Study of myocardial fiber pathway using magnetic resonance diffusion tensor imaging

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Abstract

The purpose of this study was to investigate myocardial fiber pathway distribution in order to provide supplemental information on myocardial fiber architecture and cardiac mechanics. Diffusion tensor imaging (DTI) with medium diffusion resolution (15 directions) was performed on normal canine heart samples (N=6) fixed in formalin. With the use of diffusion tensor fiber tracking, left ventricle (LV) myocardial fiber pathways and helix angles were computed pixel by pixel at short-axis slices from base to apex. Distribution of DTI-tracked fiber pathway length and number was analyzed quantitatively as a function of fiber helix angle in step of 9°. The long fiber pathways were found to have small helix angles. They are mostly distributed in the middle myocardium and run circumferentially. Fiber pathways tracked at the middle and upper LV are generally longer than those near the apex. Majority of fiber pathways have small helix angles between −20° and 20°, dominating the fiber architecture in myocardium. Likely, such myocardial fiber pathway measurement by DTI may reflect the spatial connectiveness or connectivity of elastic myofiber bundles along their preferential pathway of electromechanical activation. The dominance of the long and circumferentially running fiber pathways found in the study may explain the circumferential predominance in left ventricular contraction.

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1. Introduction

It is well known that the myocardial fiber structure of the left ventricle (LV) plays a critical role in determining mechanical properties, such as ventricular torsion, strain and stress [1–3]. Such myocardial architecture is also known as a crucial determinant of electromechanical activations in heart [4]. Therefore, precise knowledge of the myocardial fiber structure of the left ventricular wall can lead to a better understanding of cardiac mechanics and electrical conduction in normal or pathologic states.

Most research on myocardial fiber structure focuses on fiber orientation, which is one of the critical determinants of myocardial wall motions and also represents the direction in which the current spreading is the most rapid [5,6]. Myocardial fiber orientation and structure was first studied by means of histological measurements [7,8]. The myocardial wall was found to have a well-ordered distribution of fiber helix angles varying from about 60° at the inner surface to about −60° on the outer surface and no dramatic change occurred during the transition from diastole to systole, despite a 28% increase in wall thickness [8]. However, the histological procedure was laborious and time consuming. In recent years, diffusion tensor magnetic resonance imaging (DTI) has emerged as a powerful tool for rapid measurement of cardiac geometry and fiber structure at high spatial resolution. This method is based on the assumption that water diffusion along the myocardial fiber is the greatest, and...
therefore the primary eigenvector of the diffusion tensor coincides with the local fiber orientation. Previous DTI studies of the myocardium mostly focused on fiber orientation. It has been shown that there is a direct correlation between DTI and histological fiber direction measurements [9–11], validating the DTI approach to the characterization of myocardial fiber orientation. These DTI studies show that when viewed from the apex, the orientation of left ventricular fibers changes smoothly from a left-handed helix in the epicardium to a right-handed helix in the endocardium, and transmural helix angle typically ranges from –60° at the epicardium to +60° at the endocardium [9–13] though a large variation between measurements exists. This myocardial fiber architecture is found to be essentially similar in humans [14] and other mammalian species [15]. Mathematical modeling shows that such double-helical arrangement of LV myocardial fibers is efficient for dispersing strain uniformly and conserving energy expenditure [16].

However, a complete description of the contractile behavior of cardiac muscle is much complicated because of the complex interplay between myocardial fiber orientation and direction of propagation of electrical activity. The close coupling of electrical and mechanical activation determines the regional and global mechanical contraction and relaxation. In general, all the mechanical quantities — the force, muscle length, shortening velocity and the acceleration of shortening — interact with one another [17]. Individual cardiac muscle length is known to be a key factor in characterizing the contractility of a single cardiac muscle, including the maximum tension born by the muscle [17]. Globally, there is a close coupling between myocardial fiber structure and electromechanical activation in LV [18,19]. The orientation of myocardial fiber and the distribution of preferential direction of electromechanical activation–myocardial fiber pathway can play a determinant role in the overall LV contraction. Therefore, investigation on myocardial fiber pathway may provide supplementary information on fiber architecture and cardiac mechanics, contributing to the full understanding of left ventricular structure and function [20].

This study aimed to examine the myocardial fiber pathway distribution in formalin-fixed normal canine heart samples. DTI was employed to track and measure the myocardial fiber pathway in the LV myocardium. Distribution of fiber pathway length and number as a function of fiber helix angle was investigated in multiple short-axis slices located from the base to the apex of LVs. The effect of the termination criteria used in DTI fiber tracking was also examined.

2. Materials and methods

2.1. Sample preparation

Normal canines (N=6, 30–35 kg) were anesthetized with isoflurane and their hearts were arrested in diastole by intravenous injection of potassium chloride. The hearts were excised rapidly and placed in a bath of cold (4°C) cardioplegic solution. The aortas were cannulated and the hearts were perfused retrogradely with 200 ml of St Thomas cardioplegic solution, followed by 500 ml of phosphate-buffered 2.5% glutaraldehyde and 4% formaldehyde at a pressure of 100 mm Hg. All perfusion was conducted at room temperature (18°C). The hearts were stored in formalin solution for at least 18 h to let the possible early ventricular geometry changes [9] occur before imaging.

2.2. Experiments

Studies were performed on a Philips 3T Achieva scanner (Philips Medical System, Best, Netherlands) which has a maximum gradient amplitude of 80 mT/m and a gradient switch rate of 200 mT/m per second. An eight-channel head coil was used for imaging. The formalin-fixed canine heart samples were suspended in a cylinder filled with formalin to avoid tissue-air susceptibility artifacts. Scout images were first acquired for right anterior oblique and nearly four-chamber planes, which were perpendicular to each other. Left-ventricle long axis was determined as the intersecting line of the two planes that divided the LV approximately into four equal quadrants from base to apex. Left-ventricle short-axis planes were prescribed as perpendicular to the LV long axis and covered the entire heart. DTI was performed using single-shot spin echo, echo planar imaging (SE-EPI). Sensitivity encoding (SENSE) was applied to shorten image acquisition time and to alleviate image distortion caused by susceptibility artifact from single-shot EPI sequences. SENSE factor was chosen to be 2.4 in this study to trade off between image distortion due to high EPI factor and SENSE artifact caused by high SENSE factor. Imaging parameters were as follows: TE=55 ms; TR=~5 s; FOV=140×140 mm²; slice thickness=1.13 mm; slice gap=0 mm; diffusion sensitivity of b=800 s/mm²; gradient direction=15; diffusion gradient duration Δ=10.5 ms; diffusion time δ=26.5 ms; and number of averages=40.

Fig. 1. Definition of helix angle α. It is the angle between the image plane and the projection of the primary eigenvector onto the tangent plane.
The primary eigenvector determined by DTI may deviate from the real fiber orientation if very small voxel size is used, leading to high noise in DTI raw data and compromising the eigenvector, fractional anisotropy (FA) and fiber tracking calculations. Very large voxel size may be too coarse to smoothly represent fiber trajectories [21]. Furthermore, isotropic resolution is desired in order to measure the fiber tracks in all directions without bias. In this study, images were acquired with a 128×128 data matrix. These parameters yielded an isotropic resolution of $1.13 \times 1.13 \times 1.13$ mm$^3$. Depending on the heart sample size, the number of short-axis slices was typically around 45 with no gap. Total image acquisition time was about 1 h per sample.

The fiber tracking procedure was based on two arbitrary termination criteria, i.e., maximum directional threshold representing control of angle-transition from voxel to voxel, and minimum FA magnitude threshold representing extent of anisotropy [21]. To determine their optimal values in our study of myocardial fiber pathways, their influence was assessed by examining the number and length of fiber pathways tracked with different termination thresholds for the same DTI data sets.

2.3. Data analysis

Three-dimensional reconstruction of myocardial fiber pathways was obtained by using the PRIDE software package (Philips Medical Systems, Best, Netherlands). Papillary muscles and right ventricles were removed in the images and excluded from the analysis. For each of the short-axis slices, the fiber was tracked pixel by pixel within

Fig. 2. LV myocardium T2-weighted images (first column), fiber helix angle map (second column) and myocardial fiber pathway length map (third column) of four representative short-axis slices from base to apex in one normal heart sample. The entire heart was covered typically by 45 short-axis slices from base to apex. The corresponding numbers of the four slices shown are 6, 16, 26 and 36.
the slice. A MATLAB (The MathWorks, Natick, MA, USA) program was developed to compute the fiber orientation and pathway length from the data generated from PRIDE software. The myocardial fiber orientation was calculated by first transforming the primary eigenvector from the Cartesian coordinates to the local cylindrical myocardial coordinates that are centered at the LV center. The epicardial tangent planes are defined as the planes tangent to the epicardial circle and parallel to the LV long axis as proposed by Scollan et al. [11] (Fig. 1). After determining the local myocardial coordinate system for each short-axis slice, fiber helix angle was calculated for each fiber tracked within the slice. Fiber helix angle is defined as the angle between the projection of the primary eigenvector onto the tangent plane and the short-axis imaging plane (Fig. 1). The distribution of number of fibers tracked within each slice and their average pathway length was calculated respectively as a function of the helix angle using 9° steps. Unless otherwise noted, all data are presented as means ± S.D.

3. Results

3.1. Fiber distribution

Fiber distribution was investigated with FA magnitude and directional thresholds set at 0.15 and 40°, respectively,

![Graphs showing fiber distribution](image-url)
and isotropic image resolution of 1.13×1.13×1.13 mm³. Fig. 2 illustrates the representative maps of the fiber helix angle and fiber pathway length computed at four short-axis slices (Slices 6, 16, 26 and 36) from base to apex. It shows that the fiber pathways are generally long and their helix angles are small within the middle myocardium. The results show that fiber helix angle typically changes from −80° to +80° from left-handed helix in the epicardium to right-handed helix in the endocardium, consistent with previous studies [9–13]. For each heart sample, average length of the fiber pathways tracked within each short-axis slice was computed for each helix angle range in 9° steps. Fig. 3A shows the average fiber pathway length distribution in eight representative short-axis slices among the six normal heart samples from base to apex as a function of the fiber helix angle. Fiber pathways at the middle or upper ventricle (Slices 6, 11, 16, 21 and 26) are much longer than those near the apex (Slices 31, 36 and 41). In each slice, the long fiber pathways usually have small helix angles, and fiber pathways with large helix angles are usually shorter. That is, long fiber pathways likely run circumferentially rather than longitudinally. By weighting the fiber pathway length by the respective fiber number (illustrated in Fig. 3B), total fiber pathway length was plotted vs. the helix angle in Fig. 3C. For each short-axis slice, the percentage of the total fiber pathway length occupied by those tracked fibers with helix angles within ±20° was estimated from Fig. 3C. They were 72%, 70%, 70%, 69%, 67%, 67%, 69% and 64%, respectively, for the eight short-axis slices from base to apex. These results indicate that fibers with small helix angles dominate the myocardial fiber architecture in terms of total length of all fiber pathways crossing each short-axis slice, and this dominance near the apex is not as great as that observed in myocardium closer to the base.

Fig. 4 shows the top and side views of the two sets of fiber pathways tracked from slices located at the middle ventricle (Fig. 4A and B) and near the apex (Fig. 4C and D), respectively. For each set of fiber pathways, they were tracked from the same slice but had different helix angle. In Fig. 4A and B, the pathway in red with a small helix angle of −13° spirals circumferentially with fiber pathway length around 234.6 mm, while the pathway in blue with a large helix angle of 60° runs from the endocardium down towards the apex and then up back to the epicardium with fiber pathway length around 96.5 mm. Note that whether in epicardium or endocardium, for example, some fiber pathways spiral only within their original layer and do not cross the mid-wall. In Fig. 4C and D, the pathway in red with helix angle of −13° has a length of 99.0 mm while the one in blue with helix angle of 60° has a length of 76.3 mm. The observation of such complex myocardium construction was consistent with the histological findings by Ross and Streeter [22], who suspected that myocardium has a more complex three-dimensional mapping and myocardial fibers may not truly map onto nested sets of shells that are parallel to the epicardium.

![Fig. 4](image-url)
3.2. Influence of fiber tracking criteria and image resolution

Influence of FA magnitude and directional thresholds on fiber tracking were examined for their effects on fiber the pathway distribution measurements. The average FA and its standard deviation in myocardium were $0.33 \pm 0.02$ and $0.08 \pm 0.01$, measured at a mid-ventricular short-axis slice among six heart samples. The average FA of formalin suspension medium was $0.08 \pm 0.03$. The three diffusion tensor eigenvalues ($\lambda_1$, $\lambda_2$ and $\lambda_3$) were $1.78 \pm 0.06$, $1.67 \pm 0.05$ and $1.53 \pm 0.07$ (in $10^{-3}$ mm$^2$/s), respectively. These values were similar to those reported in the previous DTI studies of fixed hearts [10,12,23,24]. Fig. 5 illustrates the influence of FA magnitude threshold on fiber pathway tracking result. The fiber number decreased when FA threshold increased, while the average fiber pathway length increased slightly and then decreased. Choice of FA threshold should provide a trade-off between reducing the interference of noise in FA and diffusion tensor eigenvalues on fiber tracking and keeping fibers reasonably long. Given the average FA of $0.33 \pm 0.08$ in the myocardium of the heart samples studied, the FA threshold was chosen to be 0.15 in this study so that fibers could be tracked in most voxels and are relatively long. FA thresholds of 0.2 and 0.25 were also employed to assess fiber pathway distribution, and results similar to those shown in Fig. 3 were observed. However, large variations and inconsistencies in fiber number and length distribution were seen among the six samples with FA threshold of 0.3 or higher, most likely because the high FA threshold prevented the effective fiber tracing in presence of noise in FA and diffusion tensor directions.

Fig. 6 demonstrates that number of fiber pathway tracked decreased with more stringent requirement on angular transitions, i.e., with smaller angles. Both the fiber pathway number and the average fiber pathway length decreased sharply before $0^\circ$. Similar effects of these two thresholds on fiber number and average fiber pathway length were found on slices of other locations. Normalized average fiber pathway length distribution and normalized total fiber

![Fig. 5. Influence of varying FA threshold on the number of all fiber pathways tracked from a mid-ventricular slice pixel by pixel among the six normal canine heart samples (A); influence on the average length of all fiber pathways tracked (B). Number of fibers decreased with increase in FA threshold. The directional threshold of 40° was used in the calculations. The average normalized values are shown with error bars.](image)

![Fig. 6. Influence of varying directional thresholds on the number of all fiber pathways tracked from a mid-ventricular slice pixel by pixel among the six normal canine heart samples (A); influence on the average length of all fiber pathways tracked (B). Number of fibers decreased with increase in directional threshold. The FA threshold of 0.15 was used in the calculations. The average normalized values are shown with error bars.](image)
pathway length distribution as a function of fiber helix angle at three representative slices in one sample are illustrated in Fig. 7 with different directional thresholds of 18°, 36°, 54°, 72° and 90° used in fiber tracking. Similar patterns of fiber pathway length distribution were observed for different directional thresholds. This indicates that the directional threshold of 40° used in this canine heart sample study was adequate to track the myocardial fiber pathway curvatures with the isotropic resolution used (1.13 mm). Similarly, the effect of image resolution was also studied by examining fiber pathway length distribution with different isotropic image resolution, including 1.0 and 1.5 mm. The fiber pathway distribution patterns were found to be largely similar when voxel size was smaller than 1.5 mm. This again indicates that DTI fiber tracking with isotropic voxel size of 1.13 mm and directional threshold of 40° should

Fig. 7. Normalized average fiber pathway length distribution (A, C, E) and total fiber pathway length distribution (B, D, F) as a function of fiber helix angle at three representative short-axis slices selected from base (A, B), middle ventricle (c, d) and apex (E, F) are calculated in one heart sample using varying directional thresholds of 18°, 36°, 54°, 72° and 90°. Overall pattern of the fiber pathway length distribution is preserved.
yield a robust description of myocardial fiber pathway distribution in the canine myocardium.

4. Discussion and conclusion

DTI-tracked myocardial fiber pathways are largely of arbitrary nature. Single cardiac muscle cell or myofiber, much smaller than the imaging voxel size, is not directly examined here. Instead, the anisotropic diffusion effect of water molecules within numerous myofibers in each imaging voxel is collectively measured. Physically, the elongated thread-like heart muscle cell or myofiber consists of many long parallel-arranged myofibrils that contain serially linked sarcomere segments. Water molecules have difficulty in crossing cell membranes and the sarcomere boundaries. It is much easier for them to diffuse to different parts within the same cell and within the same sarcomere. In other words, water molecules in the myocardium diffuse preferentially along the direction of muscle fibers. Therefore, the myocardial fiber orientation measured by DTI represents the general direction of sarcomere segments, myofibrils, and heart muscle cells. In this study, we further examined the use of DTI to measure the myocardial fiber pathway which reflects the preferential direction of propagation of electromechanical activation along a group of closely coupled myofiber bundles. As a result, the myocardial fiber pathway length measured by DTI fiber tracking is likely related to the length of these closely coupled myofiber bundles that are activated in similar direction. Such fiber pathway length measurement is arbitrary because of the arbitrary termination criterion employed in DTI fiber tracking. Nevertheless, the fiber pathway length and distribution assessed by DTI may likely reflect the connectiveness of myofibers, as well as myocardial fiber bundles, that exhibit similar directional, mechanical and functional characteristics. This spatial connectiveness of elastic fiber structures along the tracked fiber pathway could reveal the intrinsic myocardial connectivity, and it might provide a potentially valuable insight into the relationship between cardiac mechanics and electromechanical activation. A disruption in the myocardial fiber pathway and/or changes in myocardial fiber pathway length may result in abnormal electromechanical activation and thus cardiac mechanics. Recent experimental studies have demonstrated that abnormal and nonhomogeneous cardiac electromechanical activation can lead to worsening of cardiac performance by causing mechanical dysynchrony [25]. Indeed, Helm et al. [26] have shown that abnormal electromechanical activation was associated with changes in the orientation of the myocardial fiber as determined by DTI.

Sarcomere is the smallest functional and structural unit of myofibril. It is also the structural unit as far as water diffusion is concerned. At long sarcomere length, the calcium affinity of the contractile proteins increases and the ability of calcium to activate contraction enhances [27]. Beyar and Sideman [28] reported that the length of sarcomere in the epicardium, mid-wall and endocardium exhibited no great difference. However, long fiber pathways in the mid-wall were observed in this DTI study. This suggests that the myocardial fiber pathway length measured in our DTI fiber tracking is not dominantly related to the sarcomere length, but rather to other factors that may contribute to the packing quality or the connectiveness of the myofibers and fiber bundles along the same myocardial fiber pathway in heart.

The results of this study demonstrated that most fiber bundles, particularly those having long pathways in the myocardium, tend to have small helix angles and spiral circumferentially in the mid-wall. Our findings are consistent with the histological measurements by Streeter et al [8], who found that the proportion of fibers lying in the sector of fiber angles oriented circumferentially (0°±22.5°) to those oriented longitudinally (67.5° to 90° and −67.5° to −90°) is approximately 10:1 and this ratio increases toward the base and diminishes toward the apex of the LV. They also demonstrated that circumferentially oriented fibers participate fully in the predominantly circumferential contraction that normally characterizes ejection. Similarly, Scott et al. [29] reported that the principal wall thickening is predominantly radially oriented throughout the myocardium in cardiac deformation. Sarcomere length changes in the mid-wall were reported to be greater than those in the epicardium or endocardium during cardiac cycle based on the report by Stevens and Hunter [30]. Vendelin et al. [16] estimated the transmural distribution of adenosine triphosphate (ATP) consumption in the cardiac wall and found that the region with the highest ATP consumption was in the middle of the left ventricular wall. These previous findings again suggest that fibers around the mid-wall play a more predominant role in cardiac contractility than those near the endocardium or epicardium. Our findings suggest that these mid-wall fibers run circumferentially, possess long pathways and dominate the fiber architecture in the myocardium. Such characteristics may contribute significantly to the highly efficient and effective myocardial contraction.

Owing to the recent advances in cardiac MR and ultrasound imaging, there is an increasing interest in investigating LV structure and function by directly associating the LV myocardial fiber geometry to the complex spatial-temporal sequence of electrical activation and mechanical contraction/relaxation in a beating heart [20,31,32]. The left ventricle is first electrically activated at the exits of the Purkinje system, close to the apical endocardium. The electrical activation then propagates from the apex to the base via depolarization and repolarization in both septum and free wall, although contractile activities may not precisely correspond with patterns of electrical excitations [18,19,32]. This is accompanied by successive mechanical shortening and intracavitary blood flow along the parallel apex-to-base direction, though highly transient and localized myocardial deformations exist and such physiologic asynchrony remains to be elucidated. During
the observation again underscores the importance of circumferential fibers for the LV contractile function.

Variation in myocardial fiber pathway length distribution among the heart samples was observed in this study. This may arise from the slightly different heart phases during which hearts were arrested and fixed. Dou et al. [33] have demonstrated that fiber helix angle changes at different phases of cardiac contraction. The histogram of fiber helix angle becomes broader from early-systole to end-systole, indicating that fibers become more longitudinally orientated at end-systole. Thus, the variation in diastole arrest during the sample fixation may contribute to the intra-sample variation in measurement of myocardial fiber pathway vs. helix angle in this study. Nevertheless, the overall patterns of myocardial fiber pathway length and number vs. helix angle distribution in short-axis slices were found to be consistent for all heart samples studied. These patterns persisted even when different termination criteria were employed in the fiber tracking. It is also noteworthy that formalin fixation may alter the DTI measurement of myocardial fiber characteristics. The effect of formalin fixation on sarcomere length was previously examined using light microscopy by Grimm and Wohlfart [34]. Results show that sarcomeres shrink from 1.3% to 5.5% with an average shrinkage of 4.2%. Such shrinkage and other structural and water content changes can influence the MRI relaxation properties, the bulk diffusion characteristics of water molecules and DTI fiber tracking. Although only slight FA changes were reported recently in fixed mouse brains and hearts [35,36], it is possible that formalin fixation can cause DTI measurement of fiber structural characteristics to be quantitatively different from those of the myocardium in vivo. However, the qualitative characteristics of the myocardial fiber structure assessed by DTI of formalin-fixed heart, including the fiber pathway length distribution examined in the present study, are likely to remain true for intact myocardium.

In conclusion, the myocardial fiber pathway distribution in fixed normal canine heart samples was investigated using DTI fiber tracking. The long fiber pathways were found to predominantly run circumferentially with small helix angles, dominating the fiber architecture in the myocardium. The fiber pathways at the middle and upper ventricle are longer than those near the apex. These specific structural findings may explain why the predominant deformation in LV is radially orientated during cardiac contraction. Such myocardial fiber pathway and connectivity characterization using DTI may provide further mechanistic insights into the relationships between myocardial electrical propagation, myocardial fiber architecture and cardiac mechanics in both normal and diseased hearts.

References