Diffusion Tensor Cardiovascular Magnetic Resonance: A Clinical Perspective

Brief title: DT-CMR Clinical perspective

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ABSTRACT
Imaging the heart is central to cardiac phenotyping but in clinical practice this has been restricted to macroscopic interrogation. Diffusion tensor cardiovascular magnetic resonance (DT-CMR) is a novel, non-invasive technique which is beginning to unlock details of this microstructure in humans in-vivo. DT-CMR demonstrates the helical cardiomyocyte arrangement that drives rotation and torsion. Sheetlets (functional units of cardiomyocytes, separated by shear layers) have been shown to reorientate between diastole and systole, revealing how microstructural function facilitates cardiac thickening. Measures of tissue diffusion can also be made; fractional anisotropy (a measure of myocyte organisation) and mean diffusivity (a measure of myocyte packing). Abnormal myocyte orientation and sheetlet function has been demonstrated in congenital heart disease, cardiomyopathy and after myocardial infarction. It is too early to predict the clinical importance of DT-CMR, but such unique in-vivo information will likely prove valuable in early diagnosis and risk prediction of cardiac dysfunction and arrhythmias.

KEY WORDS
Diffusion tensor CMR
Cardiomyocytes
Sheetlets
Microstructure
Helix angle

ABBREVIATIONS
DT-CMR: diffusion tensor cardiovascular magnetic resonance
E1: primary eigenvector
E2A: secondary vector angulation
FA: fractional anisotropy
HA: helix angle
M2-SE: second order motion compensated spin echo
MD: mean diffusivity
STEAM: stimulated echo acquisition mode
TA: transverse angle

HIGHLIGHTS
- Diffusion Tensor Cardiovascular Magnetic Resonance is a unique technique for non-invasive assessment of myocardial microstructure.
- DT-CMR has identified novel cardiomyocyte and sheetlet abnormalities in cardiac disease in-vivo.
- DT-CMR has the potential to aid clinical diagnosis and risk stratification through microscopic phenotyping.
INTRODUCTION

Myocardial phenotyping is crucial to understand the healthy heart and changes in disease. It offers insight into the cardiac structure-function relationship and is key to diagnosis, prognosis and determining therapeutic interventions. However, phenotyping with current imaging techniques is limited to a scale of millimetres and it is not possible to assess the microstructure of the myocardium without resorting to biopsy. Diffusion tensor cardiovascular magnetic resonance (DT-CMR) is a novel tool that provides non-invasive in-vivo microstructural assessment, offering information at the scale of cardiomyocyte organisation. Preliminary studies have shown DT-CMR can identify hitherto unknown microstructural derangements in cardiomyopathy, myocardial infarction (MI) and congenital heart disease. Hence there is increasing interest in its potential clinical utility and the new insight it brings to pathophysiology of cardiac disease. This clinical review describes the cardiac microstructure, the DT-CMR technique, clinical studies performed so far and the potential future role of DT-CMR.

THE CARDIAC MICROSTRUCTURE

Cardiomyocytes
Cardiomyocytes measure approximately 150μm by 20μm and intercalate with several other cardiomyocytes (1). While their branching nature means that cardiomyocytes do not strictly align in fibres, there is a directional grain to their appearance (Figure 1). This has led to variable terminology, where some use “fibre” to mean cardiomyocytes, or groups of cardiomyocytes. Here the phrase “cardiomyocyte orientation” is used to avoid confusion with the more strictly fibre-like skeletal muscle. In the left ventricle (LV), cardiomyocytes have a left-handed (LH) helical course at the epicardium, progressing through to a circumferential course in the
mesocardium and then a right-handed (RH) helical course in the endocardium, as shown in the historical sequential dissection plates in Figure 1. More recently, histology by Streeter demonstrates the transmural variation of ‘the fibre angle’ known as $\alpha$ (2). This angle, measured in the plane parallel to the epicardium and relative to the LV long axis, is positive at the endocardium and negative at the epicardium, reflecting RH and LH helical orientation respectively (Figure 2). The near zero $\alpha$ in the mesocardium indicates circumferential cardiomyocytes.

**Sheetlets**

Cardiomyocytes are aggregated to form secondary structures, termed “sheetlets” (Figure 3). Each sheetlet is approximately 4 cardiomyocytes thick, surrounded and interconnected by the collagenous perimysium, allowing shear between neighbouring sheetlets during the dynamic motion of cardiac contraction (3). It was initially thought that sheetlets formed stacked sheets, each extending the width of the LV wall (4). It is now appreciated that there are multiple local sub-populations containing counter-sloping sheetlets (5).

**Structure-function relationship**

The myocardial microstructure is crucial to the dynamics of LV function. The helical arrangement of cardiomyocytes drives myocardial rotation and torsion (6). The dominant effect of the larger epicardial radius means that cardiomyocyte shortening of the left-handed epicardial cardiomyocytes leads to net clockwise rotation basally and anticlockwise rotation apically, when viewed from the apex (6).
However, what is far less well appreciated is the contribution of sheetlets to cardiac function. Both Streeter and Spotnitz noted the ‘wall thickening paradox’ in which cardiomyocyte thickening of only 8% could not explain wall thickening of 40% (2, 7). Histological analysis in rat and dog myocardium suggested the concept of sheetlet rearrangement from a more LV epicardial wall-parallel orientation in diastole to a more wall-perpendicular arrangement in systole to facilitate LV wall thickening (4, 7). However, until the advent of DT-CMR there was no way of assessing in-vivo microstructural changes through the cardiac cycle. Recent in-vivo human and animal work strongly support the concept of sheetlet reorientation as an important, if not the dominant myocardial dynamic associated with radial thickening (8). There is still limited understanding of how myocyte shortening translates into the microstructural rearrangement that causes wall thickening. However, approaches such as DT-CMR should yield insight into this problem.

**CMR TECHNIQUE**

The theory and underlying methods behind diffusion MR were developed in the 1960s by Stejskal and Tanner (9) and advanced in the 1990s (10). The first clinical application of diffusion imaging was in neurology, where it was used for the early diagnosis of stroke (11). Cardiac diffusion imaging is considerably more difficult due to bulk motion of the heart but Edelman was able to overcome this by use of a stimulated echo acquisition mode (STEAM) technique to produce the first in-vivo diffusion cardiac image in 1994 (12).

**Diffusion**
Diffusion imaging assesses diffusion of water. Free diffusion, when water molecules may diffuse in all directions equally, is known as isotropic diffusion. However, in the myocardium where cardiomyocytes and connective tissues act as barriers, diffusion is restricted (impermeable barriers) or hindered (permeable barriers), becoming anisotropic (Figure 4). DT-CMR exploits the effects of the microstructure on diffusion to yield information about the underlying myocardial organisation.

**Diffusion weighted CMR (DW-CMR)**

Diffusion is measured by assessing the signal loss between two images; a reference image and a main image with a higher degree of diffusion weighting. The weighting is applied by diffusion encoding gradients and described by the b-value, which encompasses gradient amplitude, duration and temporal separation of the gradient pulses and is quoted in units of s/mm² (10). Typically values of 450-600s/mm² are used (8, 13, 14). At higher b-values, the signal intensity is less easily distinguished from background noise (15). At lower b-values, other sources of diffusion-like intravoxel incoherent motion, including perfusion, confound the signal attenuation (16). The measured diffusion is known as the ‘apparent diffusion coefficient’ (ADC) to accommodate factors, such as perfusion and hindered/restricted diffusion that impair accurate measures of diffusivity (17). The fact that diffusion yields directional information developed from the observation that ADC was higher when the diffusion gradient direction was aligned with axonal direction in neurological diffusion imaging (18). The modern approach to determining microstructural orientations uses diffusion gradients applied in at least 6 directions. Information from the diffusion weighted images is used to determine a diffusion tensor, which is a mathematical extension of a vector, having a 3-dimensional magnitude and direction (19).
Acquiring DT-CMR data

One method to acquire DT-CMR data is a Spin Echo (SE) sequence, known as the Stejskal-Tanner sequence (9). An excitation pulse is followed by a diffusion gradient that causes spin dephasing relative to position along the gradient. Application of a 180° refocusing pulse and a second diffusion gradient will realign the spins of static tissue to an equal but opposite net magnetisation vector. For spins within a steadily flowing medium, this pulse sequence will lead to a net phase shift. However, in diffusion imaging, the random movement of the diffusing spins results in phase dispersion within each voxel. The vector sum of the spins in the presence of phase dispersion leads to decreased net magnetisation, and thus signal loss. (Figure 5).

Diffusivity can be calculated from signal loss and diffusion weighting using the Stejskal-Tanner equation (9).

The key limitation of basic SE is cardiac motion occurring during the diffusion weighting, which being around three orders of magnitude greater than the distances diffused by water molecules, leads to large artefactual signal drop-out. This was first addressed by bipolar diffusion gradients to offer first-order motion (constant velocity) compensation (20). Second and third (acceleration and jerk) order motion compensation techniques were then developed, although these require high performance gradient systems (Figure 6a) (14, 21-22). Third order motion compensation addresses the more complex diastolic motion, but requires longer echo times, so second order motion compensation (M2-SE) is considered optimal. Thus, M2-SE data is typically acquired in systole (23-24). Validation using in-vivo and post-mortem M2-SE DT-CMR in pigs has shown good levels of agreement (25). An M2-SE approach is attractive and growing in popularity because of the higher signal-noise ratio (SNR) than alternative methods, its suitability for free-breathing acquisition and more efficient whole heart coverage and thus it has been used in
several initial human studies (14, 22, 26-27). A variation on M2-SE is a diffusion prepared method that allows a flexible choice of image acquisition (22).

A leading alternative sequence type is stimulated echo acquisition mode (STEAM) and this addresses bulk motion artefact by splitting the $180^\circ$ pulse into two $90^\circ$ pulses, so that the time over which diffusion is measured ($\Delta$) is an R-R interval and each image is acquired over two cardiac cycles (Figure 6b). Data acquisition is synchronised so that the heart is in the same position when both diffusion gradients are applied, thus minimising the effects of cardiac motion. STEAM has been the mainstay of recent clinical DT-CMR studies as it does not require specialist hardware or motion compensation. Since its initial success in producing DW-CMR images, STEAM has been readily applied to DT-CMR in both healthy and diseased hearts (8, 12, 28–30). However, the wide applicability of STEAM is offset by several disadvantages. It has low SNR, requires two regular R-R intervals per image, necessitating longer breath-holding or a navigator-led approach and excludes patients with arrhythmias (31). Another disadvantage of STEAM in measuring diffusion is the effect of strain.

**The confounding effects of strain**

Strain, a measure of myocardial deformation, has been proposed as a confounder of DT-CMR results (28, 32). Myocardial deformation occurs during the diffusion time of both sequences, but the longer diffusion time in STEAM make its effects more significant. Reese et al. originally described this phenomenon explaining how tissue compression, in the direction of and following the initial STEAM gradient phase encoding, would have the effect of increasing the spatial modulation frequency and signal attenuation and hence apparent diffusion coefficient (32). An alternative simplified visual representation of how strain will affect diffusion measures is shown
in Figure 7. Diffusion within a medium that is compressed and then stretched back to its original state results in protons that travelled further away from the starting point, compared with the stationary case thus overestimating diffusivity. Several correction methods for STEAM sequences exist including bipolar gradients (33) or acquiring data at the ‘sweet spot’, the timepoint where the effects of the strain history over the cardiac cycle are nulled (34). A post-processing correction technique uses macroscopic strain data (35). However, although strain has been considered a significant confounder to DT-CMR findings for many years (especially systolic E2A and E2A mobility), two recent studies have shown strain has only a limited effect and that uncorrected in-vivo DT-CMR data is more representative of ex-vivo findings (8, 36). Consequently, the dynamic, laminar and semi-permeable nature of the myocardium means strain has a complex, but limited effect on measured diffusion and requires further study (29, 36–37).

Analysing the data

The eigensystem

The 3D diffusion described by a tensor can be pictorially represented. Whilst isotropic diffusion in an unrestricted environment is a sphere, the restricted diffusion in the heart is an ellipsoid (Figure 8). The lengths of the 3D axes of the ellipsoid are labelled $\lambda_1, \lambda_2, \lambda_3$ (eigenvalues) in order of magnitude of diffusion, and their directions are E1, E2 and E3 (eigenvectors) respectively.

Eigenvectors

The eigenvectors can be projected onto cardiac planes (Figure 9) and several validation studies support the correspondence of E1 with average intravoxel cardiomyocyte orientation, E2 with
the predominant sheetlet orientation and E3 with the sheetlet-normal direction (table 1 in supplementary material) (5, 8, 38–43).

**Helix angle**

The primary eigenvector, the long axis of the diffusion ellipsoid, is projected onto the circumferential-longitudinal plane and reflects the mean intravoxel cardiomyocyte orientation (8, 28, 39). The angulation created by the projected primary eigenvector and the circumferential direction is E1A but is often described as the helix angle (HA), or $\alpha$ in some older literature. HA changes only a little during cardiac contraction (8).

**Transverse angle**

The transverse angle (TA) or imbrication angle reflects the tilt of the mean cardiomyocyte orientation towards/away from the central LV axis. It is derived by projecting E1 into the circumferential-radial plane and then calculating the angle with the circumferential direction (38, 43).

**Secondary eigenvector angulation**

The secondary eigenvector is projected onto the cross-myocyte plane (Figure 9). The angulation between E2 projected into this plane and the myocyte-perpendicular direction is termed E2A and is an index of sheetlet orientation (8). Unlike E1A, E2A orientation changes substantially between systole and diastole. This change is called E2A mobility and reflects sheetlet function (8).
**Tertiary eigenvector angulation**

The tertiary eigenvector angulation (E3A) is perpendicular to the sheetlet plane. E3 is projected onto the longitudinal-radial plane and is positive when directed apico-basally (upward).

**Mean diffusivity**

In DT-CMR, mean diffusivity (MD) is derived from the tensor (19). It is the mean of $\lambda_1$, $\lambda_2$ and $\lambda_3$ and measured in units of mm$^2$/s. MD reflects the packing and integrity of the myocytes, with low values indicating tight packing. MD rises with interstitial abnormality such as fibrosis as the water has more freedom to diffuse (26, 44). MD is similar to the mean ADC from DW-CMR.

**Fractional anisotropy**

Fractional anisotropy (FA) is a scalar value reflecting the degree, but not direction of anisotropy. Isotropic diffusion has an FA value of 0 and diffusion restricted to a single direction has a value of 1 (Figure 10). FA reflects the degree of organisation of a tissue, such that a higher value is more organised. Thus, a low FA might be expected with myocyte disarray, should it occur at an appropriate value relative to pixel size.

**Visualising DT-CMR findings**

Tractography provides three-dimensional visualisation, typically tracking E1 across voxels into a smooth trajectory. In reality, this is a simplification as the myocardium does not contain serially aligned cardiomyocytes. Another representation uses superquadric glyphs, which are three-dimensional bricks derived from the tensor that convey pixelwise information about the shape
and orientation of the eigensystem (45). The orientation and size of the three axes of the glyphs represent the eigenvectors and eigenvalues respectively.

Limitations of DT-CMR

Acquiring and interpreting DT-CMR data is hampered by technical issues (46). Success rates for image acquisition vary by sequence type and cardiac phase and can be affected by factors such as mis-triggering and cardiac and respiratory motion (24). Scan times can be lengthy due to multiple acquisitions in different directions.

DT-CMR is limited in both spatial and angular resolution. Current in-vivo spatial resolution is typically $2.7 \times 2.7 \text{mm}^2 \times 6 \text{mm}$ for M2-SE and $2.8 \times 2.8 \text{mm}^2 \times 8 \text{mm}$ for STEAM (8, 14). Each DT-CMR image voxel contains millions of cardiomyocytes and many sheetlets. These are not uniformly aligned, and counter-orientated sub-populations exist (5). DT-CMR techniques are not able to resolve multiple populations within a single voxel, so the derived values reflect averages. It is also possible that partial volume effects may be responsible. Techniques such as de-noising and different k-space trajectories may aid reducing voxel size but help preserve SNR.

DT-CMR OF THE HEALTHY HEART

Clinical applications of DT-CMR are in relative infancy, but the unique ability to provide in-vivo microstructural information non-invasively has led to some describing it as ‘virtual histology’. The techniques are in constant evolution, and whilst measurement of myocyte and sheetlet orientations are reproducible and stable, the more global measures such as FA and MD show variation with technical sequence parameters and acquisition, mostly due to the varying diffusion times (24, 27).
Diffusivity parameters

MD and FA are commonly reported DT-CMR parameters but vary depending upon sequence type, cardiac phase and b-value (24, 47-48). In-vivo human studies comparing M2-SE and STEAM report higher MD and lower FA using M2-SE (24, 27). This result is expected because with a shorter diffusion time, water molecules encounter fewer restrictive barriers (Figure 11). Reported values for MD and FA are provided in the Supplementary material. MD and FA tend to be provided as global values, usually across a mid-ventricular slice. However, regional differences have been described: FA peaks in the mesocardium (0.46 ± 0.04), compared to the endocardium (0.40 ± 0.04) and epicardium (0.39 ± 0.004), possibly due to the relative plateau in HA due to the circumferentially oriented mesocardial cardiomyocytes (48). For MD, there is a transmural gradient with MD of 0.87 ± 0.07 x 10^{-3} \text{mm}^2/\text{s} at the epicardium, increasing to 0.91 ± 0.08 x 10^{-3} \text{mm}^2/\text{s} in the endocardium. This may relate to laminar structure of the myocardium, as sheetlets are increasingly prevalent toward the endocardium (49).

Cardiomyocyte orientation

Various DT-CMR studies identify the transmural HA gradient in cardiomyocyte orientation (Table 1 in supplementary material) and report only minor changes in cardiomyocyte orientation towards a more longitudinal systolic orientation, shown in Figure 12 (8, 35, 50). Transverse angle lies in the region of -20° to +20°, at both cardiac phases (43).

Sheetlet orientation
E2A is used as a DT-CMR index of sheetlet orientation, although E3 is used by some authors. E2A changes substantially through the cardiac cycle (8). Figure 13 shows how sheetlets adopt a wall-parallel sheetlet orientation in diastole (blue colour), which becomes more wall-perpendicular in systole (red). This reorientation is intimately linked to the generation of radial strain. A small movement in the longitudinal direction translates into a larger radial expansion, similar to the perpendicular amplification action of a “lazy tong” (S1). This is demonstrated by glyphs in Figure 13 and shown in the Supplementary video. Biphasic differences in E2A have been described; a STEAM study of healthy humans reported diastolic E2A of 26° ± 6° and systolic E2A of 54° ± 6°, with E2A mobility of 27° ± 8° (51). A different STEAM study using E3A reports sheet angles of 65° ± 3.6° and 41.6° ± 3.7° in diastole and systole respectively (35).

MICROSTRUCTURAL CHANGES IN CARDIAC DISEASE

Myocardial infarction (MI)

DT-CMR has been applied in MI, with much interest in acute changes and post-infarct ventricular remodelling. Several animal and human studies describe increased MD and decreased FA in the infarct zone (26, 52–55). This accords with myocyte swelling and necrosis, extracellular matrix expansion and replacement by less organised collagenous fibrosis. Ex-vivo porcine DT-CMR thirteen weeks after iatrogenic MI revealed increased mean ADC (1.01 ± 0.100 x 10³ mm²/s) compared to controls (0.671 ± 0.106 x 10³ mm²/s, p<0.001) in the infarct zone (55). FA was significantly reduced in the infarct from 0.32 ± 0.01 to 0.20 ±0.03, p<0.001. In the human study by the same group 26 days post MI, DT-CMR detected increased mean ADC, decreased FA, decreased RH and increased LH helical structures (cardiomyocytes), compared to the remote zone (52). The follow-up human study at median 191 days after MI
describes increased percentage RH cardiomyocytes in the remote zone, which correlated with increased wall thickness \((r=0.66, p=0.005)\) and increased wall thickening in the adjacent zone \((r=0.61, p=0.01)\) (56). The proposed explanation was that loss of RH cardiomyocytes fits with susceptibility of the endocardium to ischaemia and gain of LH cardiomyocytes in the infarct zone and RH cardiomyocytes in the remote zone are both adaptive remodelling responses.

Most recently, a study of MI in mice, sheep and humans introduced the tractographic propagation angle (PA), defined as the angle between two adjacent primary eigenvectors along a given tract and a metric of ‘myofibre curvature’, as shown in Figure 14 (57). A PA value of \(4^\circ\) was reported as the threshold for differentiating normal from infarcted myocardium, as correlated to late gadolinium imaging and endocardial voltage mapping. Understanding microstructural changes post infarct could offer insight into the pathogenesis and prognostication of negative remodelling and arrhythmias.

**Hypertrophic cardiomyopathy (HCM)**

A microscopic hallmark of HCM is cardiomyocyte disarray, detectable currently only by histology. It is hoped that DT-CMR might detect disarray non-invasively, aiding diagnostic differentiation from HCM phenocopies and potentially identify those at higher risk of arrhythmias. A study of 5 patients suggested DT-CMR appeared to detect disarray by reduced FA and lower strain in the septum compared to the free wall of HCM patients and 5 healthy controls (58). A reproducibility study in HCM reported regional differences where FA was lowest and MD highest in the septum, though only the latter was significant (59). Whilst this may relate to septal hypertrophy and expanded extra-cellular space due to fibrosis and disarray,
technical factors such as spatial variation in SNR may also be responsible and histological validation was absent. A preliminary study suggested FA was focally reduced in HCM patients compared to controls and that DT-CMR may be able to detect myocardial disarray (60).

DW-CMR can offer another type of myocardial characterisation in HCM. In a study of 23 HCM patients, mean ADC was elevated in areas of fibrosis and correlated with ECV ($r^2 =0.72$), suggesting that DW-CMR may detect the extent of fibrosis without a contrast agent (Figure 15) (44). However, in-vivo DT-CMR has demonstrated an entirely novel abnormality in HCM. Diastolic E2A is significantly elevated and E2A mobility is reduced (Figure 16) (29). The failure of sheetlets to return to a more wall-parallel orientation in diastole can be described as a ‘failure of diastolic relaxation’. Interestingly this microstructural abnormality builds upon our knowledge of HCM pathophysiology, in which sarcomeric mutations may result in increased myofilament sensitivity to calcium, increasing relative cardiomyocyte tension (S2).

**Dilated cardiomyopathy (DCM)**

DCM has also been studied using DT-CMR. A SE study of hamsters with DCM found no change in HA or TA, but elevated MD and reduced FA compared to controls (S3). An in-vivo STEAM study of 9 non-ischaemic DCM patients and controls found MD was similar between groups and cardiac phases (30). FA was lower in DCM compared to controls (diastole $0.56 \pm 0.07$ versus $0.63 \pm 0.05$, p<0.04 and systole $0.58 \pm 0.08$ versus $0.62 \pm 0.07$, p=0.56). The transmural HA gradient in diastole was steeper in DCM patients, though this difference seems to have been driven by two patients. There was systolic HA redistribution in controls, with the expected more longitudinal orientation, but this was not seen in DCM. E2A findings are shown in Figure 17.
Biphasic E2A changes in controls (panels A and C) showed predominance of low diastolic E2A and a broader systolic distribution. However, there was relatively little change in the E2A distribution for DCM patients (panels B and D). The findings of this study must be tempered by its limitations; only 9 patients without LV dilatation and with strain correction which may have obscured altered sheetlet mobility.

Nielles-Vallespin et al studied patients with DCM and HCM, and controls and did not employ strain correction (8). They found similar HA distributions in the three cohorts. HA shifted towards more longitudinal in systole for all groups. Diastolic E2A was similar between groups, but systolic E2A findings were abnormal in DCM. In controls median systolic [IQR] E2A was 65 [6]° and in HCM 74 [7]°. In DCM, systolic E2A was significantly reduced at 40 [16]°, yielding a reduced E2A mobility and impaired sheetlet mobility, depicted in glyph form in Figure 18. Both radial and circumferential strains were significantly and similarly reduced in the cardiomyopathy groups compared to controls. However, the range of E2A (low-low in DCM and high-high in HCM) provides mechanistic insight into these findings. This study was limited by the mean EF in the DCM group of 45 ± 11%, with approximately half of the patients having only mild LV impairment. However, despite this, the altered sheetlet behaviour was statistically significant.

Having established microstructural abnormalities in DCM, the same group studied patients with recovered DCM (R-DCM) and compared them to DCM and healthy controls (61). These patients were defined by LV size and EF entirely within indexed reference ranges and New York Heart Failure Association Classification 1. Again, there was little difference in the HA histograms between the three cohorts, but significant findings were present in E2A. Diastolic E2A was similar between groups. However systolic E2A in R-DCM was 59 [14]°; significantly greater
than the DCM group (35 [17]° (p<0.0001), but lower than the controls (65 [8]°, p=0.01). When E2A mobility was plotted against the EF, R-DCM and controls were indistinguishable by EF, but could be discriminated by E2A (Figure 19).

Together these two studies suggest that DT-CMR can detect impaired sheetlet behaviour in dilated cardiomyopathy that appears to persist even when LV size and EF recover to normal by conventional measures.

**Congenital heart disease**

There is a paucity of DT-CMR data in congenital heart disease. In an adult with transposition of the great arteries corrected by arterial switch, DT-CMR showed predominance of longitudinal and oblique fibres in the systemic right ventricle, thought to be an adaptive response to the systemic pressure and load (S4). A larger study of 12 patients with situs inversus totalis (SIT), 12 healthy situs solitus (SS) controls and two ex-vivo SIT hearts has shown deranged cardiomyocyte orientation, sheetlet abnormalities and functional impairment in SIT (62). All SS hearts displayed the expected helical arrangement. By contrast, in SIT HAs were more heterogeneous, both intra- and inter-subject. However, the general pattern was an inverted HA arrangement at the base, with positive HAs in the epicardium and negative HAs in the endocardium, while towards the apex the transmural HA distribution was more similar to SS. There was a mid-ventricular transition zone. These findings were confirmed in the ex-vivo hearts. Mid-LV peak radial and circumferential strain were reduced and overall absolute torsion was significantly reduced in SIT. This was the first in-vivo demonstration of substantial departure from the typical mammalian helical cardiomyocyte arrangement (Figure 20). In
addition to the abnormal cardiomyocyte arrangement in SIT, there was also impaired sheetlet mobility with failure to achieve the expected systolic orientation. This may reflect cardiomyocyte derangement affecting the transverse shears that are integral to sheetlet reorientation (6). These findings reinforce the connection between the microstructure and cardiac mechanics. The abnormal cardiomyocyte structure in SIT raises the question of whether such patients might have a greater susceptibility to heart failure with a “second hit” to function such as hypertension, infarction or myocarditis.

**FUTURE DIRECTIONS OF DT-CMR**

DT-CMR can identify microstructural changes in disease. Further work is required to broaden the applicability and utility of the technique. Two main streams of future development are needed; technical and clinical. Technical developments would address spatial and angular resolution, scan acceleration, increased coverage and refining the understanding of strain effects. Clinical development might see DT-CMR playing a role as a diagnostic and prognostic tool. The ability to detect microstructural changes before manifest disease expression may offer a role for earlier diagnosis in the cardiomyopathies. It may also help identify patients at risk of developing heart failure, not only in cardiomyopathy, but also congenital and ischaemic heart disease. DT-CMR may inform the debate on whether DCM patients whose ejection fraction is improving have achieved recovery or remission. In HCM, the ability (if possible in the future) to detect disarray in-vivo could assist risk stratification for predicting sudden cardiac death. Similarly, identifying those at risk of potentially life-threatening arrhythmias after infarction is another area in which DT-CMR has the potential to offer novel prognostic information. Expansion to other
cardiomyopathies and congenital heart disease may offer new pathophysiological insight into these conditions.

CONCLUSION

DT-CMR is establishing itself as a technique that can interrogate the cardiac microstructure and its relation to function. For the first time, it is possible to assess cardiomyocyte and sheetlet structure and microstructural dynamics using a non-invasive approach. DT-CMR provides insight into pathological changes in disease and with this new understanding it has the potential to progress to a clinically useful tool aiding diagnosis, prognosis and guiding patient management.
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FIGURE LEGENDS

**Figure 1: Helical arrangement of cardiomyocytes in the mammalian heart**
Dissection plates showing the transmural evolution of the helical arrangement of cardiomyocytes from left-handed helix in the epicardium (top left) through circumferential in the mesocardium (bottom left) to right-handed helix in the endocardium (bottom right). Reproduced from Pettigrew. (S5)

**Figure 2: Transmural variation in cardiomyocyte orientation**
Left: Photomicrograph showing cardiomyocyte orientation smoothly progresses from a positive angle at the endocardium through to a negative angle at the epicardium. Right: Graphical representation of helix angle through the myocardial wall from epicardium (negative) to endocardium (positive). Reproduced from Streeter et al. (2)

**Figure 3: Cardiomyocytes aggregated into sheetlets**
Scanning electron micrograph of the transverse cut surface of an LV midwall section shows cardiomyocytes aggregated into sheetlets, separated by perimysial connective tissue. Adapted from Le Grice et al. [3]

**Figure 4: Diffusion is modulated by its environment**
Free diffusion is unrestricted, but in the myocardium can be restricted within cells (green), may traverse a permeable cell membrane (red) or take a hindered path within the extracellular space (blue), rendering it anisotropic. Adapted from Le Bihan (S6)

**Figure 5: Diffusion is measured by signal loss**
The DT-CMR sequence uses an excitation pulse followed by a diffusion gradient, a refocussing pulse and a second diffusion gradient. Static H⁺ spins are subject to equal dephasing and rephasing (A), whilst flowing spins experience phase shift (B). With random H⁺ displacement there is incomplete rephasing and signal attenuation (C). Reproduced with permission from Froeling et al. (S7)

**Figure 6: Diffusion tensor sequences**
Second-order motion compensated spin echo sequence (A) The sequence is triggered by the R wave and has excitation and refocussing pulses. Δ is the “diffusion time” over which the measured diffusion takes place (typically 20-30ms). Stimulated echo acquisition mode DT-CMR sequence (B) The sequence runs across two cardiac cycles; the diffusion time (Δ) is one R-R interval, whilst TM (mixing time) reflects the duration of the magnetisation storage in the longitudinal plane between the second and third pulses. Modified from Scott et al. (24).

**Figure 7: Effects of strain in an isotropic phantom**
Deformation of an isotropic substance over the diffusion time affects the measured diffusion result. In the upper panel, a systolic measurement is affected by the intervening diastolic stretch, and in the lower panel a diastolic measurement is affected by the intervening systolic compression. For both the diffusion pattern is ovoid in shape, rather than the expected isotropic sphere. Reproduced from Ferreira et al. (36)
**Figure 8: Pictorial representation of diffusion tensors**
Isotropic diffusion is shown as a sphere (left), but restricted diffusion is represented as an ellipsoid shape in DT-CMR (right). $\lambda$ signifies the eigenvalue (magnitude of diffusion) and $E$ denotes the eigenvector (direction of diffusion) along the three axes.

**Figure 9: Projections of eigenvectors on cardiac planes**
Three myocardial voxels are shown. The primary eigenvector ($E_1$) projected on the circumferential-longitudinal plane gives the helix angle (A). $E_1$ projected on the circumferential-radial plane gives the transverse angle (B). The voxels are cut in a cross-myocyte direction, showing the plane perpendicular to $E_1$ (C). Projecting the second eigenvector ($E_2$) onto this sheetlet plane gives $E_2A$ (D). Adapted from Ferreira et al. (29)

**Figure 10: Increasing fractional anisotropy**
FA in the heart reflects the underlying myocyte organisation, such that a value towards zero reflects little organisation and a value nearer 1 reflects a more organised structure (packed and anisotropic).

**Figure 11: Expected diffusion radii of free water**
Stimulated echo acquisition mode has a longer diffusion time ($\Delta$) than spin echo. The diffusion radii of free water over these timeframes are superimposed upon pig myocardium, cut perpendicular to the long axis of the cardiomyocytes. More barriers are encountered during the longer $\Delta$. Reproduced with permission from Scott et al (24)

**Figure 12: Helix angle in diastole and systole**
Diffusion tensor tractography of the lateral ventricular wall at end-diastole and end-systole. Negative epicardial helix angles are more red and the positive endocardial helix angles are more blue. At end-systole the cardiomyocytes are more longitudinally orientated. Reproduced from Mekkaoui et al (50)

**Figure 13: Secondary eigenvector $E_2A$ reflects sheetlet orientation**
Absolute $E_2A$ changes through the cardiac cycle, rising from diastole (low $E_2A$ - blue) to systole (higher $E_2A$ - red). Biphasic $E_2A$ variation is seen in-vivo (A) and corresponds to the histological findings of sheetlet orientation shown in panel B. Adapted from Nielles-Vallespin et al. (8)

**Figure 14: Propagation angle is elevated in areas of infarction**
(A) Propagation angle (PA) is a metric of myofiber curvature, (B) PA in healthy human hearts is uniformly typically $<4^\circ$, (C) in a sheep heart with myocardial infarction remote and infarcted areas are delineated by PA less and greater $4^\circ$ than respectively. Reproduced with permission from Mekkaoui et al. (S8)

**Figure 15 Diffusivity is elevated in areas of fibrosis**
In hypertrophic cardiomyopathy apparent diffusivity coefficient is elevated in regions of fibrosis, as detected by late gadolinium (LGE) and increased extra-cellular volume (ECV). Reproduced from Nguyen et al. (45)
Figure 16 E2A abnormalities in hypertrophic cardiomyopathy
Example E2A maps of a mid-ventricle slice. The healthy control has a blue (low E2A) map in diastole and a red (higher E2A) map is systole. In hypertrophic cardiomyopathy maps are predominantly red, displaying elevated E2A at both cardiac phases. The plot below shows that diastolic E2A is significantly elevated compared to the healthy controls, as is systolic E2A. Adapted from Ferreira et al. (29)

Figure 17: E2A findings in dilated cardiomyopathy
Panels A and B show E2A histograms for controls and dilated cardiomyopathy. Diastolic and systolic distributions are shown by solid and dashed lines respectively. Panels C and D show change from the expected computer model results. Reproduced from von Deuster et al. (30)

Figure 18: Abnormal sheetlet reorientation in dilated cardiomyopathy
Sheetlet planes are defined by the primary and secondary eigenvectors (E1 and E2) as shown on the top-right inset. The panels show the septal glyphs, colour-coded according to absolute E2A (blue towards wall-parallel and red towards wall-perpendicular). Healthy controls show a more wall-parallel alignment in diastole and more wall-perpendicular alignment in systole. However, in hypertrophic cardiomyopathy sheetlets retain the more wall-perpendicular arrangement of systole in both cardiac phases. Conversely in dilated cardiomyopathy the more wall-parallel arrangement of diastole persists in both cardiac phases. Replotted from Nielles-Vallespin et al. (8)

Figure 19: E2A mobility and ejection fraction plot in recovered dilated cardiomyopathy
Secondary eigenvector (E2A) mobility plotted against left ventricle ejection fraction (LV EF). Dilated cardiomyopathy (DCM) patients have low LV EF and low E2A mobility. Both controls and recovered DCM groups have normal EF, but the recovered DCM group can be discriminated by their significantly lower E2A mobility. Reproduced from Khalique et al. (61)

Figure 20: Left ventricle tractography in situs inversus
Left ventricle tractography of a situs solitus (SS) and situs inversus (SIT) heart. The contrast between the left-handed helix coloured blue in the SS heart, and the right-handed helix in red in the SIT heart is particularly striking. Modified from Khalique et al. (62)

Central Illustration: Diffusion tensor CMR non-invasively identifies microstructures in the myocardium.
(Left) DT-CMR demonstrates sheetlets of cardiomyocytes that reorient from wall-parallel in diastole to wall-perpendicular in systole, facilitating the left ventricular wall thickening that greatly exceeds that of individual cardiomyocytes. In cardiomyopathy abnormal sheetlet behaviour is identified; in hypertrophic cardiomyopathy, sheetlets fail to relax to the expected diastolic position and conversely in dilated cardiomyopathy sheetlets fail to rotate to the expected systolic position. (Right) DT-CMR demonstrates the helical arrangement of cardiomyocytes, with a right-handed helix in the endocardium, circumferential in the mesocardium and left-handed helix in the epicardium. Contraction of these oppositely oriented myocytes drives clockwise rotation basally and anti-clockwise rotation apically, resulting in left ventricular torsion. In congenital conditions, such as situs inversus this typical pattern is deranged with impaired torsion.
VIDEO LEGEND

**Microstructural dynamics through the cardiac cycle.**
Microstructural dynamics through the cardiac cycle Schematic video of sheetlets colour-coded according to the helix angle (HA), reorienting from diastole to systole. The sheetlet angle is defined as the angle between the sheetlet and the epicardial wall. While HA does not change significantly, the sheetlet angle increases in systole. These microstructural dynamics demonstrate how wall thickening far exceeds that of any single cardiomyocyte.
A IN VIVO: DT-CMR AT DIFFERENT TIMES OF THE CARDIAC CYCLE

B HISTOLOGY

RELAXED (KCl ARRESTED)  CONTRACTED (BaCl₂ ARRESTED)
A Propagation Angle

B Normal Human

C Infarcted Sheep

C₁ Remote Zone
PA < 4°

C₂ Infarcted Region
PA > 4°
DIFFUSION TENSOR CMR REVEALS IN-VIVO MICROSTRUCTURE IN HEALTH

DYNAMIC SHEETLET REORIENTATION WALL THICKENING

DIASTOLE

SYSTOLE

Histology

Histology

HEMICAL ARRANGEMENT OF MYOCYTES TORSION

clockwise rotation

Anti-clockwise rotation

Endocardium

Mesocardium

Epicardium

Histology

Histology

MICROSTRUCTURAL CHANGES IN DISEASE

HCM - FAILURE OF DIASTOLIC RELAXATION

DCM - FAILURE OF SYSTOLIC REORIENTATION

SITUS INVERSUS - DERANGED HELICAL PATTERN

Endocardial

Epicardial
Supplementary References for Main Manuscript


<table>
<thead>
<tr>
<th>Author, Year</th>
<th>Sequence</th>
<th>State</th>
<th>Species</th>
<th>Phase</th>
<th>Parameters</th>
<th>Findings</th>
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<tbody>
<tr>
<td>Hsu et al., 1998</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Dog</td>
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<td>DT-CMR: Helix angle ($\alpha_{\text{MRI}}$)</td>
<td>Excellent correlation between $\alpha_{\text{MRI}}$ and $\alpha_{\text{hist}}$. Average difference of -2.30° ± 0.98 on a point basis, and correlation coefficient of 0.942 ± 0.008 for transmural rotation of HA.</td>
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<td>Histology: Fibre angle ($\alpha_{\text{hist}}$)</td>
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<tr>
<td>Scollan et al., 1998</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Rabbit</td>
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<td>DT-CMR: Helix angle $\alpha$ (inclination angle), Transverse angle $\phi$</td>
<td>$\alpha$ is similar by DT-CMR and histology; correlation coefficient of 0.95 ± 0.15 and transmural variation consistent with previous reports. Average difference between histology and DT-CMR of 4.9°. Transverse angle of 7.9°, consistent with previous reports.</td>
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<tr>
<td>Holmes et al., 2000</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Rabbit</td>
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<td>DT-CMR: Helix angle $\alpha$ (inclination angle)</td>
<td>Good agreement between $\alpha$ from histology and DT-CMR with average difference of -3.7° and correlation coefficient of 0.979</td>
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<tr>
<td>Scollan et al., 2000</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Rabbit</td>
<td></td>
<td>DT-CMR: Helix angle $\alpha$ (inclination angle)</td>
<td>Expected transmural gradient of $\alpha$. Root mean-squared error of 5.3° ±5.1° between DT-CMR and histology. Qualitative agreement between E2 and E3 with sheet and sheet normal.</td>
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<td>Study</td>
<td>Modality</td>
<td>Imaging Type</td>
<td>Animal</td>
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<td>Tseng et al., 2003</td>
<td>STEAM</td>
<td>Ex-vivo</td>
<td>Cow</td>
<td>DT-CMR: E1, E2, E3</td>
<td>Root mean-squared angular disparity between DT-CMR findings and inked print of myocardium (approximates to 10x magnification) 11° for E1 and fibre direction, 14° for E2 and E3 with the sheet and sheet-normal directions.</td>
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<td>Ink print/ histology: fibre direction, sheet direction, sheet-normal direction</td>
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<tr>
<td>Chen et al., 2005</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Rat</td>
<td>DT-CMR: Helix angle ($\alpha_h$), transverse angle ($\alpha_t$) and sheet angle via the secondary eigenvector ($\beta_s$)</td>
<td>$\Delta\alpha_h$ (endo-epi) increased from diastole to systole, (105° ± 6° in PA, 111° ± 8° in BV+ and 134° ± 4° BV-) due to more longitudinally oriented epi-and endocardial fibres in systole.</td>
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<td>Histology: Sheet cleavage angle ($\beta_s$) (between the cleavage plane and direction normal to the epicardium)</td>
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<td>$\alpha_t$ was between -20° and +20° in all states.</td>
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<td>Kung et al., 2011</td>
<td>Spin echo</td>
<td>Ex-vivo</td>
<td>Sheep</td>
<td>DT-CMR: Myofiber angle $\alpha_{DT}$, sheet angle of $\beta_{DT}$ from E2 and $\beta'_{DT}$ from E3</td>
<td>$\alpha_{DT}$ and $\alpha_{HT}$: mean difference of 1° ± 16°</td>
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<td>Histology: Myofiber angle $\alpha_H$, sheet angle $\beta_H$ and $\beta'_{HT}$</td>
<td>Sub-populations of sheets, defined by E2 and E3 projections on the Overall mean difference of 8° ± 27° between ($\beta_{DT}$ and $\beta'<em>{DT}$) and ($\beta_H$ and $\beta'</em>{HT}$)</td>
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</table>
| Nielles-Vallespin et al., 2017 | STEAM | In-vivo, in-situ and ex-vivo | Pig | Potassium arrested (end-diastole)  
Barium arrested (end-systole) | DT-CMR: Helix angle (E1A) and E2A (sheetlet angle)  
Histology: helix angle and sheetlet angle (SA) | HA has lower magnitude of changes from diastole to systole. E1A range correlates well with HA range (r=0.92, p<0.0001)  
In-vivo: 90° → 96°, in-situ arrested 75° → 118°  
Ex-vivo: 89° → 109°, histology HA 72° → 73°  
E2A increases from diastole to systole  
In-vivo: 13° → 59°, in-situ arrested: 15° → 59°  
Ex-vivo: 18° → 62°, Histology SA: 30° → 75° |
Table 2: Diffusivity parameters obtained in-vivo in healthy subjects

<table>
<thead>
<tr>
<th>Author Year</th>
<th>Sequence</th>
<th>B value (s/mm(^2))</th>
<th>Timing</th>
<th>Mean diffusivity (x 10(^{-3})mm(^2)/s)</th>
<th>Fractional Anisotropy</th>
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<tbody>
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<td>Reese et al., 1995</td>
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<td>300</td>
<td>-</td>
<td>0.87 ± 0.13</td>
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<td>Nielles-Vallespin et al., 2013</td>
<td>STEAM-EPI</td>
<td>350</td>
<td>Systole</td>
<td>0.8 ± 0.2</td>
<td>0.6 ± 0.2</td>
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<td>Tunnicliffe et al., 2014</td>
<td>STEAM-EPI</td>
<td>350</td>
<td>Diastole</td>
<td>1.20 ± 0.09</td>
<td>0.54 ± 0.03</td>
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<td>Systole</td>
<td>1.10 ± 0.06</td>
<td>0.41 ± 0.04</td>
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<td>Lau et al., 2015</td>
<td>STEAM-EPI</td>
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<td>1.19 ± 0.16</td>
<td>0.48 ± 0.08</td>
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<td>Moulin et al., 2016</td>
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<td>1.72 ± 0.09</td>
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<td>Scott et al., 2015</td>
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<td>750</td>
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<td>McGill et al., 2016</td>
<td>STEAM-EPI</td>
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<td>0.47 ± 0.05</td>
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<td>Diastole</td>
<td>1.11 ± 0.13</td>
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<td>von Deuster et al., 2016</td>
<td>STEAM-EPI</td>
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<td>Sweet-spot</td>
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<td>0.61 ± 0.04</td>
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<td>Systole</td>
<td>1.46 ± 0.43</td>
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Supplementary Material References


