. Myocardial perfusion as measured by PET in acute MI is depressed and significantly improves following coronary intervention and may continue to improve up to two weeks later[129].

In the setting of chronic myocardial infarction, concordant reduction in signal from both perfusion and metabolism (NH₃ and FDG tracers respectively) PET is seen and readily appreciated in trans-mural infarction, however in sub-endocardial infarction PET may fail to detect small areas of sub-endocardial scar when compared to CMR[130].

2.4.5 Hibernating Myocardium

2.4.5.1 Definition

Chronically dysfunctional viable myocardium of ischaemic origin that recovers systolic function following revascularisation. The precise processes underlying the development of hibernation remain unclear, although several mechanisms have been proposed. It is thought that although resting blood flow is normal, coronary flow reserves are low[131]. This leads to repeated episodes of ischaemia, and myocardial stunning, causing a complex series of physiological and structural changes that are characteristic of hibernation.

2.4.5.2 Metabolism

There remains debate regarding the metabolic changes that occur in hibernation. However, there is some evidence to suggest that glucose uptake and utilisation are increased and fatty acid metabolism is decreased in hibernating myocardium[132] [133].

2.4.5.3 Histology

Hibernating myocardium is macroscopically similar to normal myocardium. However, at a microscopic level there are diffuse changes within the myocyte and extracellular ultrastructure. All types of collagen are seen to increase markedly in the ECM of hibernating segments and are more than twice that found in normal myocardium[136] when de-differentiation is severe. Structural changes in the extracellular matrix become more pronounced as duration of hibernation increases[137, 138].

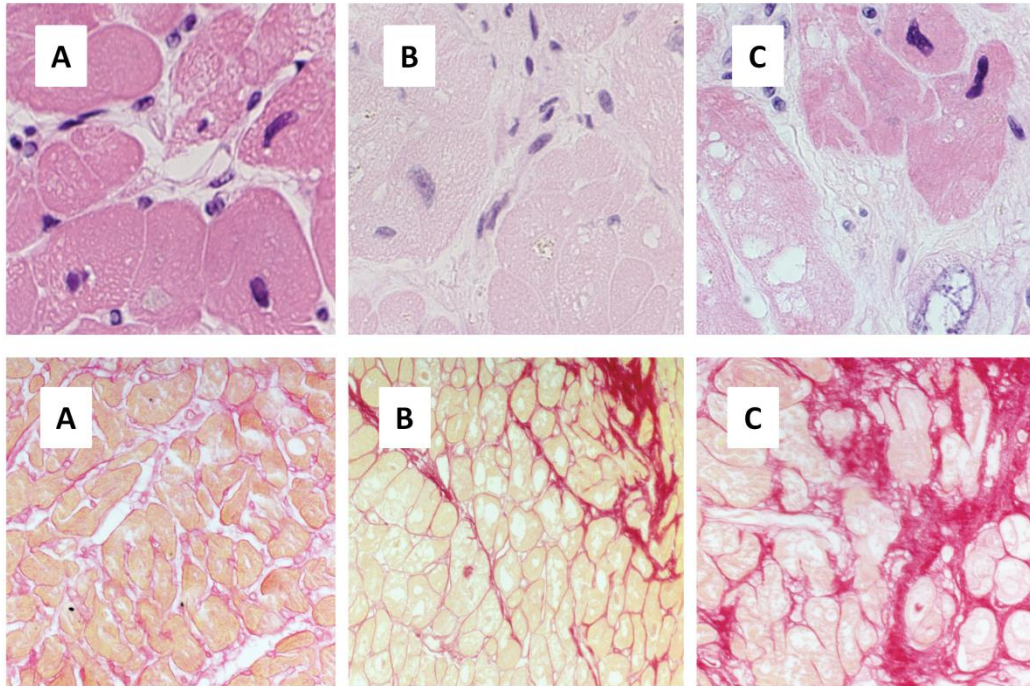


Figure 2.10 Histological change in dysfunctional ischaemic myocardium

Samples taken prior to revascularisation. Top row, hematoxylin staining: **A.** Preserved myocyte architecture in segment with preserved function. **B.** Expansion of the interstitial space and myocyte contractile depletion in hibernating tissue. **C.** More extensive matrix deposition in a segment that displayed persistent dysfunction. Bottom row, Picosirius staining: **A.** Collagen fibres are red within the ECM. **B.** Increased interstitial fibrosis in hibernating myocardium. **C.** A segment with persistent dysfunction has markedly increased collagen deposition. Taken from Frangogiannis[134]

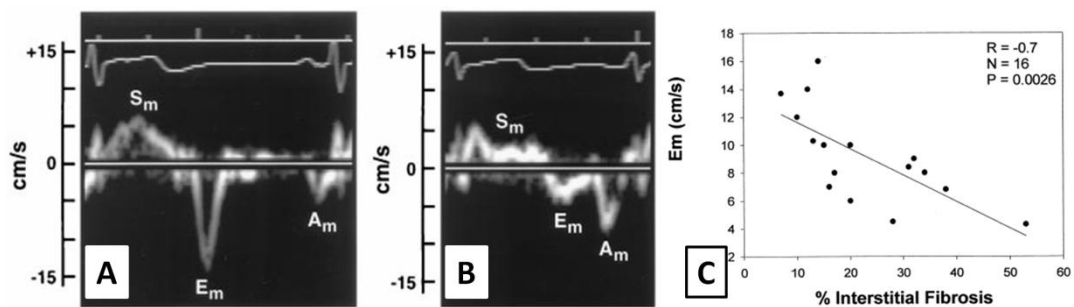


Figure 2.11 Abnormal Tissue Doppler in abnormalities in chronically ischaemic dysfunctional myocardium

E' is related to the degree of interstitial fibrosis, adapted from Shan *et al*[135].

Furthermore, there is down-regulation of mitochondria and increased glycogen storage within the myocyte when compared to both normal and remote

myocardium[136] [133]. These changes reflect alteration of mRNA expression and disorganisation of cytoskeletal proteins as a result of cellular de-differentiation.

2.4.5.4 Imaging, Morphology and Function

On functional imaging, hibernating myocardium has impaired resting systolic function, and will typically be scored as either hypo- or akinetic. Diastolic wall thickness is greater than 6mm by CMR[139] or 7mm on trans-thoracic echocardiography[140]. With inotropic stimulation, by dobutamine stress echo or CMR, hibernating myocardium shows 'contractile reserve' or a 'biphasic response', with an improvement in contractile function on low dose/effort stress prior to deteriorating at higher workloads[141]. Low dose stress tissue Doppler measurement allows quantitative echocardiographic measurement of systolic function: Doppler tissue velocities are significantly higher in hibernating myocardium than dysfunctional tissue that does not show improvement in systolic function following revascularisation[142, 143].

CMR examination of potential hibernation allows for accurate assessment of diastolic wall thickness and regional wall motion abnormalities. LGE CMR contributes only indirectly to the diagnosis of hibernation. On LGE CMR, hibernating myocardium has the same signal characteristics as normal myocardium, with signal uniformly nulled. LGE CMR therefore only excludes the presence of myocardial infarction as the cause of contractile dysfunction, suggesting hibernation as one of several potential causes. Furthermore, absence of LGE in dysfunctional segments is highly predictive of functional recovery following revascularisation[144]. CMR prediction of contractile recovery can be further enhanced by the combined use of cine, LGE and dobutamine stress imaging. Hibernating myocardium will show reduced resting function, absence of LGE and a biphasic response to dobutamine stress[141]. Finally, myocardial perfusion can be assessed and quantified by first pass CMR. Resting myocardial blood flow (ml/min/g) is decreased in hibernating myocardium, and is increased following successful revascularisation[145], whilst hyperaemic blood flow is typically reduced[146].



Figure 2.12 Increasing transmurality of LGE predicts lack of response to revascularisation in chronically ischaemic dysfunctional myocardium

Progressive transmurality of scar predicts lack of improvement in systolic function following revascularisation. A. 25% LGE of the inferior wall and infero-septum B. 50% LGE in the infero-lateral wall becoming increasingly transmural inferiorly. C. 100% LGE of the inferior wall.

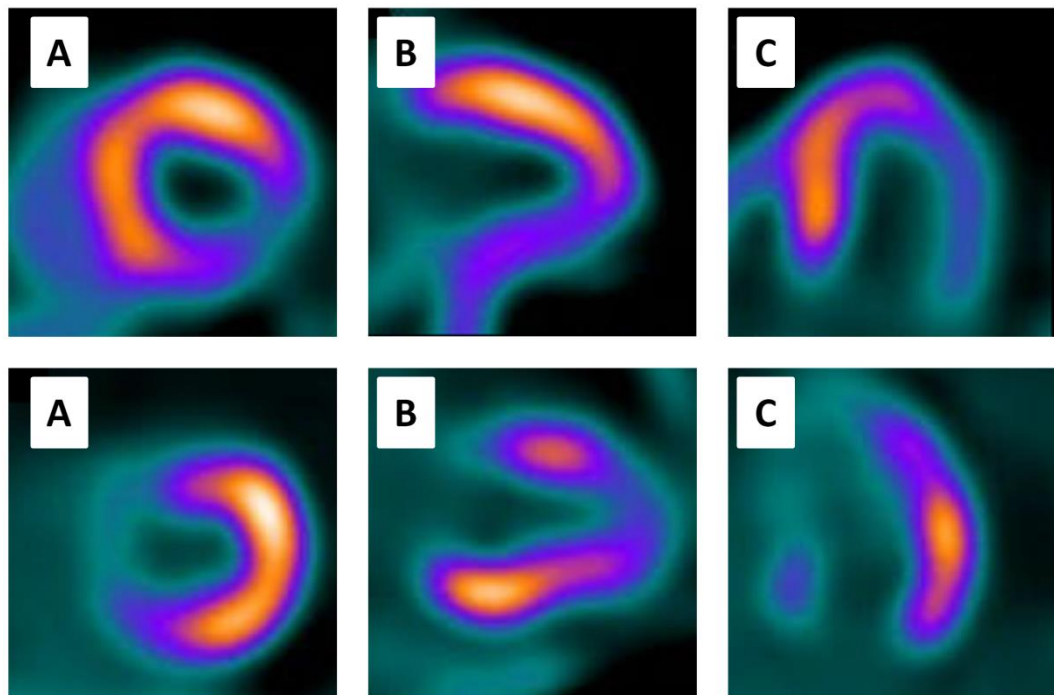


Figure 2.13 . Perfusion-metabolism mismatching in hibernating myocardium

A) Short axis. B) Vertical long axis. C) Horizontal long axis. Top row demonstrates a stress perfusion defect in the infero-lateral wall. Bottom row shows matched metabolism imaging shows preservation of metabolism in the same territory indicating potential hibernation. Adapted from Bengel *et al*, Cardiac Positron Emission Tomography[74].

Cellular uptake of Thallium and other radiotracers used in SPECT imaging is dependent upon a functional Na^+/K^+ ATPase and preserved sarcolemmal membrane function[76]. In hibernating myocardium, early acquisition following tracer administration will identify a defect, reflecting impaired blood flow on stress. On delayed acquisitions, the isotope has redistributed and been taken up by metabolically active myocytes, so the defect is reduced. Stress/redistribution SPECT thus allows to quantification ischaemia and the extent of potential recovery in hibernation assessment[147].

PET assessment of hibernation is most commonly based on the assessment of myocardial glucose uptake with FDG. Tracer signal obtained is proportional to metabolically active myocardium and likelihood of recovery may be predicted by glucose metabolic rate[148]. In addition, PET allows the detection and quantification of myocardial blood flow, which is reduced in hibernation[75]. Choice of treatment modality in patients with severe LVSD treatment may be improved utilising PET perfusion-metabolism imaging[51].

2.4.5.5 Hibernation with non-transmural scar

Hibernation commonly co-exists in the presence of sub-endocardial infarction. The likelihood of recovery of systolic function of the hibernating myocardium is modified by the presence and extent of infarction. As the transmural of infarction increases, recovery following revascularisation becomes less probable[144].

In the setting of partial infarction there is reduced response to dobutamine stress on functional imaging. Decreased circumferential strain measured by echocardiography further facilitates differentiation of normal myocardium, sub-endocardial and transmural infarction[149] [150].

As discussed previously, the spatial resolution of CMR LGE enables the detection of small volumes of infarction that may be missed by SPECT or PET. Quantification of the LGE allows the anticipated rates of recovery to be predicted: In patients with mild LVSD 60% with 1-25% LGE, 42% with 26-50% LGE and only 7% of segments with more than 50% enhancement would be expected to have recovered at three months[144]. In patients with moderate LVSD (LVEF 38%) expected recovery rates are similar (0 LGE:73%, 1-25:56%, 26-50:45%, >50%:5%)[151].

2.4.6 Myopathic Myocardium

2.4.6.1 Definition

Dysfunctional myocardium of non-ischæmic origin. Myopathy covers a wide range of pathologies. This review will focus on dilated cardiomyopathy (DCM) with only passing reference to other aetiologies.

2.4.6.2 Metabolism

Contraction and normal cardiac function relies upon matching of energy demand and consumption. This in turn is regulated by appropriate oxygen supply to myocytes, mitochondrial function, ATP transport to the site of energy consumption and a reliable feedback system to maintain appropriate metabolic rates. A problem at any step can lead to pump insufficiency, and cardiac output that does not match the physiological requirement. In patients with mild heart failure secondary to idiopathic dilated cardiomyopathy no metabolic substrate changes, or mild fatty acid metabolism up-regulation occur[79]. In severe heart failure, cellular metabolism changes significantly, with greater glucose and less free fatty acid utilisation[152, 153] although there are contradictory data[154].

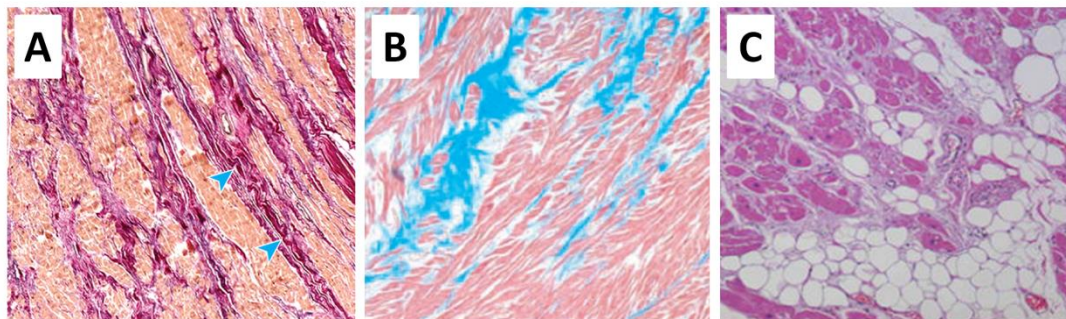


Figure 2.14 Histological abnormalities in cardiomyopathy

A) Dilated cardiomyopathy, increased interstitial fibrosis at the blue arrow. B) Hypertrophic cardiomyopathy, increased interstitial fibrosis (blue) and myocyte disarray. C) Fibro-fatty replacement in ARVC. Taken from[155, 156] [157].

2.4.6.3 Histology

Histological abnormalities differ depending on the underlying aetiology. A reduction of mitochondria in the failing heart is common[79] across the disease spectrum. Differing degrees of myocyte hypertrophy are seen depending upon the

aetiology of heart failure. However irrespective of aetiology, a common finding is the expansion of the extra-cellular matrix and fibrosis [158]. Extracellular matrix formation is influenced by local factors and circulating neuro-hormone levels. Activation of the Renin-aldosterone-angiotensin system (RAAS) leads to increased collagen synthesis[159]. Collagen type and amount in the myocardial extracellular space affects cardiac function. Collagen types I and III are the major structural components in the cardiac extracellular matrix, providing both tensile strength and elasticity[160]. In the failing heart, collagen synthesis increases, leading to accumulation of intercellular collagen, limiting ventricular compliance, myocyte function and contributing to both systolic and diastolic dysfunction[161] [162]. Specific cardiomyopathic processes have characteristic histological abnormalities, including myocyte disarray and interstitial fibrosis[163] in hypertrophic cardiomyopathy (HCM) and fibro-fatty replacement in arrhythmogenic right ventricular cardiomyopathy (ARVC).

2.4.6.4 Imaging, Morphology & Function

Diffuse fibrotic processes and reduction in contractile substrate as occur in dilated cardiomyopathy result in chamber dilation as well as wall motion and tissue relaxation abnormalities. Functional imaging by echocardiography and CMR supplies important information for prognosis and risk stratification including ejection fraction and left ventricular end diastolic dimensions [164, 165].

Comprehensive structural assessment by echocardiography and CMR may guide management and allow diagnosis of the underlying cardiomyopathic process to be determined. For example, it is possible to differentiate between morphologically similar cardiomyopathies on echocardiography: Improvement in long axis function on stress tissue Doppler allows differentiation between ischaemic and non-ischaemic cardiomyopathy[166]; In the case of left ventricular hypertrophy, 2D-strain assessment on echocardiography allows differentiation of HCM and hypertensive LV hypertrophy, whilst tissue Doppler enables differentiation of HCM and athlete's heart[167] [168].

Tissue Doppler imaging of the mitral annulus and mitral inflow velocity provides a non-invasive estimate of left atrial pressure[169]. CMR left atrial transit time also correlates strongly with LV early diastolic pressure[170], although this is not commonly applied in clinical practice.

In many cardiomyopathies, LGE CMR shows characteristic patterns of enhancement, including in ischaemic cardiomyopathy, dilated cardiomyopathy, hypertrophic cardiomyopathy and infiltrative processes. The presence and extent of

LGE on CMR predicts outcomes in a range of cardiac diseases, including DCM[171], HCM [172] and ischaemic heart disease[173].

T1 mapping and subsequent ECV calculation allows measurement of both diffuse fibrotic and infiltrative processes that are unreliably assessed with visual analysis alone[174][175]. T2* mapping allows detection and tracking of iron overload cardiomyopathy[176].

The ability of SPECT alone to accurately differentiate ischaemic from dilated cardiomyopathy is uncertain as mild stress perfusion defects are commonly seen with both aetiologies[177][178]. The defects seen have mild stress defect severity ratios (SDSR >45%), however similar abnormalities may be seen in multi-vessel coronary disease precluding the use of SPECT as the sole diagnostic tool in this situation.

Decreased FFA metabolism with increased glucose metabolism is seen on PET examination in DCM[152]. Hyperaemic blood flow by PET is lower in DCM than in healthy controls, 2.23ml/min/ml vs 4.33ml/min/ml in one report[179] and perfusion abnormalities in DCM are progressively worse in more severe heart failure[180].

2.5 Clinical Implications

Different imaging modalities assess different facets of myocardial health and disease and are often complementary. An awareness of the principles underlying acquisition, and the aspect of myocardial health and pathology assessed is crucial in both clinical practice and when considering clinical trial design.

The detection of 'normal' myocardium is straightforward, and can be accomplished using any test capable of delivering good quality anatomical images. The most appropriate method will vary depending upon patient and institute: In terms of practicality, availability and economy this will frequently be echocardiography, and is sufficient to exclude all but inducible ischaemia and sub-clinical cardiomyopathy. It needs to be noted though, that minor deviations from normal, such as small areas of infarction, may be undetected unless high resolution imaging methods such as LGE CMR are used.

Ischaemia detection or perfusion assessment may be performed using either stress/exercise echo/CMR, CMR first pass perfusion or nuclear imaging, and all are included in current practice guidelines[181]. SPECT is the mostly widely used modality worldwide, and PET remains the gold standard, however availability of

PET is limited. In some patients it may be desirable to exclude valvular disease or cardiomyopathy making DSE or CMR the most appropriate choice of test.

To detect true 'hibernating' myocardium, a test that assesses the metabolic activity of myocytes must be used i.e. SPECT or PET. However, in clinical practice the question most often posed relates to the likelihood of contractile recovery in a given coronary territory, or the potential for LV reverse remodelling following revascularisation. For this purpose, any of the non-invasive imaging techniques covered may be selected, as long as the limitations of the chosen technique are recognised in clinical decision making.

Partial infarction is best appreciated by CMR examination, with the added benefit that systolic abnormalities not associated with coronary disease may be explained by characteristic abnormalities of cardiomyopathy on LGE CMR.

Where infarction and cardiomyopathy co-exist, multi-modality imaging may be necessary, often including coronary imaging. Tissue Doppler velocities have been shown to differ in DCM and ischaemic heart disease, and this may allow discrimination of causes of LVSD, but use is not widespread in clinical practice. LGE CMR allows the extent of scarring due to either pathology to be determined, but not the benefit of any specific therapy.

Mild perfusion defects are common in DCM as discussed above. SPECT, PET or CMR first pass perfusion in combination with coronary angiography may facilitate targeted revascularisation if indicated, and potentially avoid unnecessary revascularisation.

2.6 Conclusion

Consistent adoption of standard nomenclature in clinical trials will improve the clarity of the literature, and simplify decision making regardless of imaging modality employed.

Chapter 3

Predictive Power of Late Gadolinium Enhancement for Myocardial Recovery in Chronic Ischaemic Heart Failure: A HEART sub study

3.1 Abstract

3.1.1 Objectives

To assess the predictive power of LGE for myocardial recovery in chronic severe ischaemic cardiomyopathy undergoing revascularisation as part of a clinical trial.

3.1.2 Background

The amount of myocardial scar measured by late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) imaging predicts regional recovery in wall motion following revascularisation. Previous studies have been conducted in patients with a relatively recent myocardial insult and relatively preserved left ventricular (LV) function

3.1.3 Methods

Twenty two patients with severe LV impairment of ischaemic origin were enrolled as a sub-study of a trial that randomly assigned patients to revascularisation or not in addition to guideline-indicated pharmacological therapy. Patients underwent a CMR study at baseline and six months. Scans were qualitatively and quantitatively assessed for wall motion, rest/stress myocardial perfusion and LGE.

3.1.4 Results

The median duration of heart failure since diagnosis was 13 (IQR 5 to 21) months. Patients had severe LV dilatation (EDV 280 ± 77 ml) and reduction in LV ejection fraction ($29\pm 10\%$). The percentage scar burden by LGE was $17\pm 9\%$. Patient characteristics of those undergoing revascularisation (n=7) or not (n=14) were similar. Myocardial Perfusion Reserve Index (MPRI) improved following revascularisation (MPRI 1.17 vs. 1.57, $p < 0.0001$) but not following medical therapy (1.39 vs. 1.32, $p=0.54$). However, LVEF improved in patients whether or not they had revascularisation. In the revascularisation group, 14% of dysfunctional segments with LGE $< 25\%$ and 22% of dysfunctional segments with LGE $< 50\%$ had improved contractile function. However, the transmural extent of LGE did not predict contractile recovery following revascularisation or pharmacological therapy ($p=0.19$, $p=0.42$). LVEDV improved overall ($(280\pm 77$ to 269 ± 83 ml) $p=0.05$); improvement was associated with heart failure duration ($p=0.04$).

2.1.5 Conclusions

In patients with chronic severe LV impairment of ischaemic origin, duration of heart failure is a better predictor of recovery than transmural extent of LGE, following medical therapy or successful revascularisation. This suggests that the extent of myocardial remodelling is more important for LV recovery than the presence and extent of prior infarction alone and that LGE should not be the sole determinant of treatment method in severe LVSD of ischaemic origin.

3.2 Introduction

A series of trials have demonstrated the benefits of pharmacological [39-41, 43, 44, 46] and device therapy[39-41, 43, 44, 46, 48, 182, 183] in appropriately selected patients with chronic heart failure secondary to LVSD. The benefit of revascularisation in patients with LVSD associated with an acute coronary syndrome has also been demonstrated[184, 185], but the benefit of revascularisation for chronic stable heart failure has not[12].

Early trials of revascularisation for heart failure selected patients solely on the basis of coronary anatomy and systolic function[186], whereas more recent trials have included assessment of myocardial viability and scar[49, 95]. The reference standard for measurement of myocardial infarction and scar is late gadolinium enhancement (LGE) cardiovascular magnetic resonance (CMR) imaging. In observational studies, the transmural extent of LGE has predicted the recovery of contractile function and regional wall improvement following revascularisation but some of these studies have enrolled patients shortly after an ischaemic insult[144]. The ability of LGE to predict recovery in patients with chronic, severe, ischaemic cardiomyopathy and whether revascularisation adds to the benefits of pharmacological therapy is unknown. CMR can also be used to assess the effect of revascularisation on myocardial blood flow that may explain the success or failure of the procedure.

This study investigated the relationship between the extent of LGE and myocardial recovery in patients with long-standing severe ischaemic cardiomyopathy treated either pharmacologically alone or with additional revascularisation.

3.3 Methods

Twenty-two patients were recruited from a multi-centre randomised controlled study (HEART-UK[50]) comparing best medical treatment (ACE inhibitor, beta-blocker and aldosterone antagonist) to best medical therapy plus revascularisation for patients with chronic heart failure and left ventricular systolic dysfunction secondary to ischaemic heart disease but with little or no angina.

Revascularisation was by whichever conventional means (i.e. percutaneous coronary intervention or coronary artery bypass grafting) on which the attending cardiologist and cardiac surgeon reached consensus. Patients were entered into the clinical trial on an intention to treat basis and this CMR study was a separate sub-study.

Ethical approval was obtained for the CMR study from participating regional sites and all patients gave written informed consent prior to inclusion.

3.3.1 Inclusion Criteria

Patients who fulfilled the following criteria were included; a) chronic heart failure for at least 6 weeks; b) treated with diuretics c) left ventricular ejection fraction of 35% or less on echocardiography, nuclear scintigraphy or left ventriculogram and coronary artery disease as the cause of left ventricular dysfunction; d) at least 5 of 17 segments that showed contractile dysfunction but were viable as assessed by either stress echocardiography or nuclear myocardial perfusion scanning.

3.3.2 Exclusion Criteria

Contraindications included patients who were not candidates for bypass surgery because of frailty or serious co-morbidity; unstable angina, myocardial infarction or stroke within the preceding two months or patients being considered for revascularisation for the relief of angina or valve surgery. In addition to these criteria, other contra-indications for the sub-study were a history of airways disease or conduction abnormalities precluding pharmacological stressing with adenosine and/or any contraindication to CMR scanning.

3.3.3 CMR Scanning Protocol

Recruited patients underwent two CMR examinations on a 1.5 T scanner (Philips Intera CV, Philips Medical Systems, Best, The Netherlands), equipped with a 5-element cardiac synergy surface coil and vectorcardiogram gating. The first scan was performed at baseline and the second 6 months after receiving assigned therapy. Left ventricular function was assessed via contiguous multiple slice short axis cines using a steady state free precession sequence. Rest and stress myocardial perfusion imaging was performed using a saturation recovery fast gradient echo sequence in conjunction with SENSitivity Encoding (SENSE) parallel imaging (TR 2.8, TE 1.4, Flip angle 55°. 10-14 slices covering the entire left ventricle: slice thickness 6mm, 4mm inter-slice gap, acquired resolution of 1.88*2.21*10mm³ reconstructed to 1.88*14.88*10mm³). A bolus of dimeglumine gadopentetate (Magnevist, Schering AG, Berlin, Germany) contrast medium was rapidly injected by a power injector into an antecubital vein via an 18 gauge peripheral cannula at a dose of 0.05 mmol/kg, followed by a flush of 20 ml normal saline for rest imaging; a second injection of 0.05 mmol/kg gadolinium was repeated after a 15 minute interval at point of maximum vasodilator stress during continuous intravenous adenosine infusion (4 minutes into a six minute infusion). An inversion recovery segmented k-space T1-weighted spoiled gradient echo sequence with a non-selective inversion recovery pre-pulse and trigger delay set for acquisition in mid

diastole was used for LGE imaging (6 to 8 slices to cover the entire LV. TR7.5ms, TE 3.8ms, flip angle 15°, TI adjusted to achieve optimal suppression of normal myocardium).

3.3.4 CMR Analysis

All analyses were performed by a single experienced observer blinded to all clinical data (PS). Left ventricular volumes, mass and ejection fraction were calculated from the short-axis data sets, using a disc-summation method and commercially available post-processing software (Mass 5.0, Medis, Leiden, The Netherlands). All subsequent analysis was on a segmental basis using a 16 segment modified version (apical segment not included) of the American Heart Association model [83] for tomographic imaging, with representative matching basal, mid and apical slices being selected from the short axis cine and LGE data sets.

Combined wall thickening and wall motion for each segment was graded visually as follows: 0 - normal; 1 - mild/moderate hypokinesia; 2 - severe hypokinesia; 3 - akinesia or 4 - dyskinesia, and a left ventricular wall motion index subsequently calculated. An improvement of segmental function was defined as an increase in systolic wall motion of at least one grade on follow-up, with deterioration defined as the converse. On LGE images, the extent of hyper-enhanced tissue within each segment was measured and the percentage of hyper-enhancing myocardium calculated for the left ventricle as a whole. In addition, the transmural extent of hyper-enhancement was graded on a 5 point scale: 0 - none; 1 - 1-25 % thickness of the myocardial segment, 2 - 26-50 %, 3 - 51-75 %, and 4 - 76-100 %. Semi-quantitative assessments of myocardial perfusion for each segment on initial and follow up scan were performed on separate dedicated software (View Forum, Philips Medical Systems, Best, The Netherlands). The upslope of first pass perfusion curves created from plots of changes in myocardial signal intensity over time at rest and hyperaemic stress were calculated using a five point linear fit. These were corrected for baseline myocardial signal intensity pre-contrast and arterial input as derived by blood pool signal intensity within the equivalent slice. The corrected upslope for stress was divided by the value for rest and the result expressed as a myocardial perfusion reserve index (MPRI).

3.3.5 Statistical Analysis

All data are presented as mean +/- SD (continuous) or median (IQR). Normality was determined by the Shapiro-Wilks test. The student t-test was used for continuous variables and the X^2 test for categorical comparisons. Changes over time were assessed for differences between, and within, groups by paired t-test. Data

was processed by patient or segment as stated. Two sided significance of $p < 0.05$ was considered statistically significant. All statistical analysis was performed using SPSS v20, IBM, Chicago, Illinois, USA.

3.4 Results

3.4.1 Baseline characteristics

One of the 22 recruited patients received an implantable cardio-defibrillator following intervention and was excluded from analysis. Characteristics of the 21 patients in the final analysis are summarized in table 3.1 and 3.2. Patients had severe heart failure with a mean LV EDV of 280 ± 77 ml, and mean LVEF of $29 \pm 10\%$. Mean time from diagnosis of heart failure was 16 ± 17 months, time between baseline and follow-up scans was 216 ± 62 days.

Of the 21 patients, 14 were randomised to the conservative strategy and seven to revascularization. Five patients underwent CABG, with a mean of three grafts, and two underwent percutaneous coronary intervention, both for two vessel disease. There were no significant differences in patient characteristics between the two treatment groups at baseline. Time between baseline and follow-up scans was shorter in the medical arm at 186 days (174-196) than in the revascularisation arm, at 274 days (175-373). Time from revascularisation to scan is not reported.

Table 3.1 Subject characteristics.

	All patients (n=21)	Revascularisation (n=7)	Conservative Strategy (n=14)	P
Age (years)	64 ± 8	60 ± 5	66 ± 9	0.14
Male gender	19	7	12	0.47
BMI (Kg/m ²)	28.7 ± 3.0	29.4 ± 2.2	28.3 ± 3.3	0.45
Ex-smoker or current smoker	13	6	7	0.11
Diabetes	7	3	4	0.51
Hypercholesterolaemia	15	6	9	0.31
Family History	17	5	12	0.43
Atrial fibrillation	8	0	8	0.01
NYHA Classification				
1	3	1	2	
2	14	4	10	
3	4	2	2	
4	0	0	0	
Severe Symptoms (>NYHA III)	4	2	2	0.43
Time since HF Diagnosis (months)	13 (5,21)	22.5 (16.5,40.5)	9 (2,14)	0.01
Pre-LVEDV (ml)	280 ± 77	320 ± 75	261 ± 73	0.10
LVEF (%)	29 ± 10	28 ± 13	29 ± 9	0.73
Left Ventricular Wall Motion Index (LVWMI)	2.0 ± 0.5	2.0 ± 0.7	2.0 ± 0.4	0.83
%LV mass displaying LGE	17.6 ± 9.3	19.5 ± 11.4	16.7 ± 8.2	0.53

Table 3.2 Study subject medication at enrolment & completion.

	All (n=21)		Revascularisation (n=7)		Conservative (n=14)	
	Enrolment	End	Enrolment	End	Enrolment	End
β-blocker	18	20	6	6	12	14
ACEi / ARB	17	20	6	6	11	14
AA	2	5	0	1	2	4
Digoxin	4	4	1	1	3	3
Amiodarone	0	1	0	0	0	1
Statin	14	14	5	5	9	9

ACEi/ ARB = Angiotensin converting enzyme inhibitor/angiotensin receptor blocker; AA = aldosterone antagonist

Table 3.3 Segmental pre-treatment CMR findings by study group.

	All (n=336)	Revascularisation (n=112)	Medical (n=224)	P
Transmural extent LGE (%)				
0	135 (40%)	46 (41%)	89 (40%)	0.82
1-25	51 (15%)	19 (17%)	34 (15%)	
26-50	60 (18%)	19 (17%)	41 (18%)	
51-75	52 (16%)	20 (18%)	32 (14%)	
76-100	38 (11%)	10 (9%)	28 (13%)	
Segmental Function				
Normal	24 (1%)	11 (10%)	13 (6%)	0.41
Hypokinetic	90 (27%)	24 (21%)	66 (30%)	
Akinetic	96 (29%)	35 (31%)	61 (27%)	
Dyskinetic or aneurysmal	126 (38%)	42 (38%)	84 (38%)	

3.4.2 Segmental analysis

3.4.2.1 Late Gadolinium Enhancement

For the final analysis, 336 myocardial segments were available. Contractile function was abnormal in 93% of all segments at enrolment. The severity of contractile abnormalities did not differ between groups (Table 3.3). Pre intervention regional wall motion correlated strongly with extent of LGE (Beta coefficient 0.892, $p < 0.01$).

The presence and degree of functional improvement from baseline to follow up was poorly predicted by the extent of LGE. Improvement in segmental contractile function relative to transmural extent of LGE is shown in figure 1.

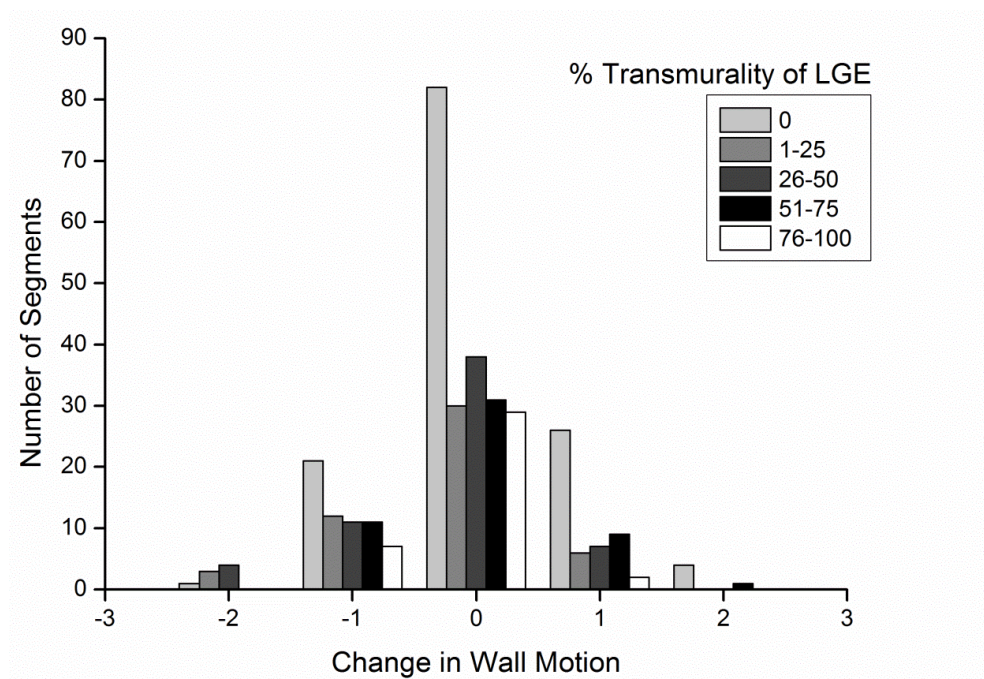


Figure 3.1 Change in segmental function by transmural extent of LGE.

A negative value indicates improvement in segmental function on the previously defined scale.

Overall lower extent of transmural extent of LGE did not predict recovery. Pre and post intervention LGE did not differ significantly and was similar between groups ($p=0.59$).

Overall, 20% of dysfunctional segments with LGE <25% improved, and 22% with LGE <50% improved systolic thickening by at least one grade (Table 3.4).

Improvement was similar in the revascularised and medical therapy groups (Tables 3.5 and 3.6).

Table 3.4 Change in segmental function overall (all segments) following intervention.

Negative change in wall motion score indicates segmental improvement (P=0.214)

Change in wall motion score	% Transmural Extent of LGE				
	0	1-25	26-50	51-75	76-100
-3	1	0	0	0	0
-2	1	3	4	0	0
-1	21	12	11	11	7
0	82	30	38	31	29
1	26	6	7	9	2
2	4	0	0	1	0

Table 3.5 Revascularisation group only: Change in segmental function following intervention (P=0.197).

Change in wall motion score	% Transmural Extent of LGE				
	0	1-25	26-50	51-75	76-100
-3	1	0	0	0	0
-2	0	1	2	0	0
-1	4	6	2	4	4
0	33	9	13	13	6
1	8	1	2	3	0
2	0	0	0	0	0

Table 3.6 Medical treatment group: Change in segmental function following intervention (P=0.413).

Change in wall motion score	% Transmural Extent of LGE				
	0	1-25	26-50	51-75	76-100
-3	0	0	0	0	0
-2	1	2	2	0	0
-1	17	6	9	7	3
0	49	21	25	18	23
1	18	5	5	6	2
2	4	0	0	1	0

3.4.2.2 Myocardial Perfusion Reserve

It was not possible to calculate MPRI in 22 segments that had either full thickness or apical infarcts with marked wall thinning. MPRI improved following revascularisation (1.17 vs. 1.57, $p < 0.0001$) but not following medical treatment (1.39 vs. 1.32, $p=0.54$), see Figure 2. There was no correlation between improvement in segmental function and MPRI in either arm.

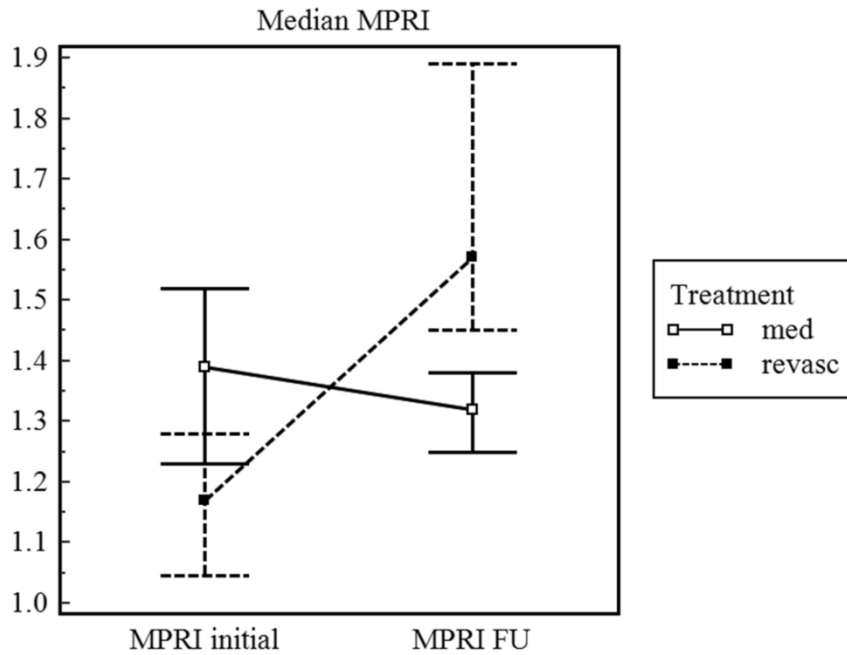


Figure 3.2 Change in myocardial perfusion reserve following revascularisation

3.4.3 Per Patient Analysis

Following intervention, LVEDV decreased by 12 ± 25 ml overall. Improvement was seen in 13 patients, 2 of 7 in the revascularisation and 11 of 14 in the medical therapy arm ($p=0.03$). EDV decreased significantly following medical therapy (261 ± 73 ml to 244 ± 74 ml, $p=0.03$), but not following revascularisation (320 ± 28 ml to 318 ± 32 ml, $p=0.87$). LVEF did not change following treatment, and no difference was seen between groups ($p=0.87$) (table 3.7). Reduction in LVEDV was associated with the duration of heart failure in the entire study group ($p=0.04$) (Figure 3.3).

Table 3.7 LV function and remodelling following intervention

	All patients (n=21)	Revascularisation (n=7)	Medical (n=14)	P
LVEF post treatment	29 ± 10%	28 ± 14%	29 ± 8%	0.82
LVEF improved	9	3	6	1.0
Change in LVEF %	0 ± 7	0 ± 10 %	0.3 ± 10.4	0.87
LVEDV after treatment (ml)	269 ± 84	318 ± 84	244 ± 74	0.05
LVEDV decreased	13	2	11	0.03
Change in LVEDV (ml)	-12 ± 25	-2 ± 24	-25 ± 44	0.2

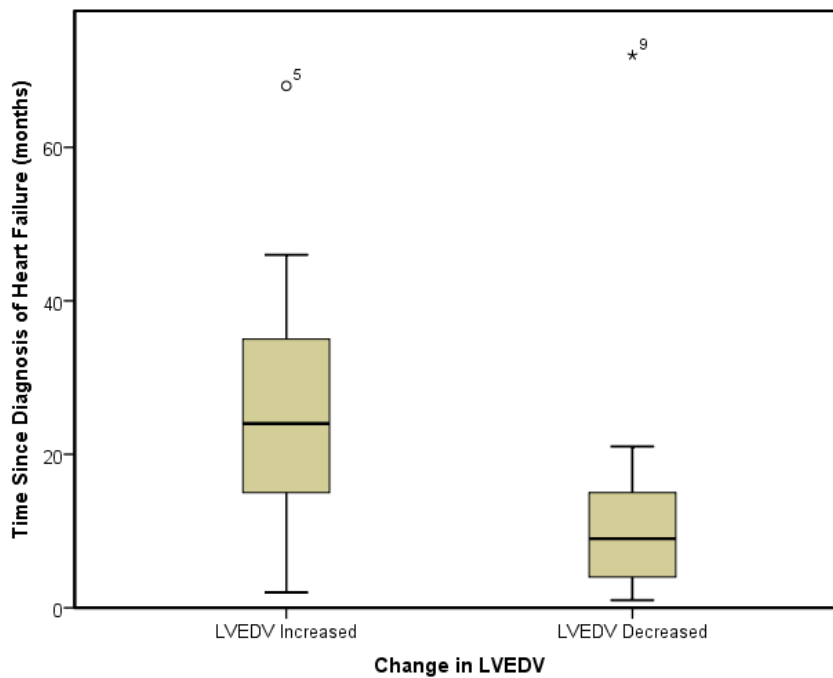


Figure 3.3 LV remodelling over study period across entire study group by heart failure duration (P=0.04)

3.5 Discussion

Dysfunctional myocardium with LGE <50% is generally considered to be ‘viable’ and expected to recover contractile function following revascularisation. Previous series have reported rates of contractile recovery of 60 to 78% in patients with no or limited LGE[144]. However, in these studies overall left ventricular systolic function was not severely impaired[144, 187], relatively few segments were dysfunctional, and time from insult to revascularisation was short[144].

In contrast, patients in this study had a longstanding diagnosis of ischaemic cardiomyopathy with widespread contractile dysfunction and severely impaired LVEF. Data for prediction of recovery of function in long-standing ischaemia and contractile dysfunction are sparse. A recent report suggested that in the absence of extensive infarction severely remodelled myocardium may recover systolic function following revascularisation[18].

In this population, functional recovery was much lower than in previous reports, with only 23/162 (14%) of dysfunctional segments with LGE <25% improving at follow-up. This was irrespective of whether patients were treated pharmacologically or with revascularisation.

The likely cause for the reduced functional recovery in patients undergoing revascularisation in this study was the long duration of ischaemia and its severity. Chronic 'hibernation' leads to changes in myocyte metabolism and the extra-cellular matrix[133, 136]. These changes become more severe with prolonged ischaemic injury and duration of hibernation[137, 188]. Delayed improvement in contractile function has previously been shown to be associated with greater duration of hibernation[132]. The low rate of recovery seen in this population is therefore likely to be due to established changes in the extra-cellular matrix and cellular metabolism and to cardiac myocyte de-differentiation.

The duration of hibernation and time to recover systolic function correlate with recovery times following revascularisation ranging for days to months. In previous studies, follow up imaging of patients was performed at approximately 3[144] to 6 months[187]. In this study patients were scanned 186 ± 7 days after commencing medical therapy and 274 ± 79 days after revascularisation; though the follow-up duration was thus longer than in many previous reports, it may still have been insufficient to detect functional recovery in a cohort of patients with long-standing and severe ischaemic heart failure.

This study is too small to derive definitive comparisons between medical treatment and revascularisation. However, several observations can be made. Segmental recovery was seen in both medical and revascularisation groups. Contemporary medical therapy with ACE inhibitor & β -blockers are known to improve LV function in heart failure, especially if the LV is not severely dilated [189, 190]. However, on average, LV ejection fraction did not improve significantly in either group. This could reflect the small number of patients. Most patients had received pharmacological treatment for a long time prior to the study and might have already received any expected benefit. However, LVEDV appeared to improve in the conservatively managed group compared to those who were revascularised. This could reflect myocardial damage caused by the revascularisation procedure.

Differences in the severity of LV dilatation and duration of disease between the two groups and a chance statistical difference due to multiple comparisons provide alternative explanations. Ultimately, in a study of this size, small differences should not be over-interpreted. However, this is a randomized study suggesting that revascularisation does not cause large, consistent benefits on LV function in patients with chronic ischaemic cardiomyopathy and is therefore an important finding.

This study also showed no correlation between the transmural extent of LGE and recovery following revascularisation, despite clear improvement in MPRI demonstrating successful revascularisation. Previous studies have not measured perfusion alongside LGE and our study provides the first evidence that improved flow does not necessarily lead to improved function when assessed at six months. The lack of functional improvement despite improved perfusion suggests established structural and metabolic change within hibernating myocardium as described above that are not reversible within the follow-up period.

3.6 Limitations

The main limitation of this study is its sample size and it therefore has to be considered as hypothesis-generating. However, it does show that improved LV function with revascularisation is not universal, consistent or to be taken for granted. Larger studies should test the ability of LGE to predict more modest or erratic recovery in function. Such studies might also use T1 mapping and extra cellular volume mapping that may better predict the likelihood of recovery following revascularisation in patients with ischaemic cardiomyopathy who do not have visually detectable LGE.

3.7 Conclusions

In patients with longstanding severe LV impairment of ischaemic origin, duration of heart failure is a better predictor of global functional recovery than transmural extent of LGE, following medical therapy or revascularisation. This suggests that the transmural extent of LGE, commonly used as the primary basis of revascularisation decision making, may fail to capture the extent of remodelling and the potential for recovery in severe longstanding LVSD.

Further studies should be performed that are powered to detect indices that predict response following revascularisation in severe LVSD, possibly using CMR techniques better at assessing diffuse myocardial change, including ECV calculation with T1 mapping. More importantly, more randomised trials should be

conducted to show whether the benefits of revascularisation justify the risk and cost even in carefully selected patients with heart failure.

Chapter 4

The Effect of Changes to MOLLI Scheme on T1 Mapping and Extra Cellular Volume Calculation in Healthy Volunteers with 3 Tesla Cardiovascular Magnetic Resonance Imaging

4.1 Abstract

4.1.1 Objectives

To establish the variation of native T1 and extracellular volume in healthy volunteers with different MOLLI schemes

4.1.2 Background

Diffuse myocardial fibrosis may be quantified with magnetic resonance by calculating extra-cellular volume (ECV) fraction from native and post-contrast T1 values. The ideal MOLLI (Modified Look-Locker Inversion Recovery) sequence for deriving T1 values has not been determined.

4.1.3 Methods

12 phantom gels were studied with inversion recovery spin echo MR at 3.0 Tesla to determine reference T1. Gels were then scanned with 6 MOLLI sequences (3s)3b(3s)5b; 4b(3s)3b(3s)2b; 5b(3s)3b with flip angles of both 35° and 50° at a range of heart rates(HR). In 10 healthy volunteers MOLLI studies were performed on two separate occasions. Mid ventricular native and post contrast T1 was measured and ECV (%) calculated.

4.1.4 Results

In phantoms, the co-efficient of variability at simulated HR (40-100) with a flip angle of 35° ranged from 6.77 to 9.55, and at 50° from 7.71 to 11.10. T1 was underestimated by all MOLLI acquisitions. Error was greatest with longer T1, and increased as heart rate increased. The 10 volunteers had normal MR studies. Native T1 time was similar for all acquisitions but highest with the 5b(3s)3b 35° scheme (1189.1ms ± 33.46). Interstudy reproducibility was similar for all MOLLI.

4.1.5 Conclusion

The 5b(3s)3b MOLLI scheme agreed best with reference T1, without statistical difference between the six schemes. The shorter breath-hold time of 5b(3s)3b scheme may be preferable in clinical studies and warrants further investigation.

4.2 Introduction

The value of detection of focal myocardial scar that characterises a number of disease processes using late gadolinium enhancement (LGE) imaging is well recognised[13, 65]. However this technique relies upon contrast between healthy and diseased myocardium and as a result it is limited in the detection of diffuse myocardial disease processes characterised by diffuse fibrosis or infiltration.

Longitudinal relaxation time (T1) mapping allows quantitative myocardial tissue characterisation; thereby enabling detection of diffuse myocardial disease processes that have previously only been detectable with cardiac biopsy[66, 67]. T1 measurement before and after the administration of gadolinium based contrast agent (GBCA) allows the relative volumes of the intra-cellular and extra-cellular components of myocardium to be quantified, as long as equilibrium between the compartments has been reached.

With increasing use in clinical practice and medical research, it is important that accurate, precise and reproducible methods for T1 mapping are employed[191]. Values obtained are dependent upon numerous scanner and pulse sequence parameters including flip angle, acquisition pulse sequence and the interval between inversions. The potential advantages of various Modified Look-Locker Inversion Recovery (MOLLI) have been investigated by simulation and in phantom studies [192, 193], however direct comparison *in vivo* has not been made between proposed sequences.

Patient factors, including heart rate and breath-hold duration, will affect the acquisition both with reference to tissue recovery and image quality. In this study we aimed to determine the reproducibility and accuracy of three published MOLLI acquisition schemes[191] utilising two different flip angles in phantom gels and healthy volunteers.

4.3 Methods

All studies were performed at a single centre equipped with a 3.0T Philips Achieva TX research MRI scanner using a 32-channel cardiac phased array receiver coil. Volunteer scanning was approved by the local ethics committee and all subjects gave written informed consent.

4.3.1 MOLLI Schemes

ECG triggered MOLLI acquisitions with different number of images were acquired according to three predefined schemes. Pause duration was defined in seconds (s),

acquisition duration by beats (b). Scan parameters were: FOV (typically 320*400mm, but modified to minimise artefact as needed), voxel size 1.98x1.98x10mm³ (reconstructed to 1.25x1.25mm), single-shot, sensitivity encoding (SENSE) factor 2, partial echo factor 0.85, water fat shift 0.4, trigger delay set for end-diastole, TFE prepulse delay 350ms. Acquisition duration was 170-185ms dependent upon FOV. TI values for the images acquired directly after inversion are spaced equally from the shortest possible value (FOV dependent) for the first inversion to 350ms for the final inversion to representatively sample magnetisation recovery. Images are then acquired at the same cardiac phase in the subsequent images.

The schemes employed were: 3b(3s)3b(3s)5b; 5b(3s)3b; 4b(3s)2b(3s)1b

All three schemes were acquired with flip angles of 50° and 35°. A flip angle of 50° was chosen as a value frequently used in the existing literature [19]. The value of 35° was chosen to maximise signal from native myocardium at 3T according to the formula:

$$\alpha_{S_{\text{max}}} = \cos^{-1} \left(\frac{T1-T2}{T1+T2} \right)$$

If myocardial native T1 is assumed to be ≈1200ms, a flip of angle of approximately 35° results in maximal signal.

4.3.2 Phantom Scanning

A total of 12 agarose gel phantoms with known T2 time were studied. T2 values were obtained using a multiple-spin-echo sequence with TR = 5000ms and TE ranging from 30-240ms in 30ms increments. A mono-exponential function was fitted on a voxel-by-voxel basis to estimate T2, and mean values calculated for each gel. Reference T1 relaxation times of each phantom were determined using standard inversion recovery spin echo pulse sequences (IRSE) using varying inversion times: 50, 100, 150, 200, 300, 500, 750, 1000, 1250, 1500, 2500, 4000ms. TR 10ms, TE 12ms, slice thickness 10mm and acquired resolution 1.5 x 1.5mm². Having determined reference T1, gels were studied using MOLLI schemes at a range of heart rates. A physiology simulator set to heart rates of 40, 60, 80, 100 beats per minute was used to trigger the MOLLI scans.

4.3.3 Volunteer Scanning

10 healthy volunteers were recruited to undergo MR studies. Subjects were excluded if they had a history of cardiac disease, hypertension, renal impairment, diabetes or contra-indication to MR. Volunteers underwent two studies, separated by a mean interval of 17.1 ± 14.2 days.

The cardiac long-axis was located as per standard practice and a stack of left-ventricular (LV) short axis images acquired using an ECG gated balanced Steady State Free Precession (bSSFP) method (echo time (TE) 1.3ms; repetition time (TR) 2.6ms; flip angle 40° , spatial resolution $1.6 \times 2.0 \times 10$ mm, 40 phases per cardiac cycle).

GBCA was administered as two split doses as part of a stress perfusion MR study. Adenosine was administered at $140 \text{mcg/kg/min}^{-1}$ via a cannula sited in the ante-cubital fossa for a minimum of three minutes and until maximal vasodilation occurred. 0.075mmol/Kg Gadovist (Bayer Schering Pharma, Berlin-Wedding, Germany) boluses were separated by twelve minutes, followed on each occasion by a 20ml saline flush. Post contrast MOLLI were performed 15 minutes after the second bolus of Gadolinium.

LGE imaging was performed at seven to ten minutes following final contrast dose (inversion recovery-prepared T1 weighted gradient echo, inversion time according to Look-Locker scout, TR/TE/flip angle $3.7 \text{ms}/2.0 \text{ms}/25^\circ$, acquired spatial resolution $1.54 \times 1.75 \times 10$ mm) as a contiguous stack with no gap, with complete coverage of the left and right ventricles.

4.3.4 Image Analysis

Left ventricular volumes and ejection fraction were analysed from bSSFP cine images using standard analysis methods. Phantom and volunteer MOLLI study images were saved as Digital Imaging and Communications in Medicine (DICOM) format. T1 values were calculated from source images using manual motion correction on CMR42 (Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). In phantoms a region of interest (ROI) was drawn in the centre of the gel away from any ringing artefact.

In volunteer MOLLI data sets, a narrow ROI in the infero-septum of the mid-ventricular slice was drawn as per Rogers *et al*[194] to replicate application of T1 mapping in clinical practice. Furthermore, segmental analysis was performed using a narrow ROI in each segment of the mid LV slice in accordance with the AHA model[195]. Conservative ROIs were drawn in each segment taking care to avoid artefact induced by epicardial vessels. Mis-registration was avoided by

visually comparing left and right ventricular anatomical features (e.g. papillary muscles, trabeculations), and any mis-registered images were discarded. The blood pool contour was drawn in the centre of the LV cavity on the same slice away from any papillary muscle. Extracellular volume fraction was calculated using the formula:

$$ECV = (1 - Hct) \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)} \text{ Where } R1 = 1/T1$$

Scans were discarded when artefact prohibited analysis.

4.3.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY). Continuous variables are expressed as means ± standard deviation (SD). Reproducibility and agreement were assessed by coefficient of variation (CoV), both comparing IR SE T1 reference values to MOLLI T1 value in phantoms, and also to assess the inter-study variability of repeat MOLLI studies in volunteers. CoV of less than 10% was considered acceptable.

4.4 Results

4.4.1 Phantom Scanning

Reference T1 values were: 228, 346, 539, 564, 784, 895, 927, 1302, 1537, 1674, 1854 and 1949ms. Co-efficient of variability with each MOLLI scheme at simulated HR of 40, 60, 80, 100 with flip angle of 35° was 6.77 to 9.55; when flip angle was 50 CoV ranged between 7.76 and 11.1 (table 4.1).

Table 4.1 Effect of simulated heart rate on MOLLI performance vs ref T1 SE in phantom gels

Flip Angle 35°	Co-efficient of Variability				Flip Angle 50°	Co-efficient of Variability			
	HR 40	60	80	100		Scheme	HR 40	60	80
<i>Scheme</i>					<i>Scheme</i>				
5,3,0	6.77	7.61	9.49	7.57	5,3,0	7.86	7.71	7.60	9.19
3,3,5	6.81	7.21	8.31	8.85	3,3,5	7.74	7.99	8.67	11.10
4,3,2	6.94	7.78	7.89	9.55	4,3,2	9.00	9.30	9.03	8.89

T1 was persistently under-estimated by all MOLLI acquisitions. Error was greatest when gel T1 was longer, and at higher heart rates.

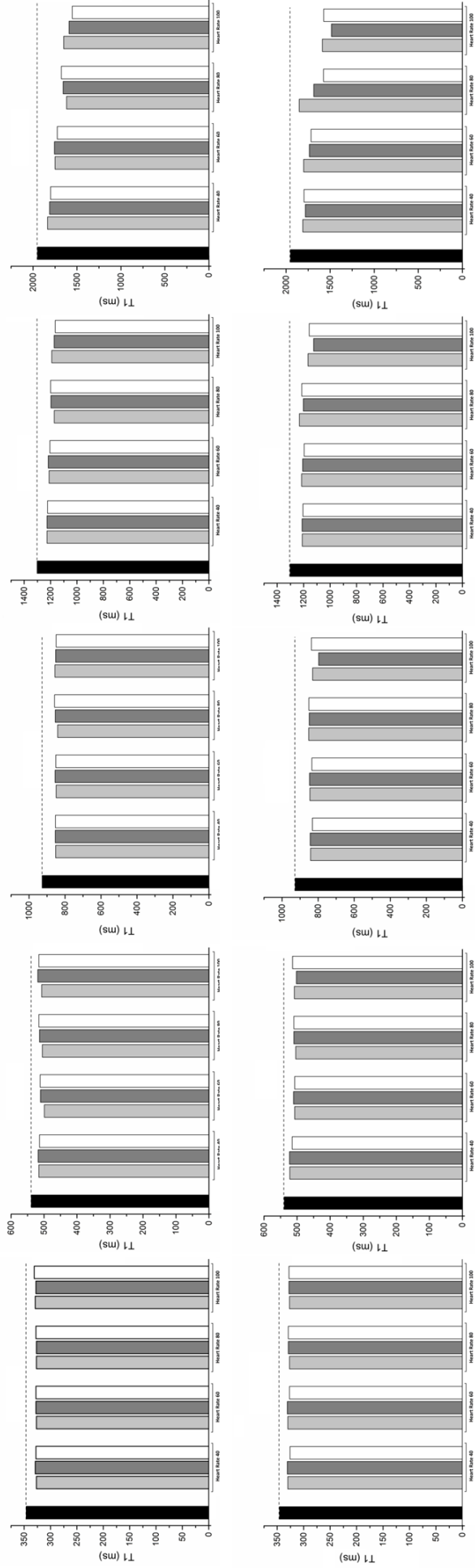
For all schemes at a simulated heart rate of less than 100 beats per minute the coefficient of variability was acceptable at less than 10%. Performance of the 3b(3s)3b(3s)5b MOLLI with 50° showed the lowest precision (CoV: 11.10). The performance of the MOLLI schemes in phantoms compared to reference T1, when T1 is of clinical relevance, (346, 539, 927, 1302, 1949ms with T2 respectively 113, 86, 99, 180, 161ms) are shown in figures 4.1 and 4.2.

Figure 4.1 (top) 35° MOLLl performance at HR 40, 60, 80 and 100 beats per minute against reference SE T1

Black ref T1; Light grey 5b(3s)3b; Dark Grey 3b(3s)3b(3s)5b; White 4b(3s)3b(3s),2.

Figure 4.2 (bottom) 50° MOLLl performance at HR 40, 60, 80 and 100 beats per minute against reference SE T1

Black ref T1; Light grey 5b(3s)3b; Dark Grey 3b(3s)3b(3s)5b; White 4b(3s)3b(3s),2.



4.2 Volunteers

4.4.2.1 Demographics

Demographic characteristics of the study population are as presented in table 4.2. The mean age was 27yrs \pm 3, (7 males). BSA corrected LVEDV ($101 \pm 12\text{ml/m}^2$), LV mass ($52 \pm 7\text{g/m}^2$) and ejection fraction ($57\% \pm 2$) were normal. All volunteers had normal right ventricular function and had normal late gadolinium enhancement images (table 4.2). 119 of 120 acquired mid-ventricular short axis MOLLI acquisitions were suitable for analysis (1 acquisition error prohibited analysis).

Table 4.2 Volunteer Characteristics

	Mean \pm SD
Age (years)	27 \pm 3
Gender (M:F)	7 : 3
Mean Systolic Blood Pressure (mmHg)	111 \pm 7
Mean Diastolic Blood Pressure (mmHg)	55 \pm 6
Indexed LVEDV (ml/kg/m^2)	101 \pm 12
Indexed LV Mass (g/kg/m^2)	52 \pm 7
LVEF(%)	57 \pm 2
Indexed RVEDV (ml/kg/m^2)	104 \pm 17
RVEF (%)	54 \pm 2

4.4.2.2 Myocardial Native T1

With a flip angle of 35° infero-septal native T1 (ms) was similar for all MOLLI acquisitions (table 4.3). Native T1 was higher when flip angle was 35° compared to 50°, and highest using the 5b(3s)3b scheme. Inter-study reproducibility of native T1 measurement was good for all pulse sequences (table 4.3).

Table 4.3 Volunteer studies: Reproducibility of infero-septal post contrast T1 (ms) by MOLLI scheme and flip angle (sd) (CoV: coefficient variability).

35° Flip Angle	Mean (sd)	CoV	50° Flip Angle	Mean (sd)	CoV
5,3,0	1189.1 (33.46)	1.78	5,3,0	1170.7 (25.90)	1.67
3,3,5	1184.5 (22.66)	1.24	3,3,5	1158.7 (33.01)	2.26
4,3,2	1181.8 (23.17)	1.28	4,3,2	1168.4 (24.05)	1.06

4.4.2.3 Extracellular Volume

ECV (%) was reproducible with each scheme employed and agreed well on visit one and two. The co-efficient of variability for all three schemes and for both flip angles was less than 7% (table 4.4).

Table 4.4 Reproducibility of extra-cellular volume fraction (%) by MOLLI scheme.

ROI in the infero-septum by MOLLI scheme (sd)

35° Flip Angle	Mean ECV	Co-efficient of Variability
5,3,0	24.8 (3.9)	5.62
3,3,5	24.5 (3.2)	5.90
4,3,2	24.7 (3.2)	4.92
50° Flip Angle		
5,3,0	25.9 (3.9)	5.97
3,3,5	25 (3.3)	5.58
4,3,2	25.3 (4.0)	6.30

4.4.2.4 Segmental Analysis

It was possible to measure native and post contrast T1 in all mid-LV segments, and measurement was not precluded by artefact. ECV in all six segments of the mid left ventricular slice was similar (calculated from pre- and post-contrast T1 maps), standard deviations were low and did not differ between segments or sequence (table 4.5).

Table 4.5 Mean extra-cellular volume fraction (%) of mid LV segments from standard 16 segment AHA model (average value of two studies).

	35° flip angle			50° flip angle		
	5,3,0	3,3,5	4,3,2	5,3,0	3,3,5	4,3,2
Mid LV slice						
Anterior	24.3 (3.1)	24.1 (3.2)	24.1 (3.0)	26.6 (3.7)	25.5 (3.3)	25.7 (3.6)
Antero-lateral	23.4 (2.3)	23.5 (2.4)	23.5 (2.4)	25.6 (3.3)	24.3 (2.5)	24.4 (2.7)
Infero-lateral	23.0 (3.2)	23.3 (3.2)	22.8 (3.3)	25.2 (4.9)	24.4 (4.1)	24.4 (4.3)
Inferior	23.2 (3.2)	23.4 (3.2)	23.1 (3.1)	24.8 (3.0)	24.4 (3.5)	24.3 (3.5)
Infero-septal	24.4 (3.7)	24.4 (3.1)	24.3 (3.3)	26.1 (3.9)	24.9 (3.6)	25.2 (3.4)
Antero-septal	24.3 (3.0)	24.1 (2.7)	24.0 (2.9)	26.2 (3.7)	24.8 (3.5)	25.1 (3.4)

4.5 Discussion

Previous studies have examined multiple sequence parameter variables in simulator, phantoms and volunteer subjects[196]. This study aimed to add to these previous studies and further define the effect of change in acquisition scheme and flip angle.

The initially proposed 3b(3s)3s(3b)5 MOLLI scheme is still commonly used[19]. However this may not be ideal for accurate T1 measurement as the final eight chronologically acquired images are influenced by previous inversion(s), so that T1 estimation is affected by incomplete tissue recovery between inversions. To shorten breath-hold time, and reduce the number of points affected by prior inversions, alternative MOLLI schemes have been suggested. These include a 3,5 acquisition, which has been found to perform similarly to 3,3,5[197, 198]. However the same problems relating to potential incomplete recovery are inherent in this scheme also. In this study we found that a 5(3s)3 scheme with flip angle of 35° agreed best with a gel T1 spin echo reference, which may be a consequence of fewer points being affected by prior magnetisations.

Many commonly used MOLLI acquisitions time pause duration and acquisition intervals in beats, and a pause of three R-R intervals (3 beats, (3b)) is commonly used. This leads to inconsistent pause duration, and potentially subsequent underestimation of long T1, as recovery may not be complete. This is particularly true of the 3b(3b)3b(3b)5b scheme at higher heart rates[199]. A fixed pause duration of at least three seconds (3s), as employed in this study, may minimise this problem and ensure more complete recovery of native blood pool and myocardium where T1 at 3T is typically 1800ms and 1200ms. However, in this study we have

demonstrated that under-estimation of native T1 remains problematic in phantom studies at high heart rates in spite of fixed pause duration. This is likely due to incomplete recovery: for example using a 5b(3s)3b the time between inversions is 9000 ms whereas at 100bpm it is only 6000ms.

Due to the effects of incomplete recovery it has been suggested to employ different MOLLI schemes for native and post contrast T1 measurement. The pause duration employed in this study was approximately 5.5 times the T1 of post contrast myocardium, which has previously been shown to have only a negligible effect on post contrast myocardial T1 measurement at heart rates up to 90 [200]. When T1 is short, for instance after GBCA administration, incomplete recovery of longitudinal magnetisation between inversion pulses is minimal and additional points sampled early following inversion pulse may improve accuracy and precision. A 4,3,2 acquisition scheme with either a single RR[191] or three beat interval recovery [201] period has been suggested as an alternative to both 3,3,5 and 5,3 in this situation. However, in this study we employed a 3 second pause, rather than the 1s pause[191], throughout to aid comparison between schemes.

Similarly, if the signal-to-noise ratio (SNR) of myocardium is not maximised, image quality may be suboptimal, which will reduce fitting quality in the T1 estimation and precision[191]. Signal is dependent on the readout pulse flip angle and is maximal for a balanced steady-state free precession sequence (as used for MOLLI) when:

$$\alpha_{S_{\text{Imax}}} = \cos^{-1} * ((T1-T2)/(T1+T2))[202]$$

Therefore a flip angle may be selected to maximise signal from any image component depending upon a tissue's T1 value. The effects of flip angle on signal have been studied previously [196]. A flip angle of 50° leads to high signal within the blood pool and an impression of good image quality due to high myocardium-blood contrast, whilst a flip angle of ~35° results in maximal SNR for native T1 measurement within healthy myocardium (assuming T1= 1200ms and T2=40ms) at the expense of a visually less appealing T1 map due to the blood pool appearing less homogenous. Following contrast administration, T1 shortens and higher flip angles result in higher SNR, however signal already increased following GBCA administration, therefore maximising signal from pre-contrast myocardial T1 becomes most relevant. In this study, native *in vivo* T1 was similar with MOLLI sequences using either 35° or 50° flip angle; however T1 was highest when using a flip angle of 35° and therefore more likely to be a true representation of tissue T1, as MOLLI tends to underestimate T1.

The shorter breath hold time that the 5b(3s)3b sequence employs means it may be better tolerated in patients with breathlessness and/or left ventricular dysfunction when compared to 3b(3s)3b(3s)5b and 4b(3s)3b(3s)2b - though this was not investigated in this study. Comparative breath hold times using 5b(3s)3b, 3b(3s)3b(3s)5b and 4b(3s)3b(3s)2b at a heart rate of 60bpm are 11s, 17s and 15s respectively. This may have positive implications for image quality by minimising cardiac motion due to respiration when T1 mapping is used as a clinical or research tool.

All schemes performed similarly regardless of the LV segment where T1 was measured. Previous studies have shown that infero-septal T1 is the most reproducible segment for T1 measurement[194]. However we did not undertake to measure signal to noise ratio (SNR) in this study. Lower SNR, especially in the lateral wall as previously demonstrated[191] will result in less precision of T1 and until this is quantified some caution should be applied to the widespread application of T1 segmental T1 mapping.

We found the studied MOLLI sequences to be similarly reproducible, with good agreement between visits. The 2013 SCMR T1 mapping consensus document[203] acknowledges that there is variation in MOLLI sequences used globally, and whilst optimal pulse sequences are being defined it is important to establish local normal values. These data suggest that these three MOLLI sequences, with two different flip angles, perform similarly; potentially suggesting that robust local practice and patient factors should be considered when deciding upon the ideal T1 mapping scheme, including scan duration and breath-hold duration.

4.6 Limitations

This small study did not include subjects with known myocardial pathology to assess the effects of change in MOLLI scheme in areas with elevated ECV. SNR was not quantified in this study.

4.7 Conclusions

Further, larger datasets may allow more comprehensive conclusions to be drawn regarding ideal MOLLI acquisition. However in this study we have shown that 5b(3s)3b performs similarly to 3b(3s)3b(3s)5b whilst offering advantages in breath hold duration and likely improvement in respiratory motion, suggesting it may be more appropriate for clinical application.

Chapter 5

Single Bolus Versus Split Dose Gadolinium Administration in ECV Calculation at 3 Tesla

5.1 Abstract

5.1.1 Objectives

To assess the effects of split dose versus single bolus contrast administration on ECV calculation

5.1.2 Background

Diffuse myocardial fibrosis may be quantified with cardiovascular magnetic resonance (CMR) by calculating extra-cellular volume (ECV) from native and post-contrast T1 values. Accurate ECV calculation is dependent upon the contrast agent having reached equilibrium within tissue compartments. Previous studies have used infusion or single bolus injections of contrast to calculate ECV. In clinical practice however, split dose contrast injection is commonly used as part of stress/rest perfusion studies.

5.1.3 Methods

Ten healthy volunteers and five patients (4 ischaemic heart disease, 1 hypertrophic cardiomyopathy) were studied on a 3.0 Tesla (Philips Achieva TX) MR system and underwent two (patients) or three (volunteers) separate CMR studies over a mean of 12 and 30 days respectively. Volunteers underwent one single bolus contrast study (Gadovist 0.15mmol/kg). In two further studies, contrast was given in two boluses (0.075mmol/kg per bolus) as part of a clinical adenosine stress/rest perfusion protocol, boluses were separated by 12 minutes. Patients underwent one bolus and one stress perfusion study only. T1 maps were acquired pre contrast and 15 minutes following the single bolus or second contrast injection.

5.1.4 Results

ECV agreed between bolus and split dose contrast administration (coefficient of variability 5.04%, bias 0.009, 95% CI -3.754 to 3.772, $r^2=0.973$, $p=0.001$). Inter-study agreement with split dose administration was good (coefficient of variability, 5.67%, bias -0.018, 95% CI -4.045 to 4.009, $r^2=0.766$, $p>0.001$).

5.1.5 Conclusions

ECV quantification using split dose contrast administration is reproducible and agrees well with previously validated methods in healthy volunteers, as well as

abnormal and remote myocardium in patients. This suggests that clinical perfusion CMR studies may incorporate assessment of tissue composition by ECV based on T1 mapping.

5.2 Introduction

Expansion and composition change of the myocardial extra-cellular matrix (ECM) is seen in a range of myocardial diseases and correlates with measures of systolic and diastolic function[158, 204][205-207]. Cardiac magnetic resonance (CMR) late gadolinium enhancement (LGE) imaging is well suited for the detection of focal myocardial scar that characterises a number of disease processes[13, 65]. However this technique relies upon the presence of healthy myocardium to detect scar, and as a result is limited in the detection of diffuse myocardial disease processes where global myocardial ECM expansion occurs.

Longitudinal relaxation time (T1) mapping allows quantitative characterisation of the myocardium, thereby enabling detection of diffuse myocardial disease processes that have previously required cardiac biopsy[66, 67]. Furthermore, the ability to accurately define myocardial composition allows for the detection of sub-clinical disease states and may enable the effects of intervention on tissue composition to be determined non-invasively [203].

T1 measurement before and after the administration of an extracellular contrast agent allows the relative volumes of the intra-cellular and extracellular components of myocardial tissue to be quantified as long as equilibrium between the extra-cellular compartments (interstitium and blood) has been reached. Equivalence of primed slow intra-venous infusion and bolus only administration of contrast agent has been demonstrated previously[16, 201]. However, stress perfusion imaging is an expanding area of CMR[3] now included in international practice guidelines[4]. During stress perfusion CMR studies, contrast agent delivery is split between rest and stress imaging. Integrating T1 mapping and ECV calculation in such a clinical protocol requires knowledge of the effects of split contrast injection on the derived measurements. Therefore we aimed to determine the effects of split versus single bolus contrast administration on ECV, and to assess the inter-study variability of ECV measured on split contrast administration CMR studies.

5.3 Methods

The research protocol was approved by the local ethics committee and all subjects gave written informed consent. All studies were performed at a single centre equipped with a 3T MRI scanner (Achieva TX, Philips Healthcare, Best, The Netherlands) using RF shimming and a 32-channel cardiac phased array receiver coil.

5.3.1 Volunteer Scanning

A total of ten healthy volunteers were recruited to undergo CMR studies. Subjects were excluded if they had a history of cardiac disease, hypertension, renal impairment, diabetes or contra-indication to CMR.

All subjects underwent a total of three CMR studies on separate days:

- Study 1. Single bolus: In one CMR study, the contrast agent (Gadovist, Bayer Schering Pharma, Berlin-Wedding, Germany) was administered as a single bolus (0.15mmol/kg) with post-contrast T1 mapping acquired 15 minutes later.
- Study 2. Split dose: Contrast was administered as a split doses (0.075mmol/kg twice) as part of an adenosine stress perfusion protocol. For stress perfusion, intra-venous adenosine was administered at 140mcg/kg/min, via an intra-venous cannula sited in the ante-cubital fossa, for a minimum of three minutes and until an appropriate haemodynamic response had occurred. Contrast agent was delivered at a dose of 0.075mmol/kg at peak haemodynamic stress. For rest perfusion, the same contrast injection regime was repeated twelve minutes later. A total Gadovist dose of 0.15mmol/kg was administered. Post-contrast T1 mapping was performed 15 minutes after the second contrast administration.
- Study 3. Split dose: Split dose stress perfusion CMR study as 2.

5.3.2 Patient Scanning

A total of five patients that had undergone a clinically indicated, non-urgent, adenosine stress perfusion CMR study were recruited if they exhibited an area of enhancement on LGE imaging on the clinical study, which included native T1 map and 15 minute post contrast T1 map as per our local protocol. Patients were then recalled for one single bolus CMR study on a separate day in keeping with the above protocols.

5.3.3 CMR Protocol

In each study, the cardiac long-axis was located as per standard practice using balanced steady-state free precession pulse (bSSFP) cine images. Cine imaging was performed using a contiguous stack of parallel short-axis slices covering the whole left ventricle (LV), with a bSSFP pulse sequence (echo time (TE) 1.3 ms; repetition time (TR) 2.6 ms; flip angle 40°, spatial resolution 1.6×2.0×10 mm, 40 phases per cardiac cycle).

For all studies, contrast was delivered via a peripheral cannula, followed by a 20ml saline flush delivered by automated injector (Medrad Inc, Warrendale, Pennsylvania, USA) at 5ml/second.

Native and 15 minute post-contrast data for T1 value estimation were obtained using breath-held Modified Look-Locker Inversion recovery (MOLLI) acquisition [19, 192, 203]. Images were acquired in the central slice of a '3 of 5' approach [208]. An ECG triggered 5b(3s)3b MOLLI balanced turbo gradient recalled echo (GRE) acquisition method was used (voxel size 1.98x1.98x10mm³ (reconstructed to 1.25x1.25mm), single-shot, sensitivity encoding (SENSE) factor 2, trigger delay set for end-diastole, flip angle 35°, acquisition duration per image 170-185ms (dependent upon FOV) a range of inversion times are calculated by the system in order to provide good sampling of T1 recovery.

Perfusion imaging acquisition used a spoiled turbo GRE sequence (echo time (TE) 2.8 ms; repetition time (TR) 1.28 ms; flip angle 15°, acquired spatial resolution 2.42x2.42x10 mm) in three 10mm short axis slices with a 148x148 matrix, FOV 300-420, sensitivity encoding factor 2.4, half scan factor of 0.65 and a saturation pre-pulse delay of 80ms.

LGE imaging was performed at 7-10 minutes following final contrast dose (inversion recovery-prepared T1 weighted gradient echo, inversion time according to Look-Locker scout, TR/TE/flip angle 3.7/2.0/25 degrees, spatial resolution 1.54x1.75x10 mm).

5.3.4 Image Analysis

Study images were saved as Digital Imaging and Communications in Medicine (DICOM) format. T1 values were calculated from source images using manual motion correction on CMR42 (Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). Mis-registration was avoided by visually comparing left and right ventricular anatomical features (papillary muscles, trabeculations) any mis-registered images were discarded. In volunteers a narrow region of interest (ROI) in the infero-septum of the mid-ventricular slice was drawn as per Puntmann[194] in an effort to minimise potential artefact induced by epicardial cardiac vessels in the anterior and lateral walls. In patient studies two separate ROIs were drawn sampling:

1. the area displaying visual enhancement on the LGE acquisition; matched using standard image planning techniques and left and right ventricular anatomical features.

2. remote myocardium (preferentially the infero-septum as in volunteer studies).

The blood pool contour was drawn in the centre of the LV cavity on the same slice away from any papillary muscle. Signal intensity was measured from each MOLLI source image and T1 estimated based on the mean signal from myocardial and blood pool ROIs. ECV was calculated using the formula:

$$ECV = (1 - Hct) \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)} \text{ Where } R1 = 1/T1$$

5.3.5 Statistical Analysis

Statistical analysis was performed using IBM SPSS® Statistics 21.0 (IBM Corp., Armonk, NY). Unless otherwise stated the results are presented as mean ± standard deviation (SD). Reproducibility and agreement was assessed by coefficient of variation and Bland Altman plot. Normality of distribution was determined with Kolmogorov-Smirnov testing, normality was assumed with a value of >0.2. Correlation was assessed with Pearson's correlation coefficient.

5.4 Results

Demographic characteristics of the study volunteers are as presented in table 5.1. The mean age of volunteers was 26.6yrs ± 2.8, 7 of 10 volunteers were men. Body surface area (BSA) corrected LVEDV (101 ± 12ml/m²), LV mass (52 ± 7g/m²) and ejection fraction (57% ± 2) were normal. All volunteers had normal right ventricular function and demonstrated no hyper-enhancement on late gadolinium enhancement images. All volunteers had an appropriate response to adenosine, with mean resting heart rate of 61.4 ± 5.2 beats per minute that increased on stress perfusion by 22.6 ± 7.6 beats per minute. Stress perfusion images were assessed qualitatively and no perfusion defects were identified. Split dose administration contrast agent doses were separated by 12.0 ± 3.7 minutes.

Patient characteristics are as displayed in table 5.1. Mean patient age was 59.0yrs ± 13, 4 of 5 patients were men, body surface area (BSA) corrected LVEDV (96 ± 36ml/m²), LV mass (64 ± 20/m²) and ejection fraction (42 ± 13). Patient pathologies were: 4 chronic ischaemic heart disease with established myocardial infarction; 1 hypertrophic cardiomyopathy. All patients had extensive LGE, and no inducible perfusion defects on stress perfusion imaging out with the area of LGE.

Table 5.1 Subject characteristics

	Healthy Volunteers (n=10)	Patients (n=5)
Age	26.6 ± 2.8	59.0 ± 13.3
Gender (M:F)	7 : 3	4 : 1
Underlying Cardiac Disease		Ischaemic Heart Disease 4 Hypertrophic Cardiomyopathy 1
Mean Rest Systolic Blood Pressure (mmHg)	111 ± 7	131 ± 19
Mean Rest Diastolic Blood Pressure (mmHg)	55 ± 6	71 ± 10
Mean Rest Heart Rate (bpm)	61.4 ± 5.2	63 ± 19
Mean Stress Heart Rate Increase	22.6 ± 7.6	86 ± 16
BSA indexed LVEDV (ml/kg/m ²)	101.3 ± 12.3	96.2 ± 35.8
BSA indexed LV Mass (g/kg/m ²)	51.5 ± 7.1	64.3 ± 20.3
LV EF %	57 ± 2	41.7 ± 13.2
RV EF %	54 ± 2	48.8 ± 5.9

29 of 30 sets of volunteer mid-ventricular short axis MOLLI acquisitions were available for final analysis; in one MOLLI an acquisition error prohibited analysis. 10 of 10 patient MOLLI acquisitions were suitable for analysis.

There was a strong positive correlation between ECV calculated following single or split bolus contrast administration in healthy volunteers, as well as abnormal and remote myocardium in patients (coefficient of variability 5.04%; bias 0.009, 95% CI - 3.754 to 3.772, $r^2=0.973$, $p=0.001$). Bland-Altman plot of the data set can be seen in figure 1.

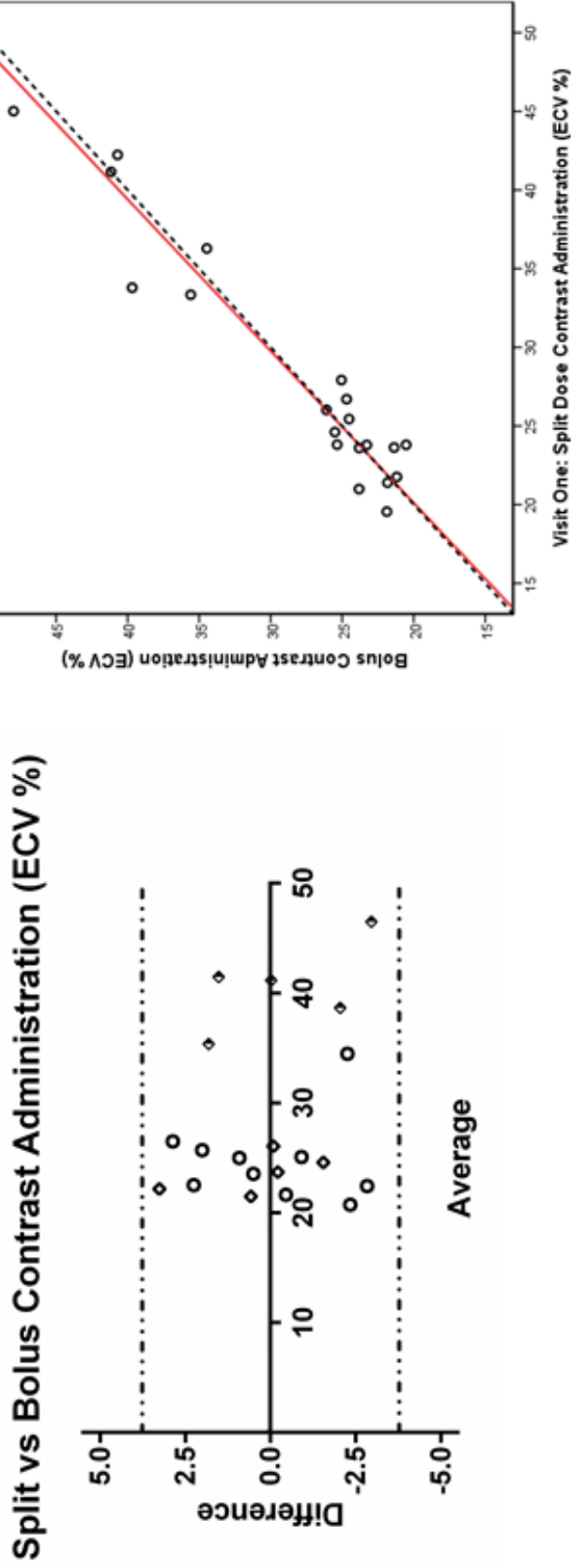


Figure 5.1 Bland Altman and scatter plots of agreement in healthy volunteers and patients between split dose and bolus contrast administration.

Inter-study agreement for ECV calculation with split dose administration visits was good in the 10 volunteers studied (coefficient of variability 5.67%, $r^2 = 0.766$, $p < 0.001$). Bland-Altman plot can be seen in figure 2 (bias -0.018, 95% CI -4.045 to 4.009).

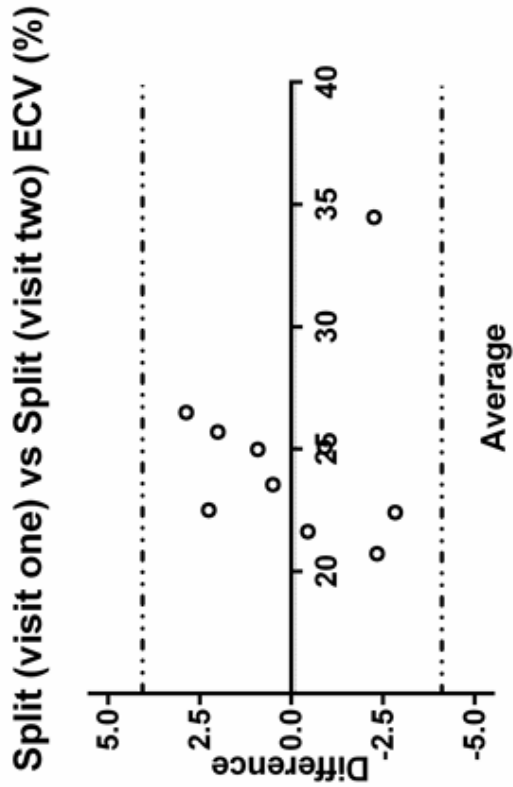
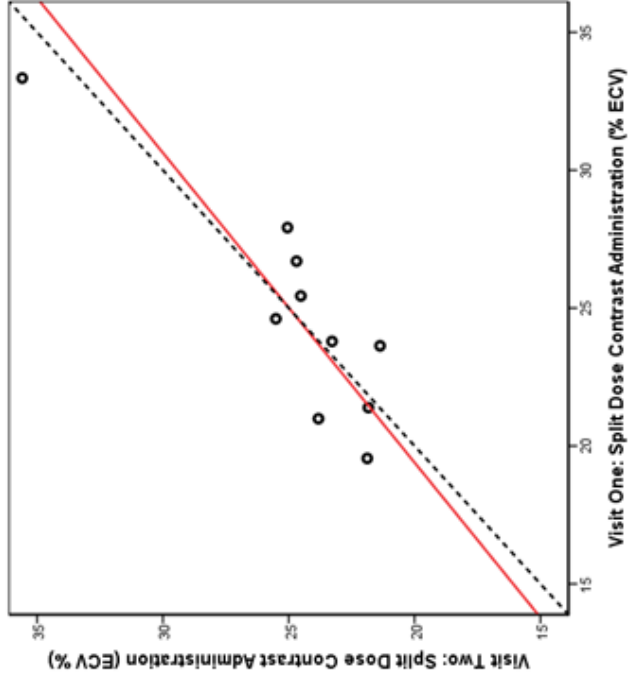


Figure 5.2 Bland-Altman and scatter plots of agreement of ECV estimates between visit 1 and 2 using split-dose administration in healthy volunteers.

(bias -0.018, 95% CI -4.045 to 4.009, $r^2=0.757$, $p=0.001$).

Inter-study agreement for ECV calculation with split dose administration visits was good in the 10 volunteers studied (coefficient of variability 5.67%, $r^2 = 0.766$, $p < 0.001$). Bland-Altman plot can be seen in figure 5.2 (bias -0.018, 95% CI -4.045 to 4.009).

5.5 Discussion

The insights that T1 mapping offers into tissue composition are increasingly applied as a research tool. T1 mapping is also being integrated into clinical protocols, particularly in the investigation of unexplained left ventricular hypertrophy. Consequently, comprehensive CMR protocols that interrogate not only cardiac structure, function and perfusion but also tissue composition in one protocol have great clinical value.

Previous studies have shown that ECV calculated using either an infusion or bolus of contrast agent[16] is reproducible, and correlates well with fibrosis measured on myocardial biopsy specimens[209]. However until this time it was not known if split dose contrast administration, as used in adenosine stress perfusion protocols affects ECV estimation and how this correlates with previously validated methods.

Given that reliable ECV calculation requires steady state of contrast agent concentration, split dose administration may have given rise to different estimates against single bolus administration or a continuous contrast infusion. Any such differences would have prevented the application of established normal ranges that have been published over recent years to subjects undergoing stress perfusion protocols[18, 66, 67, 209, 210]. It was also conceivable that vasodilator effects of adenosine stress may have led to different contrast distribution with the myocardium and peripheral tissues. Persistent vasodilation at the time of the second MOLLI, as a consequence of adenosine administration would lead to a genuine increase of ECV due to increased capillary plasma volume. However the vasodilatory effects of adenosine are both transitory and short-lived[211] and have passed by the time of rest perfusion acquisition and following that, the second MOLLI.

This study has now shown that ECV estimation with split dose contrast administration as part of a stress/rest perfusion CMR protocol agrees well with bolus administration in healthy volunteers. Reproducibility and inter-study agreement was good for split dose ECV calculations and in line with that previously published for ECV calculation following bolus contrast administration[16].

Previously published data suggested that bolus contrast administration may underestimate ECV at values of $>40\%$ [16]. However in this study we have examined 5 patients with extensive LGE enhancement and grossly elevated ECV

due to chronic myocardial infarction and cardiomyopathy and found equivalence between the techniques. This suggests that ECV calculation is reliable across a range of values using either method of contrast administration.

5.6 Limitations

This study was performed in a limited number of healthy volunteers and patients. It has previously been shown that at fifteen minutes contrast equilibrium may not have been reached for post contrast T1 mapping, especially in individuals with higher ECVs[16]. This study has attempted to address this point specifically however no patients with the most elevated ECVs (e.g. cardiac amyloidosis) were studied, due in part to the demands of returning for a non-clinically indicated research CMR study. In spite of this we have not shown difference between these techniques in ECV calculation up to an ECV of 48%. However it should be stressed that the small sample size limits definitive conclusions to be drawn and this study should be repeated in a large cohort incorporating more subjects with myocardial pathology.

This study investigated one particular MOLLI acquisition scheme which is consistent with international recommendations. However, other methods have been published and as yet there is no firm MOLLI scheme recommendation. It is difficult to select the ideal T1 mapping sequence as different sequences may perform differently depending upon the T1 of the tissue studied. Consequently the demonstration of the inter-study reproducibility of locally adopted sequences, in line with SCMR guidance, is important.

5.7 Conclusions

Split dose contrast T1 mapping, in keeping with a stress perfusion protocol, is reproducible and agrees with bolus contrast administration. This suggests ECV measurement maybe incorporated into stress perfusion protocols in both clinical and research CMR studies.

Chapter 6

Athletic Cardiac Adaptation is a Consequence of Increased Myocyte Mass

6.1 Abstract

6.1.1 Objectives

To determine if athletic cardiac remodelling is a consequence of increased myocardial cellular, rather than extracellular mass as measured by cardiovascular magnetic resonance (CMR).

6.1.2 Background

Cardiac remodelling occurs in response to regular athletic training, and the degree of remodelling is associated with fitness. Understanding the myocardial structural changes in athlete's heart (AH) is important to develop tools that differentiate athletic from cardiomyopathic change.

6.1.3 Methods

34 athletes underwent an exercise test to assess maximal aerobic capacity (i.e. VO_{2max}) and comprehensive CMR with native and post-contrast T1 mapping allowing partition co-efficient (λ) and extracellular volume (ECV) calculation.

6.1.4 Results

Participants were divided into tertiles by maximal oxygen uptake (VO_{2max}). Intracellular mass increased with VO_{2max} tertile (83.7±16.7; 101.3±21.4; 110.7±18.0 g; $P<0.01$), though extracellular mass (28.1 ±4.0; 30.1±5.7; 29.8±4.5 g; $P=0.56$) in AH remained static. CMR derived measures of tissue composition (T1, λ , ECV) differed significantly by VO_{2max} tertile, $P=0.05$, 0.03, <0.01 respectively, and were significantly correlated: Native T1 $r= -0.40$, $P= 0.02$; λ $r= -0.42$, $P=0.02$; ECV $r= -0.55$, $P<0.01$. An inverse relationship was seen between LVMi and ECV ($r= -0.56$, $P<0.01$). Indexed LV end diastolic volume (LVEDVi) and mass (LVMi) correlated with VO_{2max} ($r=0.455$, $P=0.01$; $r=0.34$, $P=0.049$).

6.1.5 Conclusions

Increased LV mass in AH occurs as a consequence of an increase in myocyte mass, whilst the extra-cellular mass remains constant. Athletic remodelling, both on a macroscopic and cellular level, is associated with the degree of an individual's fitness. ECV mapping may have a future role in differentiating AH from change secondary to cardiomyopathy.

6.2 Introduction

Regular exercise training places demands on the heart that may lead to cardiac remodelling. The rare, but prominent cases of sudden cardiac death in elite athletes have underlined the importance of understanding the adaptation underlying athlete's heart (AH)[212]. Gaining insights into the remodelling process is important to understand the nature of adaptation, and to develop tools that distinguish AH from cardiomyopathic changes, particularly those of hypertrophic cardiomyopathy (HCM).

Cardiac magnetic resonance (CMR) imaging is particularly well suited to investigate changes of structure and function in athletic cardiac remodelling due to its multi-parametric capabilities, high spatial resolution and lack of ionising radiation. The high reproducibility of the method means that anatomical change can be quantified with greater confidence and smaller sample size than with other methods[61, 213]. Emerging CMR techniques including T1 and extra-cellular volume (ECV) mapping allow the relative volumes of the extra-cellular and intra-cellular myocardial compartments to be quantified. The methods have been used to quantify extracellular expansion (higher ECV & T1) secondary to interstitial fibrosis in cardiomyopathy [67], and conversely increased myocyte volume (lower ECV & T1) in pathologies such as Anderson-Fabry disease[25][210].

In this study we sought to characterise cardiac changes in elite athletes using CMR in order to gain insights into the mechanisms underlying the AH remodelling process. We hypothesised that in athletes, LV hypertrophy occurs secondary to an increase in myocardial cellular mass rather than extracellular volume as measured by CMR and that these adaptations correlate with aerobic capacity (VO_{2max}).

6.3 Methods

Participants were approached via athletic societies and associations and recruitment was open to athletes competing at regional, national and international level. Subjects were eligible for inclusion if they took part in regular competition and trained for a minimum of 6 hours per week. Subjects were excluded if they had contraindications to CMR or systemic medical illness. The study was conducted in accordance with the declaration of Helsinki, and was approved by the local ethics committee (Research and Ethics Committee reference: 14/YH/0126). All volunteers gave informed written consent.

All subjects underwent a CMR study and maximal exercise test, performed on the same day when logistically possible.

6.3.1 Cardiac Magnetic Resonance Protocol

The CMR study was performed at rest prior to exercise testing. All studies were performed on a 3 Tesla Achieva TX system equipped with a 32 channel cardiac phased array receiver coil and multi-transmit technology (Philips Healthcare, Best, The Netherlands). The cardiac long and short axes were determined using standard scout views. Mid LV native (pre-contrast) T1 maps were generated using a previously described MOLLI sequence[214] planned using the 3 of 5 method[208], briefly comprising: ECG triggered 5b(3s)3b MOLLI, flip angle 35° , voxel size of $1.98 \times 1.98 \times 10 \text{ mm}^3$. Left ventricular (LV) mass and volumes were obtained from cine imaging covering the entire LV in the short axis: balanced SSFP, voxel size $1.2 \times 1.2 \times 10 \text{ mm}^3$, no gap, 50 cardiac phases. Right ventricular (RV) and atrial volumes were obtained from a transaxial stack covering the entire heart: balanced SSFP, voxel size $1.7 \times 1.5 \times 5 \text{ mm}^3$, no interslice gap. 0.15 mmol/kg Gadovist (Bayer Schering) was delivered by power injector (Medrad Inc, Warrendale, Pennsylvania, USA) as a single bolus via a venous cannula placed in the ante-cubital fossa, followed by a 20ml saline flush at 5ml/second. Late gadolinium enhancement (LGE) imaging (inversion recovery-prepared T1 weighted gradient echo, inversion time according to Look-Locker scout, TR/TE/flip angle $3.7 \text{ ms}/2.0 \text{ ms}/25^{\circ}$, acquired spatial resolution $1.54 \times 1.75 \times 10 \text{ mm}$) with whole heart coverage was performed seven to ten minutes following contrast administration. Post-contrast T1 maps were acquired using the same MOLLI scheme fifteen minutes after contrast administration.

6.3.2 Image Analysis

All image analysis was performed using cmr⁴² (Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). Volumetric and mass analysis was performed in the standard manner from the short axis stack[215] (LV) or long axis cine images[216] (RV, left and right atria (LA, RA)). Ventricular and atrial measurements were indexed to body surface area (BSA). The presence of focal fibrosis or scar was assessed qualitatively from LGE imaging. T1 values were calculated from source images using manual motion correction, with a region of interest (ROI) placed in the mid infero-septum as per Rogers et al [194]. Partition co-efficient (λ) and ECV were calculated using the formulae:

$$\lambda = \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)}$$

$$ECV = (1 - Hct) \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)}$$

**Where R1=1/T1 and Hct is Haematocrit.*

Myocyte and extra cellular mass were calculated using the formulae: Myocyte mass= LV mass * (100 - % ECV); Extracellular mass = LV mass * %ECV. All T1, ECV, volumetric and mass analysis was performed by two observers (AKM, BE) blinded to all subject data including sporting discipline and aerobic capacity.

6.3.3 Exercise Protocol

Participants were instructed to arrive rested (no strenuous exercise in the preceding 24 hr) having abstained from any alcohol (preceding 24 hr), food and caffeine (preceding 3 hr) ingestion. To determine maximal oxygen uptake (VO_{2max}) and anaerobic threshold (AT) participants undertook a ramp-incremental (RI) step-exercise (SE)[217], i.e. RISE test on an electronically-braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, the Netherlands), which allows for confirmation of VO_{2max} in a single test[217]. Participants wore a nose-clip and breathed through a low-dead space, low-resistance mouthpiece which was connected to a bi-directional pitot tube flow sensor and gas sample line assembly, allowing for breath-by-breath measurement of gas volumes and concentrations (O_2 , Galvanic; CO_2 infrared), and subsequent calculation of ventilatory and pulmonary gas exchange variables (Cardio2, Medical Graphics Corporation, St Paul, MN, USA). Prior to each test, the pitot tube flow sensor was calibrated over a range of flow rates using a 3 l syringe, while the gas analysers were calibrated using precision gases that spanned the inspired and expired physiological range. A 12-lead electrocardiogram was monitored throughout, and heart rate (HR) was measured from the R-R interval. The RISE test was preceded by rest period (~ 2 minutes) and unloaded cycling (20W) (~ 4 minutes), with these phases continued until a steady state was attained, after which work rate increased as a linear function of time at a rate of 20-30W/min (depending upon reported training history), with the intention of bringing participants to the limit of tolerance in ~10-12 min [218]. The RI was then followed by 5 min of active recovery (20W) after which a SE was performed at 95% of the RI work rate peak, with this SE also continued to the limit of tolerance. In both RI and SE parts of the test, the limit of tolerance was defined as the point at which cycling cadence fell below 50 rpm despite strong verbal encouragement.

Breath-by-breath data were edited using the VO_2 response to eliminate erroneous breaths (occurring outside the local mean 99% prediction limits), that were considered unphysiological [219]. AT was then estimated using the V-slope method

[220], and supporting ventilatory and pulmonary gas exchange criteria (i.e. the fractional end-tidal concentrations of O₂ and CO₂, and the ventilatory equivalents for O₂ and CO₂[221]). VO_{2peak} was identified in both RI and SE phases as the highest 12-breath rolling average (highest mean VO₂ over ~15-20 s), with this representing an appropriate sampling duration to balance identifying VO_{2peak} in the presence of breath-by-breath noise, and including data from the transient phase of the response leading to VO_{2peak} [217]. Within participants, the highest 12-breath rolling average from RI and SE phases were then compared using unpaired t-tests, with no difference (p> 0.05) between RI and SE VO_{2peak}, and thus the attainment of VO_{2max} confirmed in each test[222].

6.3.4 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0 (IBM Corp., Armonk, NY). Participants were ranked and then split in tertiles according to VO_{2max}. Unless otherwise stated the results are presented as mean ± standard deviation (SD). Differences between groups were assessed using the Chi-squared test with four degrees of freedom, or one-way ANOVA when appropriate. Post hoc analysis was performed with Tukey's HSD test. Normality of distribution was determined with Kolmogorov-Smirnov testing. Correlation was assessed with Pearson's correlation co-efficient. Significance for all tests was assumed with p <0.05. Univariable analyses were performed to identify predictors of athletic cardiac remodelling. Variables with a probability value of <0.1 in the univariable analysis were included in a multivariable analysis, based on an enter linear regression model.

6.4 Results

6.4.1 Study participant characteristics

34 athletes (26 male : 8 female) between 20 and 45 years of age (mean age 30.9 ± 7.2 yrs, height 178.5 ± 8.8 cm , weight 71.2 ± 10.5 kg) were prospectively recruited and underwent CMR study and maximal exercise testing, separated by a median of 0 days (inter-quartile range 0 – 0). Athletic disciplines of participants: 6 running; 15 cycling; 13 triathlon (table 6.1). Mean haematocrit was 0.45 ± 0.03 g/dL (by tertile from lowest to highest VO_{2max}: 0.43 ± 0.03; 0.46 ± 0.03; 0.47 ± 0.03 g/dL; p= 0.02).

Table 6.1 Subject characteristics.

	Overall	Lowest VO _{2max} Tertile (n=11)	Middle VO _{2max} Tertile (n=12)	Highest VO _{2max} Tertile (n=11)	P
Age (years)	30.9 ± 7.2	33.9 ± 6.9	30.7 ± 7.5	28.0 ± 6.5	0.16
Gender	26 M: 8F	5:6	11:1	10:1	0.01
BMI	22.5	23.4 ± 2.7	22.4 ± 1.9	21.6 ± 2.1	0.20
Haematocrit (g/dL)	0.45	0.43 ± 0.03	0.46 ± 0.03	0.47 ± 0.03	0.02
Hours training per week	11.5 ± 3.7	12.7 ± 3.7	10.0 ± 3.4	12.0 ± 3.7	0.19
Number of years training at >6 hrs per week	8.7 ± 5.8	10.0 ± 7.2	8.4 ± 4.9	7.5 ± 5.3	0.60
Sporting discipline					
<i>Running</i>	6	2	2	2	0.67
<i>Cycling</i>	15	4	5	7	
<i>Triathlon</i>	13	5	5	2	

6.4.2 Cardio-Pulmonary Exercise Testing

Subjects were divided into tertiles by VO_{2max} (n=11, n=12, n=11). Mean VO_{2max} by tertile was: 50.0 ± 2.9; 59.6 ± 1.6; 67.7 ± 6.3 mL O₂/min/kg (p < 0.01 by definition). AT, as a percentage of VO_{2max}, did not differ by tertile: 61.1% ± 8.8; 65.3% ± 5.6; 61.0% ± 8.0; p=0.31). Neither resting heart rate (59 ± 9; 54 ± 6; 52 ± 11 beats/min; p=0.17) nor maximal heart rate differed between groups (176 ± 8; 183 ± 11; 184 ± 12 beats/min; p= 0.20). However Δ HR (difference between resting and peak HR) was significantly different between tertiles (117 ± 11; 129 ± 8; 132 ± 10 beats/min; p < 0.01) (Table 6.2).

Table 6.2 Cardiopulmonary exercise testing

	Lowest $\dot{V}O_{2max}$ Tertile (n=11)	Middle $\dot{V}O_{2max}$ Tertile (n=12)	Highest $\dot{V}O_{2max}$ Tertile (n=11)	P
Resting HR (beats/min)	59.0 ± 8.8	53.9 ± 5.5	52.0 ± 11.2	0.17
Maximal HR (beats/min)	176.3 ± 8.1	183.0 ± 10.9	183.6 ± 11.7	0.20
Δ HR (beats/min)	117.3 ± 11.0	129.1 ± 8.3	131.6 ± 9.8	<0.01
Relative $\dot{V}O_{2max}$ (mL O_2 /min/kg)	50.0 ± 2.9	59.6 ± 1.6	67.7 ± 6.3	
Anaerobic threshold as % $\dot{V}O_{2max}$	61.1 ± 8.8	65.3 ± 5.6	61.0 ± 8.0	0.31
Peak Work Rate (W)	324.9 ± 44.6	388.0 ± 59.7	440.7 ± 63.1	0.01

6.4.3 CMR Findings

Typical findings may be seen in figure 6.1, and full CMR data may be seen in table 6.3.

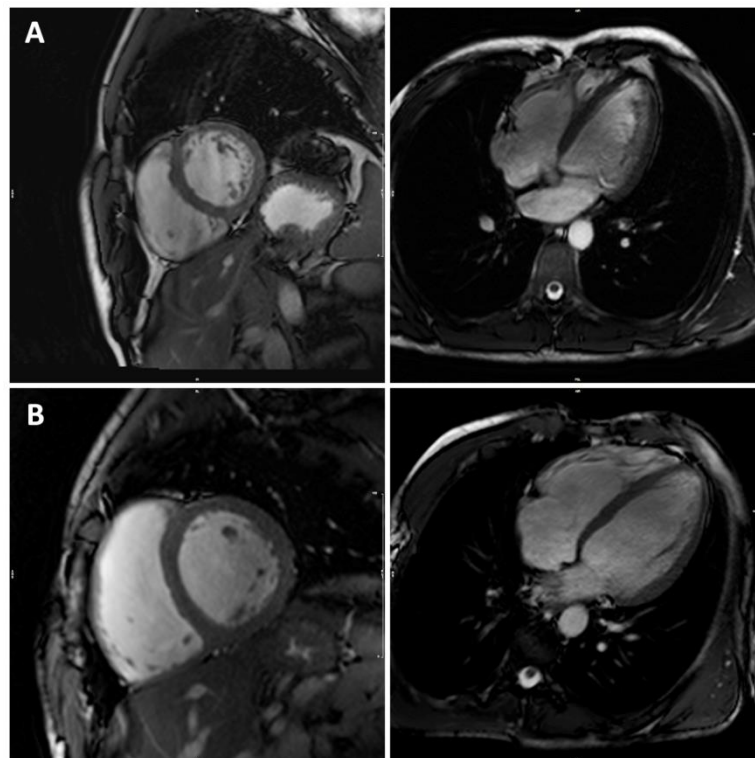


Figure 6.1 Cardiac Remodelling in athletic cardiac adaptation

a) mid left ventricular short axis and horizontal long axis CMR views of athletes in the lowest tertile, and highest tertile (b), demonstrating more pronounced remodelling in the athlete with higher $\dot{V}O_{2max}$.

Table 6.3 Cardiac MRI findings

Cardiac Geometry	Lowest VO _{2max} Tertile (n=11)	Middle VO _{2max} Tertile (n=12)	Highest VO _{2max} Tertile (n=11)	P
<i>Left Ventricle</i>				
LVEDVi (ml/m ²)	110.9 ± 13.3	117.2 ± 15.9	121.6 ± 13.4	0.30
LV Mass (g)	111.8 ± 19.7	131.3 ± 25.8	140 ± 21.7	0.02
<i>Cellular Mass (g)</i>	83.7 ± 16.7	101.3 ± 21.4	110.7 ± 18.0	<0.01
<i>Extra-Cellular Mass (g)</i>	28.1 ± 4.0	30.1 ± 5.7	29.8 ± 4.5	0.56
LVM indexed (g/m ²)	63.4 ± 10.9	67.4 ± 10.0	75.1 ± 8.0	0.03
<i>Indexed Cellular Mass (g)</i>	47.5 ± 9.5	51.9 ± 8.4	59.1 ± 6.9	<0.01
<i>Indexed Extra-Cellular Mass (g)</i>	15.9 ± 1.9	15.5 ± 2.5	16.0 ± 1.8	0.83
LVEDV/Mass ratio	1.8 ± 0.2	1.8 ± 0.3	1.6 ± 0.2	0.25
LVEF (%)	56.6 ± 4.4	54.2 ± 4.8	57.0 ± 3.8	0.27
<i>Right Ventricle</i>				
RVEDVi (ml/m ²)	106.6 ± 14.7	119.3 ± 24.3	123 ± 16.9	0.48
RVEF (%)	52.2 ± 3.5	53.3 ± 4.9	54.0 ± 5.4	0.67
<i>Atrial Size</i>				
LA Volume index (ml/m ²)	93.3 ± 11.7	101.7 ± 22.2	105.6 ± 14.5	0.40
RA Volume index (ml/m ²)	141.3 ± 29.1	147 ± 37.4	144.4 ± 28.4	0.90
<i>Tissue Composition</i>				
Individuals displaying LGE	0	0	1	0.34
Native T1 (ms)	1205.5 ± 42.3	1179.0 ± 35.9	1166.7 ± 27.6	0.05
Partition co-efficient	0.45 ± 0.04	0.42 ± 0.05	0.40 ± 0.03	0.05
ECV (%)	25.4 ± 2.6	23.1 ± 2.8	21.4 ± 2.0	<0.01

Examination of the extra-cellular and intra-cellular compartments demonstrated that the difference observed in overall myocardial mass in higher performing athletes was due to increased cellular mass (83.7 ± 16.7; 101.3 ± 21.4; 110.7g ± 18.0 g; p<0.01), rather than expansion of the extra-cellular space, which remained constant (28.1 ± 4.0; 30.1 ± 5.7; 29.8 ± 4.5 g; p=0.56) (Figure 6.2).

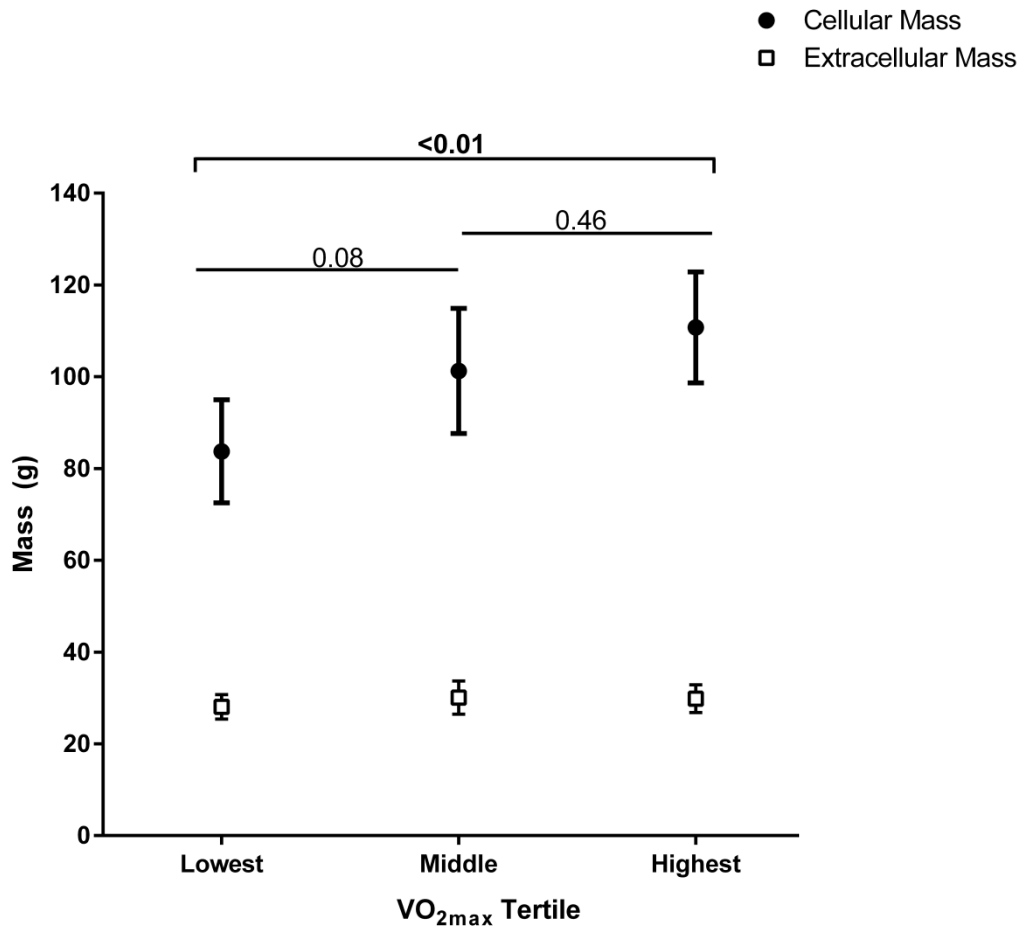


Figure 6.2 Mass of the cellular and extra-cellular myocardial compartments by VO_{2max} tertile.

Extra-cellular mass is static ($p=0.561$), whilst cellular mass increases with VO_{2max} ($p=0.007$) (error bars represent 95% CI). Inter-group differences with post hoc Tukey correction are as displayed.

CMR derived measures of tissue composition correlated significantly with VO_{2max}: Native T1 $r= -0.40$, $p= 0.02$; partition co-efficient $r= -0.42$, $p= 0.02$; ECV $r= -0.55$, $p<0.01$ (Figure 3).

When analysed by VO_{2max} tertile these changes remained significant: Native T1: 1205.5 ± 42.3 ; $1179.0\text{ms} \pm 35.9$; $1166.7\text{ms} \pm 27.6$; $p= 0.05$. Partition co-efficient: 0.45 ± 0.04 ; 0.42 ± 0.05 ; 0.40 ± 0.03 ; $p=0.05$. ECV (%): 25.4 ± 2.6 ; 23.1 ± 2.8 ; 21.4 ± 2.0 ; $p <0.01$ (Figure 4).

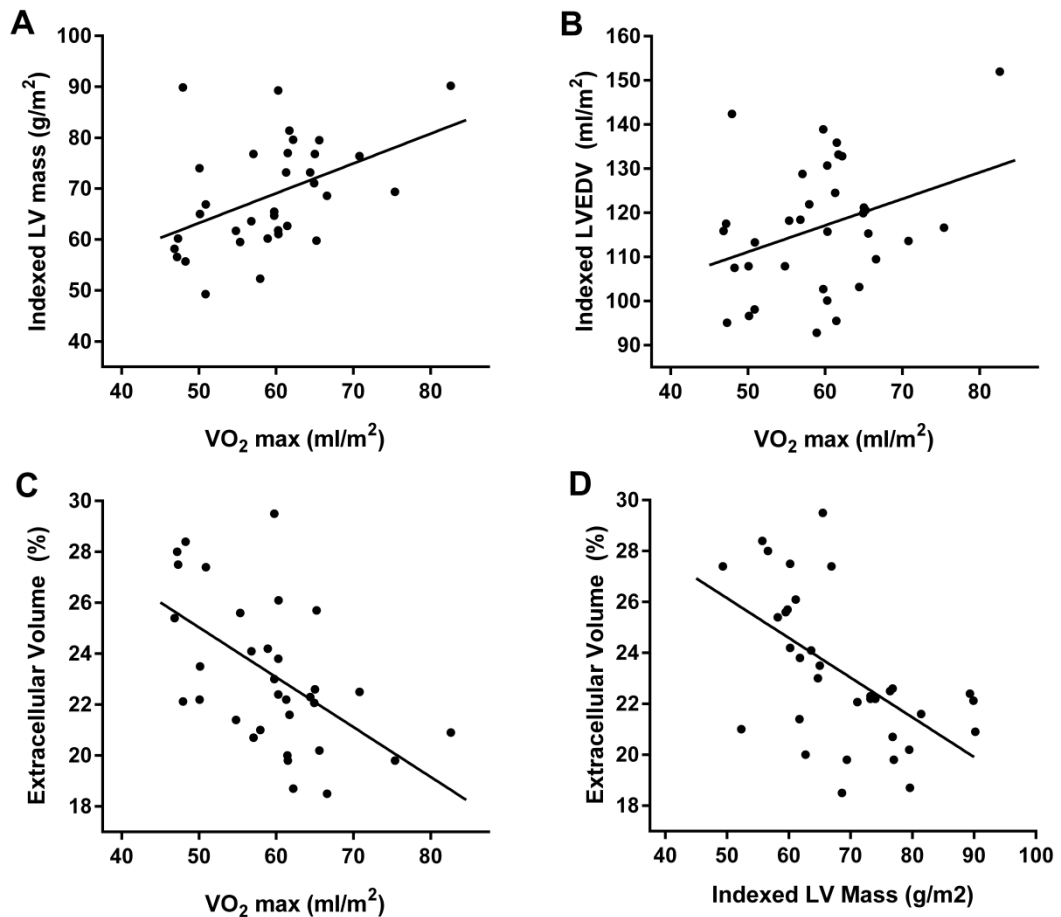


Figure 6.3 Relationship between VO_{2max} (mL O_2 /min/kg) and indices of cardiac remodelling

A) Indexed LV mass and VO_{2max} $r=0.46$, $p=0.01$; B) Indexed LVEDV and VO_{2max} $r=0.34$, $p=0.049$; C) Extracellular Volume and VO_{2max} -0.55 , $p<0.01$; D) Extracellular Volume and Indexed LV mass $r=-0.56$, $p<0.01$.

Both LVMi and LVEDVi correlated significantly with VO_{2max} ($r=0.455$, $p=0.01$; $r=0.34$, $p=0.049$). A significant inverse relationship was seen between ECV and indexed LV mass ($r= -0.56$, $p<0.01$) (Figure 6.3), and LAi correlated with LVEDVi ($r=0.47$, $p<0.01$). Indexed LV mass differed significantly by VO_{2max} tertile (63.4 ± 10.9 ; 67.4 ± 10.0 ; 75.1 ± 8.0 g/m²; $p=0.03$) (Figure 6.3). No other measures of cardiac size or function were significantly correlated.

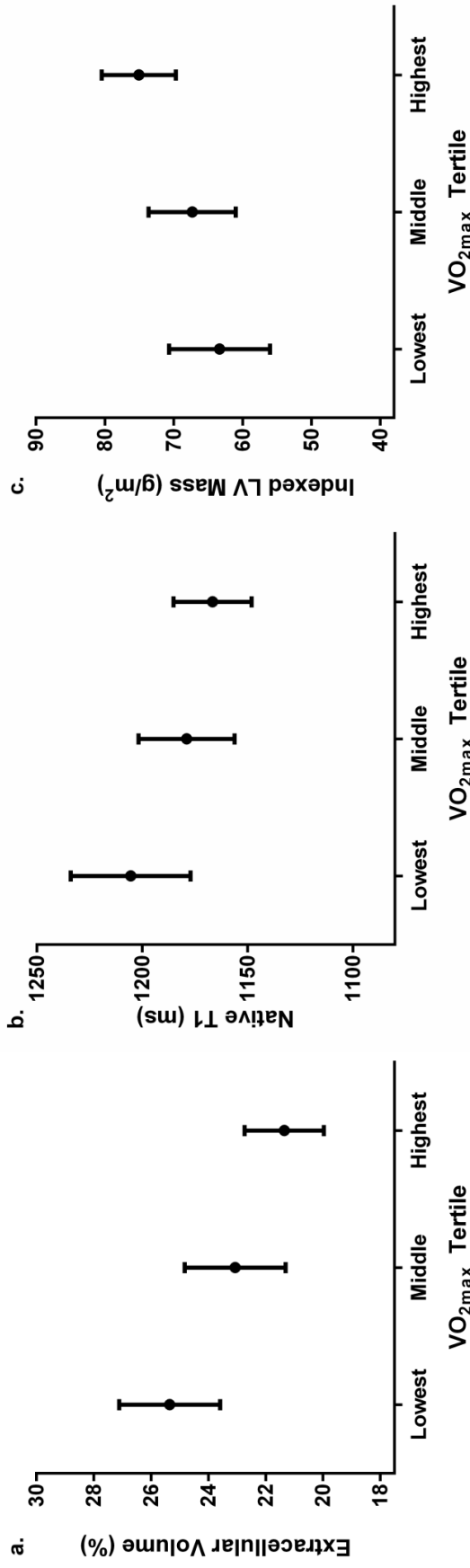


Figure 6.4 Selected Indices of cardiac remodelling by performance tertile

a) Extracellular volume fraction (%) $p < 0.01$; b) native T1 (ms) $p = 0.05$; c) Indexed LV mass $p = 0.03$ (error bars represent 95% CI). Inter-group differences with post hoc Tukey correction are as displayed.

6.4.4 Regression Analysis

On multivariate linear regression analysis increased cellular mass indexed to BSA was only significantly influenced by $\dot{V}O_{2max}$ (Beta=0.451, p=0.015), and not sex (Beta=-0.490, P= 0.109), weight (Beta=0.187, p=0.401) or haematocrit (Beta= -0.324, P=0.156) (table 6.4).

Table 6.4 Multivariate regression analysis of cellular mass (BSA indexed)

	Univariable		Multivariable	
	Beta	P	Beta	P
Age	-0.24	0.17		
Sex	-0.56	0.00	-0.490	0.109
Height	-0.149	0.400		
Weight	0.360	0.037	0.187	0.401
Discipline	0.08	0.64		
Hours of training	0.03	0.88		
$\dot{V}O_{2max}$	0.51	0.00	0.451	0.015
Hct	0.343	0.047	-0.324	0.156

6.5 Discussion

This study shows that left ventricular hypertrophy in AH occurs as a consequence of expansion of myocyte volume alone, a finding that provides novel insight into the physiological change underpinning a poorly understood phenomenon. We have further demonstrated that myocyte volume expansion is linearly related to aerobic capacity, and that there is no expansion of the extracellular space in athletic cardiac adaptation.

6.5.1 Mechanism of Myocardial Adaption & Remodelling

Cardiovascular adaptation occurs in response to haemodynamic challenge and prolonged endurance training and even in previously untrained individuals leads to marked changes in cardiac geometry[223]. Early adaption is characterised by an increase in LV mass and as a consequence change in LV mass: volume ratio, later followed by LV dilation, normalisation of the ratio and ‘eccentric hypertrophy’[223]. LV geometry and particularly LV mass have previously been shown to be related to $\dot{V}O_{2max}$ [224] [225] [226].

CMR has provided new insights into the mechanisms that underpin cardiac remodelling with exercise training. The multi-parametric assessment of the human heart and high reproducibility provided by the technique allow both accurate functional and anatomical assessment [16, 227][13]. Myocardial native T1, partition co-efficient and ECV measurement are robust and validated techniques for tissue characterisation[228], and the correlation of these parameters with histological tissue specimens is excellent[16, 227, 229]. T1 and ECV increase in myocardial fibrosis, oedema and expansion of the extracellular space, with subsequent relative decrease in myocyte mass[230]. Conversely, an expanded cellular mass reduces T1 and ECV as the distribution volume for conventional *extracellular* contrast agents is reduced.

In this study we have demonstrated that indexed LV mass is correlated with aerobic capacity, and that indexed LV mass and ECV are inversely related. Participants with a higher VO_{2max} had a similar extracellular mass as those with a lower VO_{2max} , but a significantly higher intracellular mass.

These data allow postulation of the mechanism underlying the development of AH that is consistent with known concepts: Following rapid division in foetal life, cardiac myocytes are terminally differentiated shortly after delivery. As a result, any increase in overall myocardial mass is secondary to myocyte hypertrophy or ECM expansion rather than cell hyperplasia[231]. CMR allows *in vivo* quantification of the two tissue compartments and our data show that in AH the overall extracellular compartment volume is similar to previously reported normal ranges [24], whilst there is marked cellular expansion.

Important differential diagnoses of AH include DCM and HCM, which may both display increased LV mass or LVEDV. The role of CMR in the detection of these myocardial diseases is established, and both DCM and HCM display characteristic morphological abnormalities on CMR. At a microscopic level HCM is characterised by myocyte disarray and interstitial fibrosis[163], and thus increased ECV. LGE CMR allows detection of focal replacement fibrosis and infarction in both HCM[232] and DCM[233], however diffuse processes are poorly detected with this technique. T1 mapping and ECV measurement have shown expansion of the extracellular space in HCM, occurring in both hypertrophic[24][16] and non-hypertrophic segments as well as in DCM[234][235]. Our observation that hypertrophy in AH is cellular without ECV expansion suggests that T1 mapping CMR may be an ideal tool to distinguish between athletic left ventricular adaptation and pathological hypertrophy or remodelling.

Both 'side by side' sarcomere addition in concentric remodelling, and 'end to end' addition in eccentric remodelling result in myocardial hypertrophy[236], with an

increase of muscle mass without increase in myocyte number[237]. In this study, conducted without tissue biopsy, we are unable to determine the intra-cellular sarcomere arrangement; however other CMR techniques that allow myocyte size to be quantified, as investigated by Coelho-Filho *et al*[238], may allow this to be determined in the future.

6.5.2 Late Gadolinium Enhancement & Myocardial Scar

LGE has previously been demonstrated in up to 13% of elite[239] and 50% of veteran athletes[240]. In this cohort of athletes, LGE was only displayed in one (3%), and in a myocarditis pattern rather than the typical sites of the RV insertion point or the interventricular septum. This may be as a consequence of the relatively young age of athletes studied.

6.5.3 Remodelling & Relationship with Performance

The relationship between aerobic capacity and LV remodelling is known[225][224]. In this study we have demonstrated that LVEDVi and LVMI correlate with VO_{2max} , confirming that the degree of remodelling is related to fitness. Indexed atrial volumes are not related to fitness, but as shown previously correlate with LVEDVi in athletes[241], suggesting that atrial conduit function[242], as a result of superior ventricular diastolic performance[243], is enhanced in AH.

6.6 Limitations

There was relatively little diversity in the range of athletic discipline pursued, however this has previously been shown not to affect the phenotype of cardiac remodelling[244]. Furthermore, the assumptions made regarding the mode of myocardial hypertrophy in AH in this study have not been validated by histological sample. Haematocrit, which is used in the calculation of ECV, differed between groups of athletes and may thus have introduced a bias in the results. However, native T1 and partition co-efficient, which are independent of haematocrit, also differed significantly by VO_{2max} tertile. Genotyping was not performed in any of the participants to exclude common gene mutations associated with HCM, consequently it is possible that participants may have had subclinical HCM. However no subjects displayed any characteristic resting abnormalities associated with HCM on cine or LGE imaging.

Finally, the participants were all of white origin and this study should be repeated in a range of ethnic origins in an effort to understand AH in all elite athletes, as risk and remodelling may differ between ethnicities[245].

6.7 Conclusions

Cardiac remodelling and LVH in AH is a consequence of increased myocyte mass alone, without expansion of the extra-cellular space as measured by CMR. This is unlike HCM or DCM, where CMR tissue characterisation detects expansion of the extra-cellular space, in the presence or absence of LVH. Athletic remodelling, both on a macroscopic and microscopic level is associated with the degree of aerobic capacity. T1 and ECV mapping by CMR may have a future role in differentiating AH from change secondary to cardiomyopathy, especially HCM.

Chapter 7

Preliminary Report, 'Effects of aldosterone antagonism in heart failure with preserved ejection fraction: a cardiac MRI, exercise physiology and quality of life pilot study'

7.1 Abstract

7.1.1 Objectives

To study the effect of spironolactone in heart failure with preserved ejection fraction on the myocardial extracellular volume as measured with CMR T1 mapping.

7.1.2 Background

Treatment of heart failure with preserved ejection fraction (HF-PEF) lags behind that of reduced ejection fraction (HF-REF). Aldosterone antagonists have promise in this condition and been shown to improve left ventricular filling and morbidity. In this study we investigate the mode of action and tissue effects of spironolactone in HF-PEF.

7.1.4 Methods

60 patients meeting European Society of Cardiology (ESC) criteria for the diagnosis of HF-PEF, including elevated NT-proBNP, are to be randomised 1:1 to open label six months of oral Spironolactone 25mg once daily or monitoring only. At the time of writing 30 have been recruited. A comprehensive multi-modality assessment comprising cardiac magnetic resonance (CMR), echocardiography, ambulatory blood pressure monitoring, maximal exercise test and quality of life(QoL) assessment were conducted at enrolment and at six months. The primary outcome was change in myocardial extracellular volume (ECV) as measured by CMR T1 mapping.

7.1.5 Results

30 patients (15 male: 15 female, mean age 74.2 ± 6.9) were recruited at the time of writing and 15 subjects have completed the follow-up period (7 spironolactone, 8 monitoring). The groups were well matched with the exception of native T1 (1200.4 ± 95.9 ms vs 1267.8 ± 50.4 ms, $p=0.032$). No baseline parameters correlated with ECV. After 6 months Δ ECV of $-1.8 \pm 3.9\%$ versus $+2.3 \pm 3.2\%$ ($p=0.041$) was seen with spironolactone vs monitoring. LV mass decreased with spironolactone (-11.0 ± 15.4 g vs $+6.8 \pm 13.4$ g $p=0.032$), Δ LV mass correlated with Δ ECV ($r=0.573$, $p=0.026$). No significant change was seen in echocardiographic, exercise or QoL parameter.

7.1.6 Conclusions

At this point the study is not powered to comment definitively upon the primary end-point. However treatment with spironolactone led to a reduction in LV mass is in keeping with previous studies. Spironolactone also resulted in a significant reduction in ECV: The interaction between ECV and LV mass point toward reduction in myocardial fibrosis as a possible mode of action of spironolactone in HF-PEF. This effect should become clearer upon study completion.

Note for the reader

The study is ongoing and recruitment is incomplete, as a result interpretation is limited.

Furthermore, some of the planned analysis cannot be performed until the follow-up period of all subjects is complete including analysis of stored blood samples, therefore the following should be read bearing in mind the following limitations:

1. NT-proBNP & blood biochemistry: NT-proBNP and serum biochemistry performed prior to study enrolment will be presented. This is a clinical assay and predates study entry.
2. Markers of collagen turnover & renin-aldosterone system activation: the relationship between myocardial fibrosis measured with CMR and circulating markers of collagen turnover and renin-aldosterone levels cannot be presented, analysis will be performed once all participants have completed the study period.

7.2 Introduction

7.2.1 Background

Heart failure is a clinical syndrome defined by the presence of symptoms, including decreased exercise tolerance and breathlessness, and signs such as elevated jugular venous pressure (JVP), pulmonary crackles and peripheral oedema[246]. Approximately half of all patients with a diagnosis of heart failure have preserved systolic function (HF-PEF)[247], and may affect between 1-5% of the general population [248]. Unlike heart failure with reduced ejection fraction (HF-REF), where multiple pharmacological, device and surgical interventions have changed prognosis[11], doctors are currently unable to offer disease modifying therapy to individuals with HF-PEF[11]. This is despite these patients having many shared clinical and physiological manifestations of disease[249] and poor prognosis[250, 251].

The prevalence of HF-PEF rises with age and is nearly twice as high in women than men[252]. Compared with systolic heart failure patients with HF-PEF have increased rates of atrial fibrillation, hypertension and obesity but lower rates of coronary disease. With an aging population in the developed world HF-PEF is likely to become an increasing problem with far reaching affects on health expenditure, morbidity and mortality.

7.2.2 Pathophysiology

Symptoms in HF-PEF arise due to elevated left atrial pressure, as a consequence of elevated left ventricular filling pressures due to intrinsic abnormalities of left ventricular relaxation and stiffness. Left atrial function, ventricular-arterial coupling, heart rate variability, neuro-hormonal activation and peripheral abnormalities are also important in the development of symptoms.

The key determinants of stiffness are the extra-cellular matrix (ECM) and cardiac myocytes, though abnormalities of both are not seen in all patients with HF-PEF [253]. Diastolic function may be thought to be both active, and related to cross-bridge detachment and calcium handling, and passive, related to the mechanical properties of the myocardium.

Passive: The Collagen types I, III & IV are found in the myocardial extra-cellular matrix. Passive stiffness is predominantly determined by the amount of collagen type I relative to collagen type III. Collagen synthesis is regulated by many factors including sympathetic activation, neuro-hormonal activation, preload, after-load and cardiac inflammation[159][254]. Collagen type I deposition has been

found to be elevated in HF-PEF due to down regulation of collagen degradation and up regulation of synthesis [255]. Studies in humans have demonstrated that myocardial fibrosis is related to both myocardial relaxation and contraction [135].

Active: Both calcium handling and myocyte stiffness are abnormal in HF-PEF. Active myocyte stiffness is thought to be related to changes in titin, a cytoskeletal protein and changes in its phosphorylation state [256]. Myocardial relaxation is dependent upon sarcoplasmic reuptake of calcium and cross bridge detachment. Relaxation, and cross bridge decoupling, is an active, energy intense process and myocardial energy reserves are lower in HF-PEF [257].

The resultant abnormality of active and passive stiffness is diastolic dysfunction. Diastolic dysfunction is defined as 'the inability to fill the ventricle to an adequate preload volume at acceptably low pressures' [258]. Active and passive myocardial relaxation abnormalities result in slowing and prolongation of left ventricular filling which is especially important on exertion. On exertion the available ventricular filling time shortens and exercise induced abnormalities of left ventricular untwisting develop, in turn leading to a marked rise in left atrial (LA) pressure [259][260].

In health approximately 80% of left ventricular filling occurs in early diastole due to rapid myocardial untwisting and left ventricular suction. Impairment of myocardial relaxation decreases early LV filling, with a subsequent increase in the importance of the active atrial contribution [261]. Loss of normal LA function is an important factor in the development of symptoms in HF-PEF, with the presence of heart failure symptoms predicted by reduced LA strain and increased LA stiffness in patients with similar LVH and LA size [262]. The development of atrial fibrillation (AF), as a consequence of LA dilation and remodelling, is a sign of advanced HF-PEF and associated with more fibrosis, necrosis and neurohormonal activation [263]. Chronic elevation of LA pressure may also lead to the development of pulmonary hypertension and subsequent right ventricular dysfunction.

Abnormalities of systolic function are found in HF-PEF potentially reflecting an early myocardial process resulting in a progressive decline of systolic performance [264]. Systolic abnormalities of longitudinal and radial contractile function in HF-PEF may be measured with tissue Doppler and strain assessment.

Multiple peripheral and central abnormalities lead to an inability to increase cardiac output in HF-PEF, with subsequent development of exertional symptoms. Loss of atrial contribution to LV filling in AF, seen in more severe HF-PEF, leads to marked impairment of exercise capacity with marked loss of cardiac efficiency [263]. In addition minor abnormalities of systolic performance are amplified on exertion

as stroke volume fails to increase as in health[265], and when occurring with the chronotropic incompetence often seen in HF-PEF leads to a failure to augment cardiac output[266]. In addition increased afterload on exercise, and diminished preload reserve due in part to increased aortic stiffness, leads to unfavourable ventricular-coupling and exercise limitation[267][268].

7.2.3 Diagnosis

The European Society of Cardiology (ESC) state that a diagnosis of HF-PEF may be made when there are signs and symptoms present compatible with heart failure, normal or mildly reduced left ventricular ejection fraction and associated structural cardiac abnormalities including left ventricular hypertrophy (LVH), left atrial dilation (LA) and or diastolic dysfunction[246].

7.2.3.1 Non-invasive

7.2.3.1.1 Echocardiography

The primary function of echocardiography in the diagnosis HF-PEF is to confirm that systolic function is normal, there are structural abnormalities present in keeping with HF-PEF and in excluding other cardiac causes of heart failure. The addition of continuous wave Doppler, tissue Doppler and echo strain imaging allows the assessment of diastolic function.

For a diagnosis of HF-PEF to be made the LVEF must be >50% on echo, with an indexed of LVEDV <97ml/m²[246]. There are multiple echocardiographic measures that predict LV filling pressure and diastolic function, although all are not in routine clinical practice. Continuous wave Doppler (CW) measurement of mitral inflow allows assessment of LV relation and LV filling pressure with use of the E/A ratio as well as the E wave deceleration time, however this technique is more prone to variation due to fluid status than tissue Doppler imaging (TDI)[269]. Decreased septal and mitral lateral wall diastolic velocities (E') are seen in HF-PEF, and if tissue relaxation is normal a diagnosis of HF-PEF is unlikely. TDI measurement of myocardial systolic and diastolic velocities either alone, or in combination with mitral inflow CW Doppler identifies raised LV filling pressures [269], though accuracy is limited whilst patients are decompensated[270]. Measurement of diastolic indices on exertion may unmask previously minor diastolic abnormalities. When myocardial relaxation (E') is abnormal it fails to increase in proportion to mitral inflow (E), leading to elevation of the E'/E ratio and LV filling pressure[271].

Echo strain imaging allows the detection and quantification of subtle motion abnormalities and strain rate, a parameter that predicts LV filling pressure better

than E/E' [272]. Diastolic strain rate has been shown to correlate with LV relaxation [272], as well as interstitial fibrosis in animal models[273], suggesting potential use in HF-PEF patients.

7.2.3.1.2 CMR

Cardiac magnetic resonance imaging (CMR) provides excellent image quality without the use of ionising radiation and is well suited to the assessment of LV function, mass and atrial volumes. As well as volumetric data grid-tags are well established in the assessment of radial, longitudinal or circumferential LV deformation. Systolic and diastolic strain, strain rate and torsion are all indices of systolic and diastolic function useful in the diagnosis of HF-PEF, although the availability and clinical application of tagging quantification is not widespread[274-276].

The use of gadolinium containing contrast agents in CMR allows for focal myocardial scar[144] and fibrotic[277] processes to be delineated with late gadolinium enhancement (LGE) imaging. However LGE is limited in diffuse myopathic processes as a contrast between 'normal' and abnormal tissue is required to detect pathology.

Extra-cellular volume fraction (ECV), measured by CMR T1 mapping, allows measurement of the myocardial interstitial expansion without biopsy; allowing the relationship between ECV expansion, diastolic function and outcomes in HF-PEF to be investigated[278][279].

7.2.3.2 Invasive

7.2.3.2.1 Cardiac Catheterisation

LV end diastolic pressure (LVEDP) may either be measured from direct measurement at left heart catheterisation or assumed from pulmonary capillary wedge pressure (PCWP) at the time of right heart catheterisation. LVEDP or PCWP of >16 or >12 mmHg are respectively diagnostic of diastolic dysfunction in the presence of a normal LVEDI[280], on occasions that resting haemodynamics are normal exercise right heart catheterisation may be performed and reveal abnormal LV filling[260]. Pressure volume measurement readily detect abnormalities in LV filling and ventricular-arterial coupling, though they are not routinely applied in clinical practice[281][282].

7.2.3.2.2 Biomarker Measurement

The use of B-type natriuretic peptides (BNP and NT-proBNP) is well established in the diagnosis of heart failure, with both high negative and positive predictive value[283]. BNP levels are a strong predictor of outcome in both HF-PEF and

systolic HF[284, 285]. BNP/NT-proBNP above the trial median value has persistently been associated with worse outcomes in a number of randomised controlled trials[286, 287]. There are a number of situations where BNP values alone may be misleading, though when combined with echocardiographic indices test performance improves[288]. 'False positive' BNP measurement is associated with atrial fibrillation, increasing age and female gender, whilst 'false negative' measurements are seen in obesity and fluctuating atrial pressure elevation. The prevalence of the previous factors in the HF-PEF population mean that the development of other, more specific biomarkers, is important.

Alternative biomarkers are being investigated in HF-PEF including markers of collagen and extra-cellular matrix turnover and may be associated with clinical outcomes[289, 290].

7.2.4 The Role of Mineralocorticoid Antagonists in HF-PEF

Inhibition of the Renin-angiotensin system (RAAS) is theoretically beneficial in HF-PEF. Many of the contributory and pathophysiological changes seen in HF-PEF are directly affected by RAAS activation including myocardial fibrosis, vascular stiffness, hypertension and afterload.

The mineralocorticoid antagonists (MRA) spironolactone and eplerenone improve survival and quality of life in selected patients with HF-REF[45, 46]. The beneficial effects of MRAs in HF-REF are likely due to altered sodium and potassium handling and decreased myocardial fibrosis[291, 292].

Fibrosis is a key mediator of myocardial stiffness and diastolic dysfunction in HF-PEF[162, 253, 293]. Recent studies have demonstrated that in HF-PEF MRAs improve left ventricular diastolic function[234, 294], decrease HF hospitalisation[295] and in selected patients may decrease mortality[296]. The mode of action of MRAs in HF-PEF is uncertain. However spironolactone has been shown to decrease serum markers of collagen turnover and improve tissue relaxation[294, 297]. If spironolactone's action is shown to be related to remodelling of the myocardial extracellular matrix this study will improve understanding of action of a disease modifying therapy, in a condition likely to become an increasing health burden[247].

7.2.5 Aims

In this study we employ CMR T1 mapping to quantify myocardial ECV before and after six months spironolactone therapy to determine if the effects of MRAs in HF-

PEF are mediated by a decrease of diffuse myocardial fibrosis. Having not previously been used as a primary end-point ECV measurement provides new opportunities for examining response to intervention.

7.3 Methods

This study was funded by British Heart Foundation Project Grant (PG/14/10/30641), received appropriate ethical approval (13/NE/0292) and was conducted in accordance with the declaration of Helsinki.

60 subjects are to be recruited from a single tertiary hospital following primary care referral with symptoms compatible with HF and if they met European Society of Cardiology (ESC) guidance for the diagnosis of HF-PEF[11] with the addition of elevated NT-proBNP.

7.3.1 Inclusion and exclusion criteria

Inclusion criteria are: age 18-90, left ventricular ejection fraction (LVEF) >50% by echocardiogram (with relevant structural abnormality e.g LVH or dilated LA), symptoms compatible with HF or signs of HF, and elevated NT-proBNP (>400pg per ml) at time of referral.

Exclusion criteria were: inability to undergo CMR examination, severe renal dysfunction (eGFR <30ml/min/1.73m²), severe valvular abnormality, uncontrolled hypertension (systolic >140mmHg or >160mmHg on three agents) on clinic measurement, diabetes mellitus (DM) (whether insulin dependent or not), contra-indication to spironolactone therapy and severe co-morbidity with expected life expectancy <5 years.

7.3.2 Study medication & randomisation

Patients underwent 1:1 open-label randomisation. Randomisation was performed using a randomised permuted block strategy by an online service provider (Sealed Envelope, London, UK). Spironolactone was started at the subjects choosing following all enrolment investigations to allow monitoring of renal function to be scheduled in primary care. Spironolactone was commenced at 25mg orally once daily with no planned titration. Renal function was monitored in primary care or at the study centre depending on patient preference. Monitoring was performed at regular intervals in accordance with national guidance[298] for use of the drug in systolic heart failure (renal function checked one week, one month, two months and three months after initiation). If renal dysfunction or hyperkalaemia developed the

dose was decreased to alternate day or stopped. Safety follow-up was continued for 28 days after study completion.

7.3.3 Multimodality Assessment

7.3.3.1 Cardiac Magnetic Resonance Protocol

Examinations were performed with a 3 Tesla Philips Achieva system equipped with a 32 channel cardiac phased array receiver coil and MultiTransmit® technology (Philips Healthcare, Best, The Netherlands). The cardiac long and short axes were determined using standard scout views. Mid LV native (pre-contrast) T1 maps were generated using a previously described MOLLI sequence [214] planned using the 3 of 5 method, briefly comprising: ECG triggered 5b(3s)3b MOLLI, flip angle 35° , voxel size of $1.98 \times 1.98 \times 10 \text{ mm}^3$. Left ventricular (LV) mass and volumes were obtained from cine imaging covering the entire LV in the short axis: balanced SSFP, voxel size $1.2 \times 1.2 \times 10 \text{ mm}^3$, no gap, 40 cardiac phases. Right ventricular (RV) and atrial volumes were obtained from a transaxial stack covering the entire heart: balanced SSFP, voxel size $1.7 \times 1.5 \times 5 \text{ mm}^3$, no interslice gap, 40 cardiac phases. 0.15mmol/kg Gadovist (Bayer Schering) was delivered by power injector (Medrad Inc, Warrendale, Pennsylvania, USA) as a single bolus via a venous cannula placed in the ante-cubital fossa, followed by a 20ml saline flush at 5ml/second. Late gadolinium enhancement (LGE) imaging (inversion recovery-prepared T1 weighted gradient echo, inversion time according to Look-Locker scout, TR/TE/flip angle $3.7 \text{ ms} / 2.0 \text{ ms} / 25^{\circ}$, acquired spatial resolution $1.54 \times 1.75 \times 10 \text{ mm}$) with whole heart coverage was performed seven to ten minutes following contrast administration. Post-contrast T1 maps were acquired using the same MOLLI scheme fifteen minutes after contrast administration.

7.3.3.2 CMR Analysis

All image analysis was performed using cmr⁴² (Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). Volumetric and mass analysis was performed in the standard manner from the short axis stack (LV) or long axis cine images (RV, left and right atria (LA, RA)). The presence of focal fibrosis or scar was assessed qualitatively from LGE imaging. T1 values were calculated from source images using manual motion correction with the region of interest (ROI) placed in the mid infero-septum to minimise variability[17, 194]. ECV was calculated using the formula:

$$ECV = (1 - Hct) \frac{R1(myo\ pre) - R1(myo\ post)}{R1(blood\ pre) - R1(blood\ post)}$$

**Where R1=1/T1 and Hct is Haematocrit.*

All T1, ECV, volumetric and mass analysis was performed by two observers (AKM, PS) blinded to time point, subject data and study arm.

7.3.3.3 Trans-thoracic echocardiogram

All echocardiograms were performed by British Society of Echocardiography (BSE) accredited echocardiographer. Measurements were averaged over 3 cardiac cycles for subjects in sinus rhythm and 5 cardiac cycles for those in atrial fibrillation. Acquisitions included continuous wave (CW) and tissue Doppler imaging (TDI) measurements of mitral inflow peak early (E), peak late (A) flow velocities, E/A ratio and mitral flow deceleration time (DT). TDI measurement (systolic (S'), early (E'), late (A')) were performed from the apical 4 chamber view and measured at the lateral and septal mitral annulus. E/E' was calculated from averaged mitral annulus TDI. Diastolic function was graded from 0-3[269]. All echocardiographic measurements were made by the investigators blinded to time point, subject data and study arm.

7.3.3.4 Ambulatory blood pressure monitoring

24 hour ambulatory blood pressure (ABPM) was performed with standard clinical equipment (Del Mar Reynolds NIBP, Sentinel Space lab 7.0.3.737).

7.3.3.5 Maximal exercise testing

Exercise test and determination of maximal oxygen consumption (Peak VO₂) were performed using RISE-95 protocol and performed with an electromagnetically braked cycle ergometer (Excalibur Sport, Lode BV, Groningen, The Netherlands). Heart rate, pulse oximetry and electrocardiogram were monitored continuously. Respirated gas was sampled and measured at 50Hz for N₂, O₂ and CO₂. Calculation of pulmonary gas exchange (V_{O₂}, V_{CO₂}, RER) and ventilator variables (Ve; tidal volume, Vt; breathing frequency) were performed on a breath by breath basis. Anaerobic threshold was calculated using the V slope method. All exercise physiology measurements were made blinded by experienced cardiac physiologists blinded to subject study arm.

7.3.3.6 Quality of life & functional status

Validated self-assessment questionnaires (EQ5D-3L, SF-12) were used to determine quality of life[299]. Functional status was assessed by the investigator and graded using the New York Health Association (NYHA) scale from I to IV.

7.3.3.7 Serum analysis

A 20 ml blood sample was taken under standard conditions following a 20 minute seated period prior to CMR study. Samples were cooled, centrifuged and stored at -80°C within 30 minutes for later analysis. Markers of collagen turnover and renin-angiotensin-aldosterone system (RAAS) activation will be performed upon completion of study follow-up period to minimise variability. Consequently they are not presented in this paper. Future analysis will include: serial PINP, PIIINP, MMP-9, NT-proBNP, aldosterone and renin levels, urea & electrolytes, albumin and bilirubin.

7.3.4 Endpoints

7.3.4.1 Primary endpoint

The primary outcome was to determine if spironolactone therapy leads to a decrease of diffuse myocardial fibrosis as assessed by CMR T1 mapping.

7.3.4.2 Secondary Endpoint

Exploratory analysis of the relationships between the extracellular volume and exercise capacity and diastolic function.

7.3.5 Statistical Analysis

This study was powered to detect a 3% change from baseline to 6 month follow up in extracellular volume fraction between groups. Assuming a 10% drop-out rate a sample size of 60 subjects was required[300]. All data are presented as mean +/- SD unless otherwise stated. Continuous variables were compared with independent t-test and categorical variables with χ^2 testing. Normal variance was assumed. Correlation was assessed with Pearson's correlation co-efficient. Significance was assumed throughout with $P < 0.05$.

7.4 Results

Between June 2014 and April 2015, 232 subjects meeting eligibility criteria were approached to take part in the study. 30 subjects were recruited and randomised to 6 months spironolactone or monitoring. At the time of writing (end March 2015) 15 subjects had completed the follow up period and four had withdrawn, or been withdrawn from the study. Of those no longer under follow-up: 2 withdrew consent, 1 was withdrawn having been unable to complete the CMR study and 1 was withdrawn by the study investigators due to poor compliance. 3 withdrawals were in the treatment group, 1 in monitoring group.

7.4.1 Findings at Study Enrolment

7.4.1.1 Demographics

Study subject characteristics can be seen in table 7.1. The subjects had a mean age of 74.4 ± 7.0 years and equal gender split. 77% of patients had NYHA II heart failure symptoms, with the remainder having NYHA III symptoms. Diagnoses of hypertension and atrial fibrillation were common and found in 80% and 90% of subjects respectively. β -Blockers were prescribed in 71% and angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker in 68%, and prescribed for systemic hypertension or atrial fibrillation. Blood pressure control at enrolment was satisfactory with mean daytime systolic blood pressure of 130.1 ± 15.2 mmHg, diastolic 71.8 ± 10.4 mmHg on mean of 1.9 ± 0.9 agents. NT-proBNP was elevated at 1718.0 ± 177.8 pg/ml, haemoglobin, haematocrit, sodium and potassium were normal, renal function was mildly impaired with mean eGFR of 63.0 ± 14.3 ml/min/1.73m².

7.4.1.2 Baseline CMR Characteristics

Left and right ventricular chamber measurements and function were within normal limits. Left and right atrial volumes were elevated (148.6 ± 29.2 ; 163.3 ± 39.7 ml respectively). Only one patient displayed LGE, which was in a typical myocarditis pattern (though no history of prior cardiac disease was elicited). Native T1 was similar to those previously recorded in volunteers studied in our unit. The extracellular volume was elevated at $28.3 \pm 3.8\%$ when compared to previously published normal ranges[18] (table 7.2).

Table 7.1 Baseline demographics and subject characteristics

	Overall (n=30)	Completed follow up		P
		Monitoring (n=8)	Spirolonolactone (n=7)	
Age	74.2 ± 6.9	73.0 ± 6.8	76.7 ± 3.9	0.26
Male:Female	15:15	3:4	3:3	0.82
BMI (Kg/m ²)	29.5 ± 5.3	31.2 ± 5.9	29.6 ± 6.2	0.63
Medical History				
<i>Hypertension (%)</i>	24 (80)	6(75)	5 (86)	0.88
<i>Atrial Fibrillation (%)</i>	27 (90)	8 (100)	7 (100)	-
<i>MI or coronary revascularisation (%)</i>	1 (3)	0	0	-
<i>Vascular disease (%)</i>	3 (10)	2 (25)	1 (14)	0.61
Medication				
<i>ACE inhibitor/ARB (%)</i>	19 (68)	6 (75)	3 (43)	0.21
<i>B-blocker (%)</i>	20 (71)	8 (100)	5 (71)	0.10
<i>Loop diuretic (%)</i>	12 (43)	3 (38)	4 (57)	0.45
<i>Calcium antagonist (%)</i>	9 (32)	4 (57)	1 (14)	0.14
<i>Digoxin (%)</i>	7 (25)	1 (13)	2 (29)	0.44
<i>Lipid-lowering med (%)</i>	13 (46)	4 (50)	4 (57)	0.79
Clinical findings				
<i>Breathlessness</i>	27 (96)	8 (100)	7 (100)	-
<i>Orthopnoea</i>	7 (25)	1	1	0.92
<i>Oedema</i>	19 (68)	4	5	0.40
<i>Systolic blood pressure</i>	130.2 ± 15.9	130.9 ± 13.1	129.2 ± 14.4	0.829
<i>Diastolic blood pressure</i>	72.8 ± 10.4	74.4 ± 12.6	75.2 ± 9.4	0.908
<i>Pulse pressure</i>	55.5 ± 18.2	56.4 ± 10.6	54.0 ± 15.2	0.741
Laboratory Tests				
<i>Haemoglobin</i>	12.4 ± 3.2	11.2 ± 4.6	14.1 ± 1.3	0.17
<i>Haematocrit</i>	40.7 ± 0.04	0.40 ± 0.03	0.44 ± 0.05	0.10
<i>Sodium</i>	140.2 ± 3.6	141.3 ± 4.4	141.2 ± 2.2	0.95
<i>Potassium</i>	4.3 ± 0.3	4.3 ± 0.3	4.3 ± 0.2	0.47
<i>eGFR(ml/min/1.73m²)</i>	63.0 ± 14.1	53.1 ± 12.2	59.7 ± 14.9	0.40
<i>NT-proBNP</i>	1669.3 ± 1428.2	1727.0 ± 895.0	1224.3 ± 416.8	0.23

Table 7.2 Baseline CMR Characteristics

	All	Completed follow up		P
	(n=30)	Monitoring(n=8)	Spironolactone(n=7)	
Chamber volumes & masses				
<i>LVEDV (ml)</i>	149.4 ± 40.9	158.9 ± 56.7	141.8 ± 21.2	0.50
<i>LV mass (g)</i>	108.2 ± 325.3	100.3 ± 40.3	104.7 ± 21.5	0.81
<i>Male</i>	126.7 ± 27.5			
<i>Female</i>	92.0 ± 34.0			
<i>Indexed LV mass (g/m²)</i>	55.8 ± 17.1	50.7 ± 16.9	54.3 ± 16.1	0.71
<i>Male</i>	61.1 ± 13.1			
<i>Female</i>	51.2 ± 19.2			
<i>LV ejection fraction (%)</i>	53.6 ± 5.8	53.7 ± 3.9	55.8 ± 5.6	0.46
<i>RVEDV (ml)</i>	158.9 ± 52.2	155.6 ± 40.8	172.1 ± 49.1	0.35
<i>RV ejection fraction(%)</i>	49.0 ± 5.8	48.6 ± 7.3	48.9 ± 7.0	0.92
<i>Left atrial volume (ml)</i>	148.6 ± 29.2	156.4 ± 30.8	144.5 ± 35.4	0.55
<i>Right atrial volume(ml)</i>	163.3 ± 39.7	90.3 ± 21.1	84.2 ± 10.9	0.51
Tissue Characteristics				
<i>LGE</i>	1*	0	1	-
<i>Native T1 (ms)</i>	1234.3 ± 82.7	1276.4 ± 24.4	1148.5 ± 120.1	0.18
<i>ECV (%)**</i>	28.3 ± 3.8	28.0 ± 2.4	27.5 ± 4.0	0.78

*One individual displayed LGE in a typical myocarditis pattern without prior history and no associated regional wall motion abnormality.

** normal range (25.4 ± 2.5%)

7.4.1.3 Echocardiography, quality of life & exercise physiology

Baseline characteristics can be seen in table 7. 3. Diastolic function was abnormal in all subjects though comprehensive assessment was limited by the presence of atrial fibrillation. Mild, moderate and severe diastolic dysfunction were seen in 10 (36%), 15 (50%) and 5 (17%) subjects respectively. Mean E/E' was outwith the normal range at 10.5 ± 3.0.

9 participants were unable to perform maximal exercise testing for a range of reasons (knee pain, hip pain, and insufficient strength to move pedals). Peak VO₂ was 12.5 ± 4.3ml/kg/min, anaerobic threshold was reached at 10.7 ± 2.5ml/kg/min though VE/CO₂ slope was normal. Physical QoL and health self-rating were decreased while mental QoL was normal.

Table 7.3 Baseline echocardiography, exercise physiology and quality of life characteristics

	All (n=30)	Completed follow up		P
		Monitoring(n=8)	Spironolactone(n=7)	
Echocardiography				
<i>E wave velocity (cm/s)</i>	100.0 ± 22.0	103.1 ± 24.2	102.3 ± 27.8	0.96
<i>Deceleration time (ms)</i>	188.6 ± 47.5	180.6 ± 40.9	175.0 ± 29.7	0.77
<i>Lateral E' (cm/s)</i>	10.6 ± 2.8	10.3 ± 2.4	10.8 ± 2.5	0.71
<i>Septal E' (cm/s)</i>	9.3 ± 2.3	8.7 ± 1.9	9.6 ± 1.9	0.42
<i>Mean E' (cm/s)</i>	10.0 ± 3.5	9.5 ± 1.9	10.2 ± 1.7	0.49
<i>E/E'</i>	10.5 ± 3.0	11.2 ± 4.4	10.1 ± 2.2	0.58
<i>Right ventricular S'</i>	11.7 ± 3.0	11.0 ± 2.	9.9 ± 1.9	0.42
Diastolic dysfunction grade				
<i>I (normal) (%)</i>	0 (0)	0 (0)	0 (0)	0.96
<i>II (mild) (%)</i>	10 (33)	3 (38)	3 (43)	
<i>III (moderate) (%)</i>	15 (50)	4 (50)	3 (43)	
<i>IV (severe) (%)</i>	5 (17)	1 (13)	1 (14)	
Functional Status				
<i>NYHA II</i>	23 (77)	6	4	0.88
<i>NYHA III</i>	7 (23)	2	3	
Cardiopulmonary Exercise Testing (2 monitoring and 3 spiro could not perform CPEX)				
<i>VO2 max</i>	12.5 ± 4.3	12.4 ± 2.8	16.4 ± 1.8	0.06
<i>VO2 at AT</i>	10.7 ± 2.5	10.3 ± 1.7	11.9 ± 3.7	0.41
<i>VE/VCO2 slope</i>	32.0 ± 5.0	29.4 ± 3.3	28.5 ± 3.3	0.72
Quality of Life				
<i>SF 12 physical</i> (44.2 ± 11.7)	30.7 ± 9.7	32.5 ± 7.4	31.6 ± 7.6	0.83
<i>SF 12 mental</i> (47.7 ± 10.5)	48.9 ± 14.3	47.1 ± 13.5	53.4 ± 10.1	0.33
<i>EQ 5D TTO</i>	0.85 ± 1.21	0.53 ± 0.44	0.68 ± 0.28	0.43
<i>EQ 5D visual scale</i> (75.3 ± 18.2)	0.66 ± 0.24	0.58 ± 0.30	0.68 ± 0.21	0.47

7.4.1.4 Relationship between left ventricular extracellular volume & study parameters

No significant relationships were seen between ECV and cardiac geometry, tissue relaxation, functional status, quality of life or well-being at baseline.

7.4.1.5 Relationship between other study parameters

Systolic blood pressure and pulse pressure were significantly associated with LV mass ($r=0.399$, $p=0.032$; $r=0.624$, $p=0.000$), though diastolic blood pressure was not ($r=-0.306$, $p=0.107$ (figure 7.2)). NT-proBNP correlated with cardiac chamber volumes, most strongly with RVEDV ($r=0.581$, $p=0.001$) and eGFR ($r=-0.402$, $p=0.025$). eGFR correlated with LVEDV, RVEDV and RA volume ($r=-0.379$, $p=0.039$; $r=-0.430$, $p=0.018$; $r=-0.458$, $p=0.016$) but otherwise no significant relationships were identified (Table 7.4).

Table 7.4 Interaction between NT-proBNP, eGFR and selected study parameters

NT-proBNP	Correlation	P value
<i>LVEDV</i>	0.388	0.034
<i>LVEF</i>	-0.553	0.002
<i>LV Mass</i>	0.355	0.054
<i>RVEDV</i>	0.581	0.001
<i>RVEF</i>	-0.399	0.067
<i>left atrium</i>	0.221	0.269
<i>right atrium</i>	0.407	0.035
<i>Peak VO₂</i>	-0.462	0.046
<i>eGFR</i>	-0.402	0.025
eGFR		
<i>LVEDV</i>	-0.379	0.039
<i>LVEF</i>	0.119	0.531
<i>LV Mass</i>	-0.288	0.122
<i>RVEDV</i>	-0.430	0.018
<i>RVEF</i>	0.161	0.394
<i>Left atrium</i>	-0.346	0.077
<i>Right atrium</i>	-0.458	0.016
<i>Peak VO₂</i>	0.076	0.757

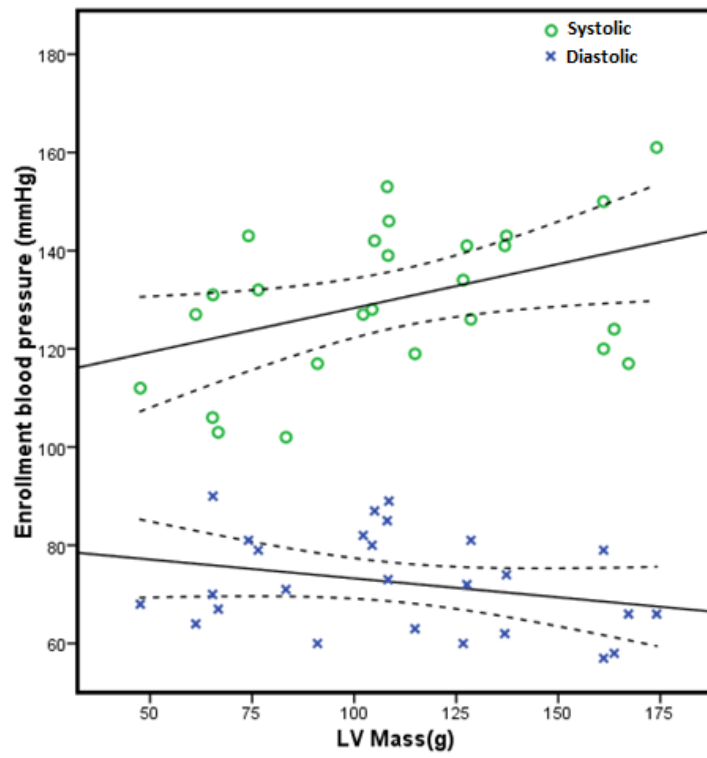
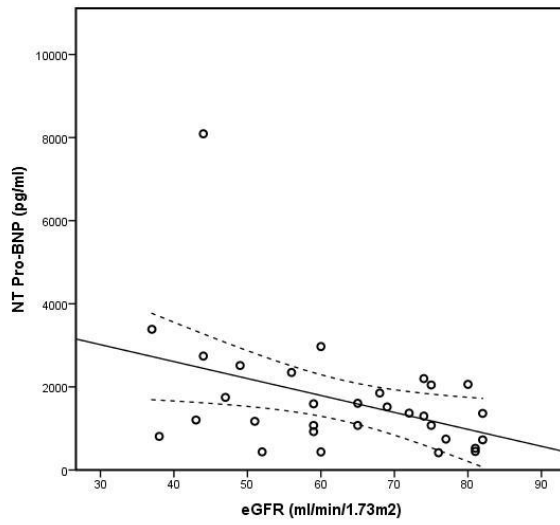


Figure 7.1 Correlations of baseline systolic and diastolic blood pressure with LV mass

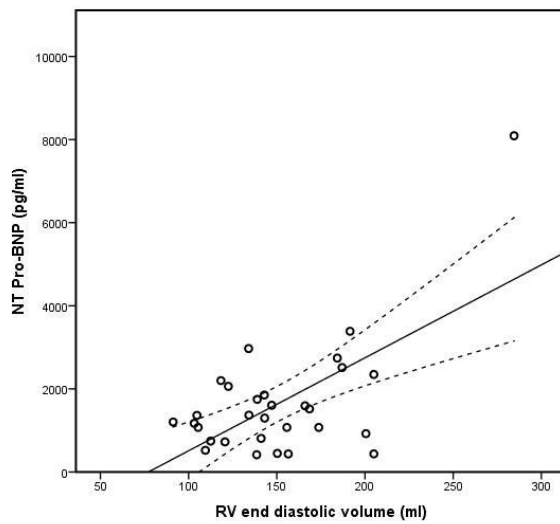
systolic $r= 0.424$, $p=0.031$; diastolic $r= 0.282$, $p=0.162$

Figure 7.2 Relationship of NT-proBNP and selected baseline characteristics

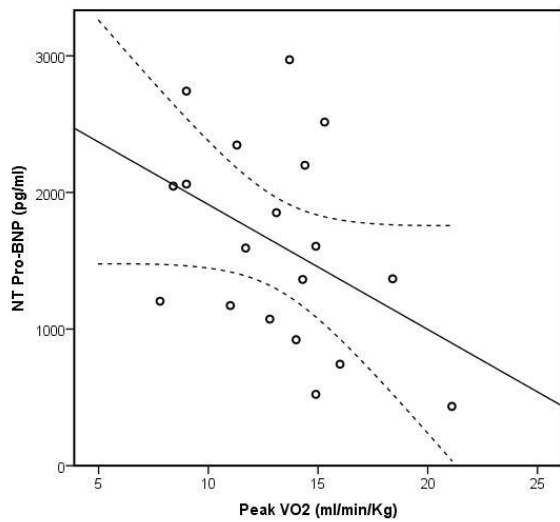
A) eGFR ($r=-0.402$, $p=0.025$)



B) RV end diastolic volume ($r=0.630$, $p=0.000$)



C) Peak VO₂ ($r=0.462$, $p=0.025$)



7.4.2 Intervention Effect

15 subjects have completed the follow-up period: 8 monitoring, 7 spironolactone. The interval between baseline and follow up CMR studies was 207 ± 20 days. One individual required down titration of spironolactone due to mild hyperkalaemia, but the study drug was continued. No adverse events occurred.

7.4.2.1 Results

Group composition, including age and gender were not statistically different. Δ ECV of $-1.8 \pm 3.9\%$ versus $+2.3 \pm 3.2\%$ ($p=0.041$) was seen in spironolactone and monitoring groups respectively. A significant decrease in LV mass following treatment with spironolactone versus controls was seen ($p=0.032$) (figure 7.3 & table 7.5).

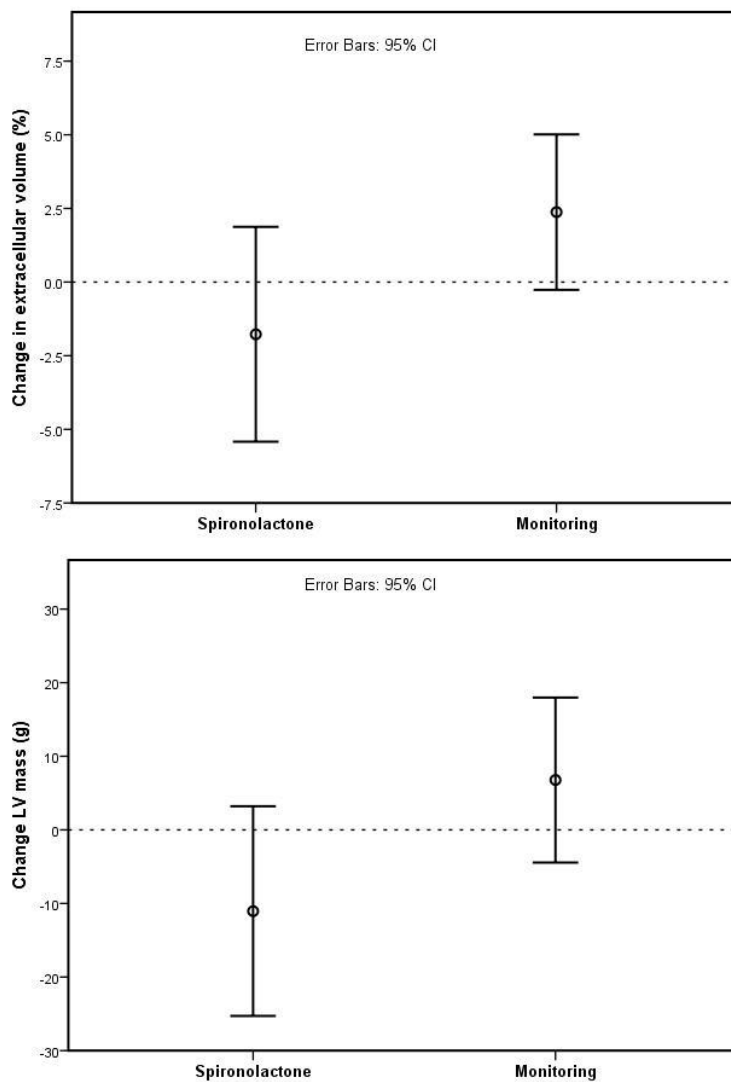


Figure 7.3 Change in ECV and LV mass over the study period

A) Δ ECV after 6 months follow-up ($p=0.041$) B) Δ LV Mass (g) after 6 months follow-up ($p=0.032$)

Table 7.5 Change of CMR characteristics over the study period

	Monitoring(n=8)	Spirolactone(n=7)	P
Chamber volumes & masses			
<i>LVEDV (ml)</i>	-9.7 ± 14.6	-15.4 ± 10.9	0.41
<i>LV mass (g)</i>	6.8 ± 13.4	-11.0 ± 15.4	0.32
<i>LV ejection fraction (%)</i>	1.2 ± 5.5	0.4 ± 2.3	0.73
<i>RVEDV</i>	-0.3 ± 12.9	-15.3 ± 14.1	0.04
<i>RV ejection fraction (%)</i>	4.2 ± 7.2	0.0 ± 5.3	0.23
<i>Left atrial volume (ml)</i>	4.9 ± 15.4	2.6 ± 20.1	0.80
<i>Right atrial volume (ml)</i>	-9.9 ± 25.1	-3.3 ± 22.7	0.62
Tissue Characteristics			
<i>Native T1 (ms)</i>	-43.5 ± 22.5	46.5 ± 115.5	0.049
<i>Extracellular Volume (%)</i>	2.4 ± 3.3	-1.8 ± 3.9	0.041

Table 7.6 Change in echocardiography, exercise physiology and quality of life measures

	Monitoring(n=8)	Spirolactone(n=7)	P
Blood Pressure			
<i>Systolic</i>	3.5 ± 13.7	-9.0 ± 15.1	0.12
<i>Diastolic</i>	6.0 ± 11.4	-2.0 ± 6.6	0.13
<i>Pulse pressure</i>	-2.5 ± 11.4	-3.0 ± 6.4	0.44
Echocardiography			
<i>E wave velocity (cm/s)</i>	2.1 ± 19.0	-6.6 ± 13.4	0.39
<i>Deceleration time (ms)</i>	-16.3 ± 25.4	-11.3 ± 34.0	0.75
<i>Lateral E' (cm/s)</i>	0.1 ± 3.0	0.6 ± 1.2	0.66
<i>Septal E' (cm/s)</i>	-1.3 ± 1.5	-0.7 ± 1.7	0.49
<i>Mean E' (cm/s)</i>	-0.6 ± 2.0	-0.0 ± 0.7	0.48
<i>E/E'</i>	1.1 ± 2.0	-0.7 ± 1.3	0.06
<i>Right ventricular S'</i>	3.2 ± 5.6	-1.3 ± 4.9	0.13
Cardiopulmonary Exercise Testing (3 spiro and 2 monitoring could not perform CPEX)			
<i>Peak VO₂</i>	-0.7 ± 2.6	0.0 ± 1.9	0.70
<i>VO₂ at anaerobic threshold</i>	-0.5 ± 0.8	0.7 ± 2.5	0.42
<i>VE/VCO₂ slope</i>	1.1 ± 3.7	-7.4 ± 16.1	0.42
Quality of Life			
<i>SF 12 physical</i>	-4.6 ± 10.0	1.9 ± 5.3	0.15
<i>SF 12 mental</i>	-4.0 ± 26.7	-7.3 ± 10.6	0.77
<i>EQ 5D TTO</i>	0.1 ± 0.5	-0.1 ± 1	0.37
<i>EQ 5D VAS</i>	-0.8 ± 2.7	-0.1 ± 0.1	0.46
<i>EQ 5D visual scale</i>	7.3 ± 12.4	-4.6 ± 12.7	0.09

RVEDV decreased significantly following treatment with spironolactone versus monitoring, though no other right sided cardiac parameters changed.

No significant change was seen in any echocardiographic or quality of life parameters. A non-significant change of E/E' was seen, with positive change observed in the treatment group (1.1 ± 2.0 vs -0.7 ± 1.3 ; $p=0.06$). Maximal exercise testing was performed by 4 and 6 subjects in the spironolactone and monitoring groups respectively, no significant change in peak VO_2 , anaerobic threshold or VE/VCO_2 slope was identified (table 7.6).

ΔECV mass correlated with ΔLV ($r=0.573$, $p=0.026$) but not with Δ diastolic, systolic or pulse pressure ($r=0.419$, $p=0.120$; $r=0.495$, $p=0.061$ $r=0.314$, $p=0.255$) (table 7.7). A non-significant relationship was seen between ΔECV and $\Delta E/E'$ ($r=0.499$, $p=0.069$).

Table 7.7 Relationship between ΔECV and Δ of selected study parameters

ΔECV	Correlation	P
<i>LVEDV</i>	0.443	0.098
<i>LVEF</i>	0.136	0.630
<i>LV Mass</i>	0.573	0.026
<i>RVEDV</i>	-0.21	0.941
<i>RVEF</i>	0.112	0.690
<i>Left atrium</i>	-0.02	0.996
<i>Right atrium</i>	-0.71	0.810
<i>Systolic blood pressure</i>	0.495	0.061
<i>Diastolic blood pressure</i>	0.419	0.120
<i>Pulse pressure</i>	0.314	0.255
<i>E wave velocity</i>	0.129	0.660
<i>Mean E'</i>	-0.287	0.319
<i>E/E'</i>	0.499	0.069
<i>Peak VO_2</i>	0.055	0.888

7.5 Discussion

7.5.1 Treatment Effect

This is the first drug study to employ ECV as the primary end point, and provides insights into the mode of action of aldosterone antagonism in HF-PEF. Recent data suggest that in carefully selected patients spironolactone may improve mortality and morbidity in HF-PEF [296]; however it remains a condition without randomised data supporting disease modifying therapy. In this preliminary analysis the sample size is insufficient to definitively test the primary hypothesis.

Despite this we have found a significant change in the myocardial extracellular volume following six months of spironolactone in HF-PEF.

In keeping with previous data LV mass fell following spironolactone treatment in HF-PEF[234]. However for the first time we have demonstrated that change in LV mass was associated with Δ ECV. Simultaneous decrease of ECV and LV mass potentially points to the physiological basis underlying remodelling: suggesting that the decrease of LV mass is secondary to regression of extracellular expansion and diffuse fibrosis.

Myocardial fibrosis is a major contributor to myocardial stiffness, diastolic dysfunction and subsequent heart failure[293, 301][302]. In this preliminary analysis the data suggest that improvement in cardiac relaxation may be associated with regression of fibrosis. A change in E/E' of 1.5 and 3 has previously been reported following 12 and 6 months of MRA treatment[234, 294]. We did not observe a significant change in echocardiographic measures of cardiac relaxation; however previous studies would suggest that a sample size of at least 10 is needed to detect an E/E' change of 2.

The relationship between LV mass, systolic and diastolic blood pressure is established[303][304][305], as are the anti-hypertensive effects of spironolactone[306]. Improved diastolic function has been shown to be independent of change in blood pressure[234], but instead occurs as a consequence of inhibition of neuro-hormonal activation.

Previous studies have demonstrated cardiac remodelling following aldosterone antagonism in HF-PEF; however the direct myocardial effects have previously not been known. The multi-parametric assessment of the heart possible with CMR means that this study is ideally placed to determine the interaction between neuro-hormonal activation and myocardial tissue characteristics.

7.5.2 Enrolment Characteristics

Our population was similar in age, gender, NYHA classification and BNP to previously published studies[295]. Importantly, rates of atrial fibrillation (89%) in the present study are much higher than previously published studies. As DM was an exclusion criteria rates this study population differed from other published studies were rates were as high as 60%[294].

Baseline myocardial fibrosis did not significantly correlate with any study parameter, though a non-significant relationship was seen between Peak VO₂ and

ECV. A relationship between myocardial fibrosis and peak oxygen consumption has not previously been described.

BNP has powerful prognostic value in HF-PEF[284, 307] and has previously been reported to be related to RV dysfunction[308] and renal impairment[309]. RV dysfunction develops as a consequence of pulmonary hypertension and afterload mismatch due to chronically elevated left atrial pressure, and is itself related to the presence of atrial fibrillation[309]. Renal dysfunction, RV dysfunction and elevated heart failure biomarkers are a triad of adverse predictors outcome in HF-PEF. Spironolactone as a mild diuretic and neuro hormonal antagonist may directly interact with two aspects of this triad, whilst indirectly lowering left atrial pressure as diastolic performance improves. This positive interaction may explain the survival benefit reported by Pfeffer *et al*[296], in patients from the Americas recruited to TOPCAT that more frequently had elevated BNP, atrial fibrillation and renal impairment. The inclusion criteria in this study will result in this cohort being more similar to the population that benefited from spironolactone, and as a result should offer important insights into spironolactone's action in HF-PEF.

7.6 Limitations

As preliminary results the interpretation of the findings in this study is limited. A sample size of 60 was determined at the start of the study, as of my period of study ending half that number have been recruited. As before, some findings thus far are interesting but definitive conclusions may not yet be drawn despite findings being in line with published data and study hypothesis.

Stored serum has not yet been analysed, to minimise variation of results all specimens are to be analysed as a single batch. The lack of NT-proBNP and markers of collagen turnover following the study period precludes insight that may have been gained by examining the interaction of serum biomarkers, the effect of spironolactone on the renin-angiotensin-aldosterone system and tissue composition.

7.7 Conclusions

In this preliminary analysis few subjects have completed the study period and a fuller analysis will be performed when the study is complete. Despite this the initial analysis points to the mode of action of aldosterone antagonism in HF-PEF, and for the first time measures the tissue effect of the intervention.

In addition, on completion this study will help further understanding of the interaction between tissue composition, blood pressure, tissue relaxation and markers of collagen turnover.

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List of Abbreviations

A	Peak late mitral inflow
ABPM	Ambulatory blood pressure
ACE	Angiotensin converting enzyme
AF	Atrial fibrillation
AH	Athlete's heart
ARVC	Arrhythmogenic right ventricular cardiomyopathy
AT	Anerobic threshold
ATP	Adenosine triphosphate
bpm	Beats per minute
BSA	Body surface area
bSSFP	Balanced steady-state free precession
CMR	Cardiovascular Magnetic Resonance
CoV	Coefficient of variation
CW	Continuous wave
DCM	Dilated cardiomyopathy
DSE	Dobutamine stress echo
DT	Deceleration time
E	Peak early mitral inflow
ECM	Extracellular matrix
ECV	Extracellular volume
EDV	End diastolic volume
eGFR	Estimated glomerular filtration rate
GBCA	Gadolinium based contrast agents
HCM	Hypertrophic cardiomyopathy
Hct	Haematocrit
HF-PEF	Heart failure with preserved ejection fraction

HF-REF	Heart failure with reduced ejection fraction
IHD	Ischaemic Heart Disease
LA	Left atrium
LGE	Late Gadolinium Enhancement
LV	Left ventricle
LVEDP	Left ventricular end diastolic pressure
LVEF	Left ventricular ejection fraction
LVSD	Left ventricular systolic dysfunction
MCE	Myocardial contrast echocardiography
MI	Myocardial infarction
MO	Micro-vascular obstruction
MOLLI	Modified Look-Locker Inversion recovery
MPRI	Myocardial perfusion reserve index
MRA	Mineralocorticoid antagonists
MRS	Magnetic Resonance Spectroscopy
ms	Milliseconds
NT-proBNP	N-terminal prohormone brain natriuretic peptide
NYHA	New York Heart Association
PCWP	Pulmonary capillary wedge pressure
PET	Positron Emission Tomography
QoL	Quality of life
RA	Right atrium
RAAS	Renin-aldosterone-angiotensin system
RI	Ramp incremental
ROI	Region of interest
RV	Right ventricle
	Saturation Pulse Prepared Heart Rate Independent Inversion
SAPPHIRE	Recovery
SASHA	Saturation Recovery Single Shot Acquisition

SE	Spin echo
SENSE	SENSitivity Encoding parallel imaging
ShMOLLI	Shortened Modified Look-Locker Inversion Recovery
SI	Step incremental
SNR	Signal to noise
SPECT	Single Photon Emission Tomography
STIR	Short-TI triple inversion recovery prepared fast spin echo
TDI	Tissue Doppler imaging
TE	Echo time
TI	Inversion time
TID	Transient ischaemic dilation
TR	Repetition time
Ve	Tidal volume
Vt	Breathing frequency

Appendix A Ethical, University & Trust Permissions

A.1 Athlete's Heart Study

A1.1 Research and Ethics Approval



Health Research Authority

NRES Committee Yorkshire & The Humber - Leeds West

Room 002, Jarrow Business Centre
Rolling Mill Road
Jarrow
Tyne and Wear
NE32 3DT

Telephone: 0191 428 3387

30 May 2014

Prof Sven Plein
Consultant Cardiologist, British Heart Foundation Senior Clinical Research Fellow
University of Leeds
Department of Cardiology
Sunshine Corridor
Leeds General Infirmary
LS1 3EX

Dear Prof Plein

Study title: The Athletes Heart Study: Multi-modality assessment of athletic cardiac adaptation
REC reference: 14/YH/0126
IRAS project ID: 151213

Thank you for your email of 30 May 2014, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Miss Sarah Grimshaw, nrescommittee.yorkandhumber-leedswest@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research

A1.2 Trust Approval

The Leeds Teaching Hospitals 
NHS Trust

Cardiology & Respiratory Directorate
Leeds General Infirmary
Great George Street
Leeds LS1 3EX

Derek Norfolk
Associate Director
Department of Research & Development
34 Hyde Terrace
Leeds
LS2 9LN

6th May 2014

Dear Dr Norfolk

Re: The Athletes Heart Study: Multi-modality assessment of athletic cardiac adaptation

I confirm that I am aware of the above research project and agree that it can take place within the Cardio-Respiratory CSU. I am satisfied that there is the capacity within the Directorate to host this study and the financial implications of the study have been considered and agreed.

With best wishes

Yours sincerely



Dr Greg Reynolds
Clinical Director

A.2 Effects of aldosterone antagonism in heart failure with preserved ejection fraction: a cardiac MRI, exercise physiology and quality of life pilot study

A.2.1 Research and Ethics Approval



Health Research Authority

NRES Committee North East - York

Room 002
TEDCO Business Centre
Viking Business Park
Rolling Mill Road
Jarrow, Tyne & Wear
NE32 3DT

Telephone: 0191 4283563

13 November 2013

Professor Sven Plein
University of Leeds
Cardiovascular and Diabetes Research
Leeds Institute of Genetics
Leeds
LS2 9JT

Dear Professor Plein

Study title: Effects of Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction (HF-PEF): Cardiac MRI, Echocardiography, Exercise Physiology & Quality of Life Assessment
REC reference: 13/NE/0292
Protocol number: CD13/10671
EudraCT number: 2013-000867-10
IRAS project ID: 121727

Thank you for your letter of 06 November 2013, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by myself as Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the REC Manager Mrs Helen M Wilson, nrescommittee.northeast-york@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting

A.2.2 University Approval

Faculty of Medicine and Health
Research Office

Room 10,110, Level 10
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Leeds LS2 9RL

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UNIVERSITY OF LEEDS

Sven Plein
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www.leedsteachinghospitals.com

Dear Prof Plein

Sponsor Authorisation to apply for a CTA

Short Project Title: Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction (AA HF-PEF)
Sponsor ID: CD13/10671
Chief Investigator: Prof Sven Plein

I can confirm that, in principle, the University of Leeds is willing to take on the duties of Sponsor for the above study as defined under **The Medicines for Human Use (Clinical Trials) Amendment (No.2) Regulations 2006***.

We are able to grant you authorisation, on behalf of the sponsor, to apply to the MHRA for a Clinical Trial Authorisation and any subsequent amendment to the CTA / protocol.

Once available, please forward to the sponsor QA office a copy of:

1. the signed / dated application form and covering letter
2. a copy of the MHRA letter acknowledging receipt of the original application
3. the MHRA Notice of Acceptance letter
4. any correspondence you have with the MHRA regarding responding to any *Remarks* they may have included in their Notice of Acceptance

Please note that you will be required to notify us immediately, as your sponsor, of any amendments to the protocol / supporting documentation / Clinical Trial Authorisation using sponsor template CTT05 / CTT06. We will then be able to provide advice on the notification of this amendment to Ethics and to the MHRA.

Yours sincerely

13/Nov/2013

Ms Clare Skinner
Faculty Head of Research Support
Faculty of Medicine and Health
University of Leeds

* These amend the principal Regulations (Medicines for Human Use (Clinical Trials) Regulations 2004, SI 2004/1031) which implemented the Council of the European Union Directive 2001/20/EC and the Commission of the European Communities Directive 2005/28/EC.

cc: Caroline Bedford, Clinical Trials Manager, Pharmacy Department

A.2.3 Trust Approval

The Leeds Teaching Hospitals NHS Trust

MS/AR/SPT/MS

Research & Development

28 April 2014

Leeds Teaching Hospitals NHS Trust

34 Hyde Terrace
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Professor Sven Plein
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Dear Professor Plein

Re: NHS Permission at LTHT for: Effects of Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction: A Cardiac MRI, Exercise Physiology and Quality of Life Study [Cardiac MRI in HF-PEF]
LTHT R&D Number: CD13/10671
EuDRAC: 2013-000867-10
REC: 13/NE/0292

I confirm that *NHS Permission for research* has been granted for this project at The Leeds Teaching Hospitals NHS Trust (LTHT). NHS Permission is granted based on the information provided in the documents listed below. All amendments (including changes to the research team) must be submitted in accordance with guidance in IRAS. Any change to the status of the project must be notified to the R&D Department.

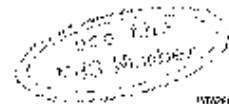
Permission is granted on the understanding that the study is conducted in accordance with the *Research Governance Framework for Health and Social Care*, ICH GCP (if applicable) and NHS Trust policies and procedures available at <http://www.leedsth.nhs.uk/academic/research-development/>

This permission is granted *only* on the understanding that you comply with the requirements of the *Framework* as listed in the attached sheet "Conditions of Approval".

If you have any queries about this approval please do not hesitate to contact the R&D Department on telephone 0113 392 2878.


Chairman Mike Collier OBE, Chief Executive Maggie Bayle

The Leeds Teaching Hospitals incorporating:
Chapel Allerton Hospital, Leeds Dental Institute, Seacroft Hospital,
St James' University Hospital, The General Infirmary at Leeds, Wharfedale Hospital



www

A.2.4 Directorate Approval

The Leeds Teaching Hospitals 
NHS Trust

Cardiology & Respiratory Directorate
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Great George Street
Leeds LS1 3EX

Derek Norfolk
Associate Director
Department of Research & Development
34 Hyde Terrace
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LS2 9LN

April 11th 2014

Dear Dr Norfolk

Re: "Effects of aldosterone antagonism in heart failure with preserved ejection fraction: a cardiac MRI, exercise physiology and quality of life pilot study"

I confirm that I am aware of the above research project and agree that it can take place within the Cardio-Respiratory CSU. I am satisfied that there is the capacity within the Directorate to host this study and the financial implications of the study have been considered and agreed.

With best wishes

Yours sincerely



Dr Greg Reynolds
Clinical Director

A.2.5. MHRA Approval



MHRA

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London SW1W 9SZ
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Mr A McDiarmid
UNIVERSITY OF LEEDS
LEEDS INSTITUTE OF GENETICS, HEALTH & THERAPEUTICS (LIGHT)
CARDIOVASCULAR & DIABETES RESEARCH DIVISION, WOODHOUSE LANE
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UNITED KINGDOM

13/02/2014

Dear Mr A McDiarmid

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference: 16767/0270/001-0001
Eudract Number: 2013-000867-10
Product: Spironolactone
Protocol number: CD13/10671

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 11/02/2014.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

**Clinical Trials Unit
MHRA**

Appendix B

Subject & GP Information Sheets

B.1 Athlete's Heart Study

B.1.1 Patient information sheet

The Athletes Heart Study: Multi-modality assessment of athletic cardiac adaptation

PARTICIPANT INFORMATION SHEET

1. Invitation

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish.

Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

2. What is the purpose of the study?

In people that undertake prolonged resistance and endurance sport and train to a very high level the heart may undergo a series of changes that allow the heart to pump more. When the heart changes the main pumping chamber (left ventricle) increases in size and becomes more muscular.

When these changes are very pronounced it can be difficult to tell an athletes' heart from some forms of heart disease including cardiomyopathy. This is important because prolonged strenuous exertion and physical training in people with heart disease may be dangerous. This means that it is very important that we can tell when a muscular heart is due to physical training or a potentially dangerous heart condition.

Currently the best method of doing this is for an athlete to undergo a period of 'detraining'. This may last up to six months, and as well as being highly frustrating for the professional or keen amateur athlete may also have professional repercussions.

In this study we are using a new cardiac magnetic resonance (CMR) technique to compare changes that occur in highly trained heart muscle against people with known heart disease. If this technique is able to tell apart athletes' heart and people with heart disease we will be able to help sportsmen and women exercise safely, avoid detraining and help avoid people with heart disease exposing them to harm.

3. Why have I been chosen?

You have been chosen because you are either a professional or keen amateur athlete **OR** a patient with cardiomyopathy that has volunteered to take part in the study.

We hope to have 80 people like you taking part so we can learn as much as possible about the changes that occur in heart muscle in very vigorous exercisers and potentially protect athletes from harm in the future.

4. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. If there is a possibility that you might be pregnant, you should not take part in the study. Our research team will be happy to discuss any other questions that you may have concerning your suitability for the study, before you decide whether to take part.

5. What will happen to me if I take part?

If you want to take part you will come to the LGI on two separate occasions. On the first visit you will have a cardiac MRI scan and blood sample taken, on the second visit you will perform an exercise test and have an echocardiogram and an ECG performed.

CMR scan: This will last approximately one hour, but you should allow an hour and a half for your total time in the department. During the MRI you will lie flat in the scanner and be asked to hold your breath. We will give you a contrast agent that allows us to learn more about your heart and is used commonly in this type of scan.

Exercise test: The duration of the CPEX (cardio-pulmonary exercise test) will depend on your level of fitness and will be performed on a treadmill. During the exercise test we will monitor your heart rhythm and blood pressure. We will also ask you to wear a mask when you are exercising so that we can accurately measure how efficient your circulation is. In some people the exercise test will be short and be finished after 10 minutes, in some it may last up to an hour.

Echocardiogram: This will last approximately ten minutes and will be done before your exercise test. It uses ultrasound to take pictures of the heart, and other than a little bit of jelly on your chest is very comfortable and uneventful.

ECG: Using an ECG we can measure the electrical impulses within the heart. This provides important information about the heart's 'wiring' and how muscular the heart is.



6. What do I have to do?

If you decide to take part you will undergo a few extra tests including one blood test. If you are one of the people taking part that has heart disease many of the tests will

form part of your normal care, and the results will be sent to your medical team. Over and above the two short visits you need not do anything else.

7. What are the side effects of any treatment received when taking part?

Your treatment will not be affected by taking part in this study in any way, but you will have a one blood test performed as part of the study.

8. What are other possible disadvantages and risks of taking part?

MRI scan: Magnetic Resonance Imaging (MRI) is safe and no radiation is used for this scan. There are no known risks from the technique.

Some people may experience claustrophobia (fear of confined spaces). Our MRI staff will do all that they can to make you feel comfortable during the scan, and will be monitoring you via a video camera and an audio link. If we are unable to make you feel comfortable in the scanner, we will not go ahead with scanning.

We will need to insert a small tube (cannula) into your arm for the contrast dye. The contrast medication we use during the scan is very safe but, as with any injection, reactions may occur. These include a warm sensation at the injection site, nausea or vomiting and transient skin rash.

People with a history of allergy are more likely to suffer a more severe reaction to the medication used, but this is rare (less than 1 in 3000). The department is equipped to cope with allergic reactions if they happen.

For Women:

If you are a woman who might become pregnant, you will be asked to have a pregnancy test (urine or blood) before taking part. If you become pregnant during the study you will have to leave the study.

9. What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get might help improve the prospects of young athletes and individuals with cardiomyopathy in the future. There is also a small chance that a non-cardiac abnormality may be detected during a follow-up scan that may lead to you undergoing investigation for an unrelated abnormality leading to prompt treatment.

We have a standardised procedure where incidental findings will be formally reported to your general practitioner. If appropriate one of the study doctors will advise you of any incidental findings on the day of your scan.

10. What happens when the research study stops?

After your second visit your involvement with the study will cease but your normal care will continue, under your clinical team, uninterrupted.

11. What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements, however in certain circumstances arrangements may differ. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

12. Will my taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

13. Contact Details

Your Doctor

Name : Dr Peter Swoboda or Dr Adam McDiarmid
Cardiac MRI department,
Clarendon wing,
Leeds General Infirmary
Tel. Number: 01133925909

Your Research/Specialist Nurse

Name : Research Nurses
Cardiovascular Research
Sunshine Corridor
Leeds General Infirmary
Tel. Number: 0113 392 5481 or 392 6286

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

16. What if new information becomes available?

Sometimes during the course of a study, new information becomes available on the condition being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

17. What will happen if I don't want to carry on with the study?

The data collected up to the time you withdraw from the study will be kept in fully anonymised form.

18. Will my part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study treatment, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 15 years. Arrangements for confidential destruction will then be made.

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

19. Informing your General Practitioner (GP)

We will inform your GP that you are taking part, but the study will not have an impact on your care.

20. What will happen to any samples I give?

Blood samples will be stored within the LGI for the duration of the study to allow for some specialist tests to be performed in one batch. After this samples will be destroyed.

21. Will any Genetic testing be done?

No

22. What will happen to the results of this medical study?

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the participants involved in the trial will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study doctor.

23. Who is organising this medical study?

This study is being organised by the Leeds Institute of Genetics, Health and Therapeutics (LIGHT) within the University of Leeds.

24. Who has reviewed the study?

This study was given favourable ethical opinion for conduct in the NHS by Yorkshire & Humber – Leeds West Research Ethics Committee.

25. Contact for further information

You are encouraged to ask any questions you wish, before, during or after your research investigations. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact one of the following people:

Dr Peter Swoboda or Dr Adam McDiarmid
Cardiac MRI department,
Clarendon wing,
Leeds General Infirmary
01133925909

Research Nurses
Cardiovascular Research
Sunshine Corridor
Leeds General Infirmary
Tel: 0113 392 5481 or 392
6286
p.bijsterveld@leeds.ac.uk
l.m.clark@leeds.ac.uk

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to

keep. A copy of the consent form will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

B.1.2 General Practitioner Information Sheet

The Athletes Heart Study: Multi-modality assessment of athletic cardiac adaptation

General Practitioner Information sheet
Version 1.0 February 2014.

25/02/2014

Dr

Re:

Dear colleague,

Your patient has agreed to take part in the **'The Athlete's Heart Study: Multi-modality assessment of athletic cardiac adaptation'** study. This is a study where we look at the change in heart muscle morphology and function in individuals that have developed physiological changes following intensive physical training. We would like to give you some relevant information about this study.

Purpose of the study:

Following prolonged resistance and endurance training the heart may undergo a series of physiological adaptations. These changes, including left ventricular dilation and hypertrophy, can be difficult to differentiate from changes seen in some forms of cardiomyopathy including hypertrophic cardiomyopathy. Prolonged strenuous exertion and physical training in patients with cardiomyopathy may be dangerous and result in sudden death, as a result it is essential that when overlap in physical appearances exist the two are differentiated.

Currently the best method of differentiating the two is for an athlete to undergo a period of 'detraining'. This may last up to six months, and as well as being highly frustrating for the professional or keen amateur athlete may also have professional repercussions.

In this study we plan to use a new cardiac magnetic resonance (CMR) technique to detect changes that occur in highly trained heart muscle versus patients with known cardiomyopathy displaying similar morphological changes. If this technique is able to differentiate between athletes' heart and patients with cardiomyopathy it will allow us to accurately define the cause of the changes in heart structure and function, avoiding detraining and protecting patients with cardiomyopathy from potential harm.

Recruitment: 40 subjects will be recruited from national and local sporting associations with 40 controls recruited from local tertiary specialist hypertrophic cardiomyopathy clinic.

What will happen to your patient?

Athletes and controls will undergo the same range of non-invasive multi-modality tests.

After having given informed consent subjects will then:

- Have a perfusion CMR examination (including a blood test)
- Perform a cardio-pulmonary exercise test to determine peak oxygen consumption
- Have a 12 lead surface electrocardiogram and brief echocardiogram

By entering into the study subjects will have a series of additional tests. However these are safe, well tolerated and performed as part of routine clinical care in patients with hypertrophic cardiomyopathy.

In this study the CMR scan will last approximately one hour, which is similar to a normal examination. The echocardiogram and ECG should last approximately 15 minutes in total whilst the duration of the cardiopulmonary exercise test will be dependent upon the subject's level of fitness and is anticipated to last less than one hour.

Confidentiality: All information, which is collected about your patient during the course of the research, will be kept strictly confidential. Patients will not be identified in any publication that may result from this research. We will inform you if an incidental abnormality is found on any of the scans.

Indemnity/Compensation: If patients are harmed as a direct result of taking part in this study, there are no special compensation arrangements however in some special circumstances compensation may be provided. If patients have any cause to complain about any aspect of the way they have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to them.

The research organisation: This study is organized by the CMR Clinical Research Group at the University of Leeds.

For further information please contact:

Dr Adam McDiarmid or Peter Swoboda
Clinical Research Fellows
Cardiac MRI Department
B Floor Clarendon Wing
Leeds General Infirmary
LS1 3EX
Tel 0113 392 5909

Research Nurses
Cardiovascular Research
Sunshine Corridor
Leeds General Infirmary
LS1 3 EX
Tel: 0113 392 5481 or 392 6286
p.bijsterveld@leeds.ac.uk
l.m.clark@leeds.ac.uk

B.2 Effects of aldosterone antagonism in heart failure with preserved ejection fraction: a cardiac MRI, exercise physiology and quality of life pilot study

B.2.1 Patient Information Sheet

Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction

PATIENT INFORMATION SHEET V 2.2 1st August 2014

1. Invitation

You are being invited to take part in a research study. Before you decide whether or not to take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully, and discuss it with others if you wish. Ask us if there is anything that is not clear, or if you would like more information. Take time to decide whether or not you wish to take part.

2. What is the purpose of the study?

Heart failure with Preserved Ejection Fraction (HF-PEF) affects many hundreds of thousands of people in the UK. In HF-PEF the heart becomes stiff and becomes a less effective pump, this can lead to breathlessness and fluid retention.

We diagnose this condition by taking a history (listening to you tell us about your symptoms), examining you and using tests that tell us about heart function including a cardiac ultrasound (echocardiogram) and blood samples.

The treatments we currently have helps to get rid of any fluid that has accumulated but doesn't improve the way the heart functions. One reason that we don't yet have disease altering treatments in this condition is that we don't fully understand what makes the heart become stiff to begin with. Some recent research has suggested that a commonly prescribed drug called Spironolactone might help the heart relax and make filling easier. We want to use cardiac magnetic resonance imaging (CMR) to learn more about changes in the heart in HF-PEF and the effects that Spironolactone has on it. A CMR is a detailed, very safe scan where we use a very powerful magnetic to make very accurate pictures of the heart.

We hope that by learning more about these changes we might be able to develop treatment for this condition in the future.

3. Why have I been chosen?

This study is looking at patients like you that have HF-PEF. We hope to have 60 people like you taking part so we can learn as much as possible about HF-PEF and the effects that Spironolactone may have. We don't have a good treatment for HF-PEF at the moment and we hope that what we learn in this study might allow us to help people like you in the future.

4. Do I have to take part?

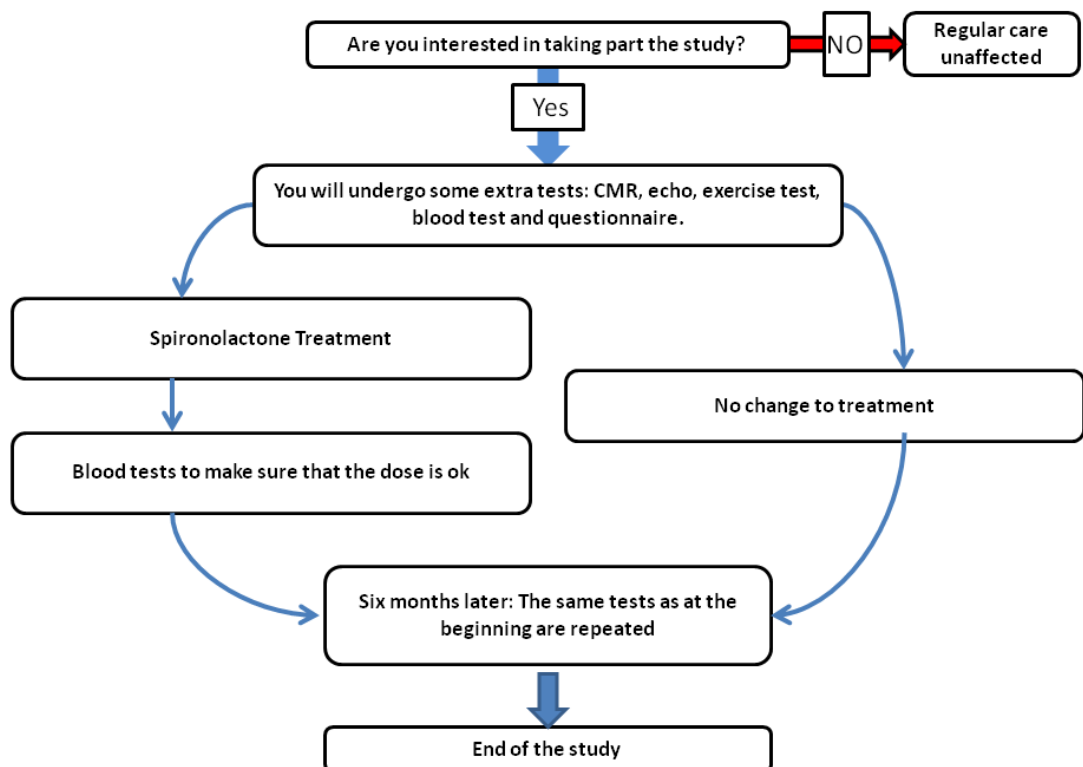
No. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. This will not affect the standard of care that you receive from the NHS. If there is a possibility that you might be pregnant, you should not take part in the study. Our research team will be happy to discuss any other questions that you may have concerning your suitability for the study, before you decide whether to take part.

5. What will happen to me if I take part?

If you want to take part you will first have a CMR study, an echocardiogram, an exercise test, a quality of life questionnaire, 24hr blood pressure cuff and a blood sample taken. After that you will randomly put into one of two groups: one group is the 'Spironolactone' group and the other the 'Control' group.

If you are assigned to the control group we won't need to do anything further immediately but will be asked to come back to the study centre at the end of month one and three to see how you are feeling. If you are assigned to the Spironolactone group you will be asked to start taking a new tablet called Spironolactone, we will prescribe this in the hospital. You will remain on this treatment for six months during which time you will have a few extra blood tests through your GP to make sure that you are experiencing no adverse effects. We will ask you to come back at the end of month one and three to pick up your prescription and check how you are feeling.

Once the six months are up we will ask both groups to come back for the same tests as at the beginning of the study to see if Spironolactone has any positive effects in HF-PEF.

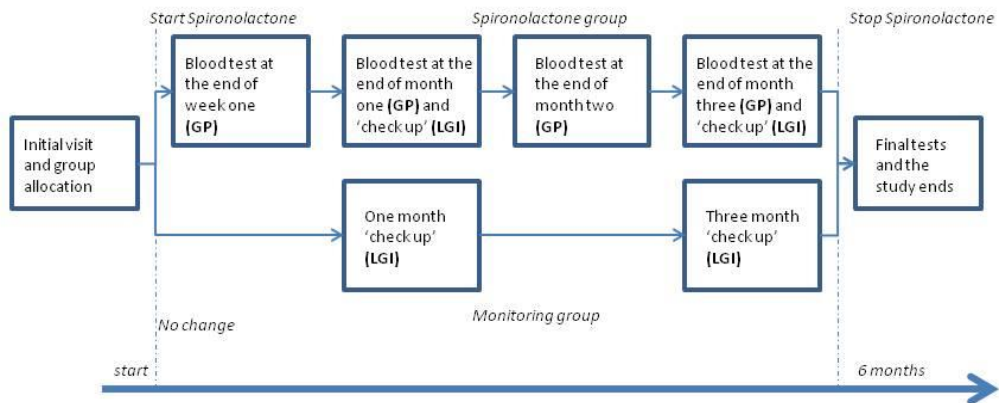


**Please note that if you are allocated to either the treatment or monitoring groups we'd like to see you at the end of month one and three to see how you are feeling*

6. What do I have to do?

If you decide to take part you will undergo some extra tests that you would not have otherwise had including 24hr blood pressure, CMR and exercise test on two separate occasions, as well as the 'check-ups' at the end of month one and three. This means that you will have four trips to the LGI that you would otherwise not have had.

If you are randomized to the Spironolactone group you will be taking a medication that you would not otherwise be prescribed, this medicine means that you need to have a few extra blood tests to make sure it doesn't interfere with your kidneys. Below you can see the typical blood testing that we would expect needs to be performed during the study. Sometimes, if your results are abnormal, we will need to do occasional extra blood tests.



7. What is the drug that is being tested?

Spironolactone. Is a commonly used medication for breathlessness and fluid retention caused by heart failure due to a weak pump. Some studies have shown that it can have positive effects in HF-PEF, we hope that by examining the effects of Spironolactone in HF-PEF we can learn more about how these positive effects come about.

8. What are the alternatives for diagnosis or treatment

Currently there are no proven alternatives for this condition. This treatment may not make you feel better but it will hopefully enable us to devise better treatment for patients with this condition in the future.

9. What are the side effects of any treatment received when taking part?

In some cases Spironolactone can cause problems with kidney function. Your Doctor will be aware of this potential problem as it is a commonly prescribed medicine. We will ask them to perform blood tests in the standard manner. We will monitor these results and be happy to offer advice to your doctor if need be. If you do decide to take part in the study, you must report any problems you have to your study nurse or doctor and your GP.

There is also a contact number given at the end of this information sheet for you to phone if you become worried at any time.

If you become suddenly unwell whilst you are with us we will contact your nominated next of kin.

10. What are other possible disadvantages and risks of taking part?

The medication: There is a small risk of kidney problems with Spironolactone however the regular blood tests that your GP will be performing should minimise any risk of harm from this. Other side-effects including gynaecomastia are relatively minor and should resolve on stopping the treatment. Gynaecomastia is tender breast swelling that may affect both sexes and can occur as a side effect of the medication. Gynaecomastia typically resolves once the medication is withdrawn.

CMR scan: Magnetic Resonance Imaging (MRI) is safe and no radiation is used for this scan. There are no known risks from the technique.

Some people may experience claustrophobia (fear of confined spaces). Our MRI staff will do all that they can to make you feel comfortable during the scan, and will be monitoring you via a video camera and an audio link. If we are unable to make you feel comfortable in the scanner, we will not go ahead with scanning.

We will need to insert two small tubes (cannulae) into your arms; one for the contrast dye and another for the stress medication. The contrast medication we use during the scan is very safe but, as with any injection, reactions may occur. These include a warm sensation at the injection site, nausea or vomiting and transient skin rash.

During the scan you will be given Adenosine and a Gadolinium containing contrast agent, these are both well studied medications that we use regularly for CMR. The effects of Adenosine last for a few minutes and allow us to perform a 'stress' test. It can cause flushing, breathlessness and chest discomfort. However, all of these feelings usually subside within one or two minutes or even more quickly when the medication is stopped.

People with a history of allergy are more likely to suffer a more severe reaction to the medication used, but this is rare (less than 1 in 3000). The department is equipped to cope with allergic reactions if they happen. Adenosine, the medication we use to increase the blood flow to the heart,

For Women:

The treatment might harm the unborn child; therefore you should not take part in this study if you

are pregnant, breast-feeding or you intend to become pregnant during the study.

If you are a woman who might become pregnant, you will be asked to have a pregnancy test

(urine or blood) before taking part. You must agree to use a reliable form of contraception during

the trial, e.g.

- Oral contraceptive + condom
- Intra-uterine device (IUD)+ condom
- Diaphragm with spermicide + condom

This should be continued for at least 6 months after the treatment has finished.

- If you become pregnant during the study it is essential that you inform the study centre and your GP

For Men:

Please share this information with your partner if it's appropriate:

It is (or is not) known if the study medicine will affect sperm or semen and therefore you must not father a child during this study or for a safety period of 6 months after treatment. If your partner might become pregnant you must use reliable forms of contraception during the trial and for 6 months afterwards, e.g.

- Oral contraceptive +condom
- Intra-uterine device (IUD)+ condom
- Diaphragm with spermicide + condom

11. What are the possible benefits of taking part?

We cannot promise the study will help you but the information we get might help improve the treatment of people with Heart Failure with Preserved Ejection Fraction (HF-PEF) in the future.

There is also a small chance that an abnormality, not relating to the heart, may be picked up by the MRI scan. This could mean that a totally unexpected health problem is investigated and treated more promptly than otherwise expected with some potential health benefits for you.

12. What happens when the research study stops?

When the research study stops people assigned to the Spironolactone group will stop taking the study medication. Follow up will continue for 30 days after the study if there are any problems after medication has stopped.

13. What if there is a problem?

If you have a concern about any aspect of this study, you should ask to speak with the researchers who will do their best to answer your question. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from the hospital.

In the event that something does go wrong and you are harmed during the research study there are no special compensation arrangements, however in certain circumstances arrangements may differ. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

14. Will my taking part in this study be kept confidential?

Yes. All the information about your participation in this study will be kept confidential. The details are included in Part 2.

15. Contact Details

Your Doctor

Name **Dr Adam McDiarmid**

Tel. Number: **0113 243 2799** extension **25909**

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making any decision.

PART 2

16. What if new information becomes available?

Sometimes during the course of a clinical trial, new information becomes available on the drugs that are being studied. If this happens, we will tell you about it and discuss with you whether you want to or should continue in the study. If you decide to withdraw, we will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

On receiving new information, we might consider it to be in your best interests to withdraw you from the study. If so, we will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

17. What will happen if I don't want to carry on with the study?

If you do not wish to continue with the study normal clinical care will be arranged for you.

18. Will my part in this study be kept confidential?

If you consent to take part in this study, the records obtained while you are in this study as well as related health records will remain strictly confidential at all times. The information will be held securely on paper and electronically at your treating hospital under the provisions of the 1998 Data Protection Act. Your name will not be passed to anyone else outside the research team or the sponsor, who is not involved in the trial. You will be allocated a trial number, which will be used as a code to identify you on all trial forms.

Your records will be available to people authorised to work on the trial but may also need to be made available to people authorised by the Research Sponsor, which is the organisation responsible for ensuring that the study is carried out correctly. A copy of your consent form may be sent to the Research Sponsor during the course of the study. By signing the consent form you agree to this access for the current study and any further research that may be conducted in relation to it, even if you withdraw from the current study.

The information collected about you may also be shown to authorised people from the UK Regulatory Authority and Independent Ethics Committee; this is to ensure

that the study is carried out to the highest possible scientific standards. All will have a duty of confidentiality to you as a research participant.

If you withdraw consent from further study treatment, unless you object, your data and samples will remain on file and will be included in the final study analysis.

In line with Good Clinical Practice guidelines, at the end of the study, your data will be securely archived for a minimum of 5 years. Arrangements for confidential destruction will then be made.

With your permission, your GP, and other doctors who may be treating you, will be notified that you are taking part in this study.

19. Informing your General Practitioner (GP)

For you to take part it is essential that we inform your GP of your involvement. This is to insure that the medication is introduced safely and that we can arrange monitoring blood tests close to home to save you travelling to the LGI as regularly.

20. What will happen to any samples I give?

Blood samples will be stored within the LGI for the duration of the study to allow for some specialist tests to be performed in one batch. After this samples will be destroyed.

21. Will any Genetic testing be done?

No

22. What will happen to the results of this clinical trial?

The results of the study will be available after it finishes and will usually be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the patients involved in the trial will be identified in any report or publication.

Should you wish to see the results, or the publication, please ask your study doctor.

23. Who is organising and funding this clinical trial?

This study is being organised by the Leeds Institute of Cardiovascular and Metabolic Medicine (LICAMM) within the University of Leeds. Funding has been awarded by the British Heart Foundation.

24. Who has reviewed the study?

This study was given favourable ethical opinion for conduct in the NHS by North East - York Research Ethics Committee.

25. Contact for further information

You are encouraged to ask any questions you wish, before, during or after your treatment. If you have any questions about the study, please speak to your study nurse or doctor, who will be able to provide you with up to date information about the drug(s)/procedure(s) involved. If you wish to read the research on which this

study is based, please ask your study nurse or doctor. If you require any further information or have any concerns while taking part in the study please contact one of the following people:

If you decide you would like to take part then please read and sign the consent form. You will be given a copy of this information sheet and the consent form to keep. A copy of the consent form will be filed in your patient notes, one will be filed with the study records and one may be sent to the Research Sponsor.

You can have more time to think this over if you are at all unsure.

Thank you for taking the time to read this information sheet and to consider this study.

Dr Adam McDiarmid
Cardiovascular Magnetic Resonance
Leeds General Infirmary
Leeds
LS1 3EX

0113 243 2799 extension **25909**

B.2.2 General Practitioner Information Sheet

AA - HFPEF STUDY

Effects of Aldosterone Antagonism in Heart Failure with Preserved Ejection Fraction: A Cardiac MRI, Exercise Physiology and Quality of Life Study

General Practitioner Information sheet
Version 2.2 1st August 2014.

01/08/2014

Dr

Re:

Dear colleague,

Your patient has agreed to take part in the **AA HF-PEF** study, which is a randomised controlled trial of Spironolactone use in the treatment in HF-PEF. We would like to give you some relevant information about this study.

Your patient has been randomised to: (Please monitor renal function as per NICE guidance for systolic heart failure if on SPIRONOLACTONE)

Purpose of the study:

Heart failure is a common problem affecting approximately 900,000 people in the UK. Nearly half of these will have a normal, or near normal, ejection fraction and are said to have Heart Failure with Preserved Ejection Fraction (HF-PEF). Unlike systolic dysfunction there have been no improvements in the treatment of HF-PEF over the past decades, and prognosis remains poor.

In the HF-PEF the heart becomes stiff and diastolic function is impaired, this is believed to be due to diffuse fibrosis throughout the left ventricle. There have been some recent studies in patients with HF-PEF that have shown improvement in diastolic function following treatment with Spironolactone. Using cardiac MRI (CMR) we hope to find out why this is. In this study we will measure diffuse cardiac fibrosis

using CMR techniques that were originally developed in Leeds over 25 years ago, to help answer this.

If the improvements seen in diastolic function are associated with regression of diffuse fibrosis it might point to future interventions that will make a big difference the care of patients with in HF-PEF.

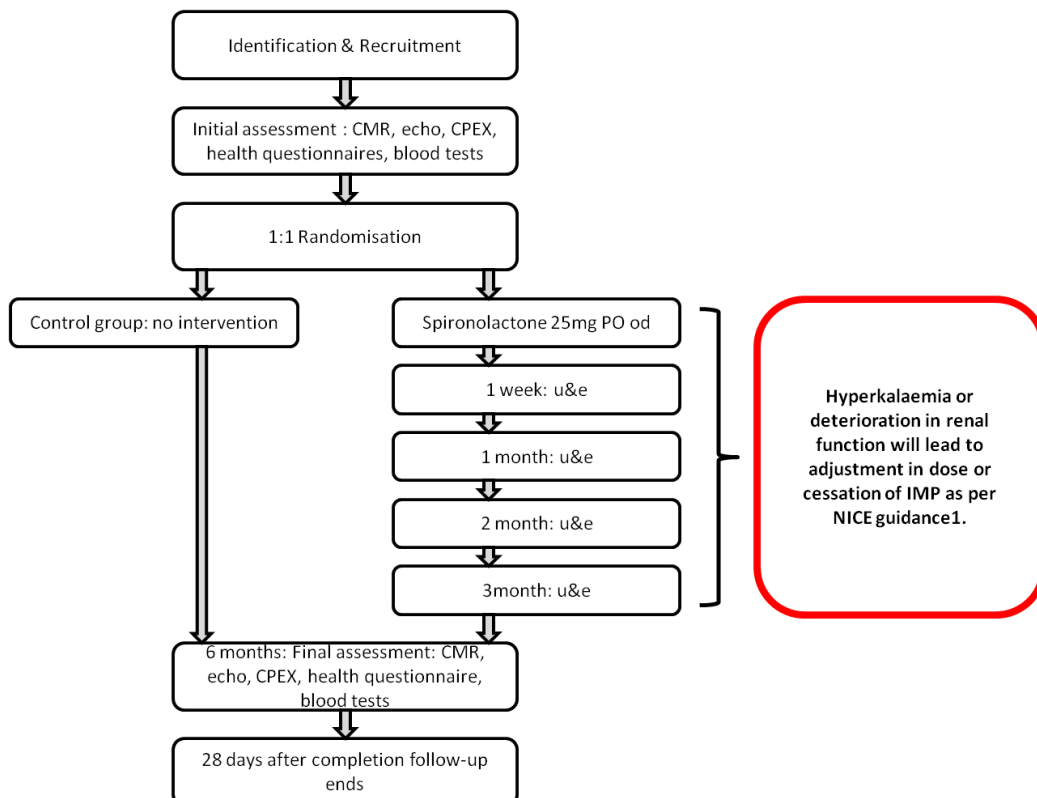
Recruitment: 60 patients will be recruited and randomised to either a treatment group or a control group.

What will happen to your patient?

All patients will undergo a comprehensive assessment of cardiac function, quality of life and functional status and serum bio-makers of heart failure. This will include:

- CMR study
- Echocardiogram
- Cardio-pulmonary exercise tests
- 2 Health questionnaires (MLHF & EQ5D-2) validated in cardiovascular disease
- Blood tests

Patient journey flowchart: please note there will be study centre visits at the end of month one and three to assess any issues with the study medication, medication compliance or adverse events.



Patients will be randomised 1:1 to either Spironolactone 25mg PO od or to a control group. Individuals randomised to the control group will undergo no further intervention.

Those randomised to treatment with Spironolactone will be commenced on 25mg PO od in accordance with NICE guidelines for the initiation of Spironolactone in congestive cardiac failure by the study centre.

What we would like you to do: We would be grateful if you could monitor renal function and electrolytes as recommended by NICE in the treatment of in congestive cardiac failure *, all results will be seen by the research centre. If performing venesection in the practice is difficult we will make arrangements for your patient to attend the LGI for monitoring.

If your patient is unwell, has any problems with the medication or has any illness we would be grateful if you would inform the study centre. We will see study participants at the end of month one and three at the study centre to see how they are doing and make sure they are suffering no adverse events. We will re-issue prescriptions for the study medication at these visits.

At six months all patients will be asked to re-attend for repeat tests, at this point the medication will be stopped and patients will be discharged from follow-up.

Adverse Events & Pregnancy: Your patient has been asked to contact the study centre if they become unwell during the study.

Likewise if your patient becomes pregnant during the study we would be grateful if you would contact the study centre.

Confidentiality: All information, which is collected about your patient during the course of the research, will be kept strictly confidential. Patients will not be identified in any publication that may result from this research. We will inform you if an incidental abnormality is found on any of the scans.

Indemnity/Compensation: If patients are harmed as a direct result of taking part in this study, there are specific compensation arrangements in place. If patients have any cause to complain about any aspect of the way they have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to them.

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