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In Vivo Diffusion Tensor MRI of the Human Heart: Reproducibility of Breath-Hold and Navigator-Based Approaches

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Abstract

The aim of this study was to implement a quantitative in vivo cardiac diffusion tensor imaging (DTI) technique that was robust, reproducible, and feasible to perform in patients with cardiovascular disease. A stimulated-echo single-shot echo-planar imaging (EPI) sequence with zonal excitation and parallel imaging was implemented, together with a novel modification of the prospective navigator (NAV) technique combined with a biofeedback mechanism. Ten volunteers were scanned on two different days, each time with both multiple breath-hold (MBH) and NAV multislice protocols. Fractional anisotropy (FA), mean diffusivity (MD), and helix angle (HA) fiber maps were created. Comparison of initial and repeat scans showed good reproducibility for both MBH and NAV techniques for FA ($P > 0.22$), MD ($P > 0.15$), and HA ($P > 0.28$). Comparison of MBH and NAV FA ($FA_{MBHday1} = 0.60 \pm 0.04$, $FA_{NAVday1} = 0.60 \pm 0.03$, $P = 0.57$) and MD ($MD_{MBHday1} = 0.8 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$, $MD_{NAVday1} = 0.9 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$, $P = 0.07$) values showed no significant differences, while HA values ($HA_{MBHday1Endo} = 22 \pm 10^\circ$, $HA_{MBHday1Mid-Endo} = 20 \pm 6^\circ$, $HA_{MBHday1Mid-Epi} = -1 \pm 6^\circ$, $HA_{MBHday1Epi} = 17 \pm 6^\circ$, $HA_{NAVday1Endo} = 7 \pm 7^\circ$, $HA_{MBHday1Mid-Endo} = 13 \pm 8^\circ$, $HA_{MBHday1Epi} = -2 \pm 7^\circ$, $HA_{MBHday1Epi} - 14 \pm 6^\circ$.) were significantly different. The scan duration was 20% longer with the NAV approach. Currently, the MBH approach is the more robust in normal volunteers. While the NAV technique still requires resolution of some bulk motion sensitivity issues, these preliminary experiments show its potential for in vivo clinical cardiac diffusion tensor imaging and for delivering high-resolution in vivo 3D DTI tractography of the heart.

Keywords

cardiovascular magnetic resonance imaging; prospective navigators; cardiac diffusion tensor imaging; cardiac diffusion-weighted imaging

Diffusion MRI has the unique ability to characterize the microstructure of tissues noninvasively. Histological studies have shown that the myocardium consists of an array of crossing helical fiber tracts (1,2), which evolve smoothly from a left-handed helix in the subepicardium to a right-handed helix in the subendocardium. This structure contributes significantly to efficient ventricular function and is subject to remodeling and disarray in the presence of disease, such as myocardial infarction and cardiomyopathies. In humans, in vivo cardiac diffusion tensor imaging (DTI) has been used to noninvasively depict the fiber structure in the healthy human heart (3–10), in hypertrophic cardiomyopathy (11), and in patients with myocardial infarction (12,13).

Microstructural changes in the myocardium can be quantified by measuring mean diffusivity (MD), fractional anisotropy (FA), and the orientation (helix angle, HA) of the myofibers. These indices have been widely used in ex vivo cardiac DTI studies of both healthy (14–29) and diseased myocardium (30–35). Moreover, these indices have been used in humans in vivo to characterize the microstructural integrity of the myocardium after infarction (12,13). However, the reproducibility of these metrics (MD, FA, and HA) in the human heart in vivo is unknown.

Achieving reproducible DTI measurements in the heart in vivo is particularly challenging because the bulk motion of the myocardium is four orders of magnitude greater than the diffusion coefficient. The biggest challenge for in vivo cardiac DTI is motion both within and between cardiac cycles. Two sequence designs have been reported to perform DTI in vivo. A diffusion-encoded stimulated echo (STEAM) approach, which runs over two heart beats, and makes the assumption that the heart is in the same position at both diffusion-encoding times on consecutive cardiac cycles (3,4,6,7), and a spin-echo single cardiac cycle approach with bipolar (velocity compensated) diffusion-encoding gradients (9). Several techniques have been used to compensate for respiratory motion including breath-hold (3), synchronized breathing (4), and retrospective navigators (NAV) using an intensity-based correlation postprocessing method to select the images to be used for modulus averaging (9). These implementations required acquisition times of 7–10 min per slice. In this form, DTI remains practical only in relatively healthy patients and well-motivated volunteers. A prospective NAV-based approach combined with biofeedback mechanisms to increase scan efficiency, however, has not previously been implemented. This may facilitate clinical implementation and enable higher resolution DTI acquisitions with more complete coverage of the heart within a clinically acceptable acquisition time.

The purpose of the current study was thus to implement and perform DTI of the human heart in vivo with both a multiple breath-hold (MBH) and a NAV-based version of a diffusion-weighted STEAM sequence. For that purpose, a novel modification of the prospective diaphragmatic NAV technique was implemented and combined with a biofeedback mechanism to increase scanning efficiency. Furthermore, we aimed to compare the accuracy of the NAV-based approach with the MBH approach, and to assess the reproducibility of the two techniques. To the best of our knowledge, this is the first study to systematically assess the reproducibility of DTI in the human heart in vivo.

METHODS

Technique Development

The diffusion-weighted STEAM single-shot echo-planar imaging (EPI) sequence was implemented on a clinical scanner (3 T, MAGNETOM Skyra, Siemens AG Healthcare Sector, Germany) equipped with an anterior cardiac 18-element matrix coil, a 48-element spine matrix coil, and a standard 45-mT/m gradient set with a slew rate of 200 m/ T/s. This sequence, shown in Figure 1, runs over two heart beats and makes the assumption that the

heart is in the same position at both diffusion-encoding times (end systole) on consecutive cardiac cycles as described in (3,4). To minimize the length of the single-shot EPI readout, parallel imaging with external reference lines and zonal excitation were implemented, such that the first two 90° radio-frequency (RF) pulses were set perpendicular (along the phase-encoding direction) to the third 90° RF pulse so that only spins lying in the intersection of both planes contributed to the echo formation (36). The slice thickness of the first two RF pulses defines the dimension of the field-of-view (FOV) in the phase-encoding direction and can be adapted to the size of the heart. Crusher gradients around the second and third RF pulses were needed to suppress the other echo paths created by the STEAM encoding, and to prevent flow artifacts in the b0 image. This allowed for a phase FOV of 25–35% resulting in an EPI echo train length of about 16–20 readouts.

To monitor and correct for respiratory motion, both MBH and prospective diaphragmatic NAV were implemented for this technique. The prospective NAV were based on spin echoes with crossed 90°/180° slices and could be applied before and/or after the STEAM module. In this study, NAV before and after the STEAM module were used in an attempt to guarantee that the first and second halves of the STEAM module were in the same breathing position, as shown in Figure 1. The crossed pair NAV was positioned over the dome of the right hemidia-phragm and a 5-mm gating window positioned around the end-expiratory pause position. Slice tracking was not implemented as the tracking factor is highly subject- and location-specific (37,38) and difficult to measure.

The NAV accept/reject algorithm was modified to prevent bulk respiratory motion artifacts in the diffusion-encoded images. As the STEAM technique runs over two heartbeats, the straight-forward application of prospective NAV with an acceptance window of, for example, ± 2.5 mm means that the position difference between the first and the second heartbeat could be as large as 5 mm, in which case the diffusion sequence would detect bulk respiratory motion instead of diffusion. Reducing the acceptance window to less than ± 2.5 mm would make the scan too inefficient to be clinically feasible. Instead, we modified the accept/reject algorithm such that only scans that were within the acceptance window would be accepted, but added an extra restriction that required the diaphragm position during first and second heartbeats in the STEAM acquisition to be within a 1 mm range (see Supporting Information Fig. 8). This decreases NAV efficiency, but not as much as reducing the whole scan acceptance window to ± 0.5 mm, and improves image quality substantially compared with an overall acceptance window of ± 2.5 mm (39,40).

To increase scanning efficiency, a biofeedback mechanism (40) was implemented by which the volunteers were able to visualize the time evolution of the NAV signal and therefore were able to adapt their breathing pattern to optimize data acceptance. An magnetic resonance (MR) compatible monitor was installed at the back of the scanner room, aligned to the MR scanner, which was displaying the NAV signal evolution over time. A mirror inside the magnet allowed the volunteers to see the monitor.

Data Acquisition

Ten volunteers were each scanned on two separate days (subsequently referred to as initial and repeat scans) within a period of 2 weeks, to perform interstudy reproducibility analysis. Both the MBH and navigated (NAV) protocols were run in each scanning session. The study was approved by the institutional research ethics committee and the subjects gave written informed consent.

After the localization steps to determine the short axis of the left ventricle (LV), a retrograded cine sequence with a temporal resolution of 40 ms was used to find the timing and duration of the subject-specific end systolic pause. The average cycle length of the volunteers was

960 ± 57 ms. The average systolic pause was found to begin at 303 ± 22 ms and on average its duration was 43 ± 13 ms. Localized first-order and second-order shimming and frequency adjustment were performed with an adjustment box fitting the whole heart.

The following sequence parameters were used: $b = 0$ s/mm² plus 6 diffusion-encoding directions, fat saturation, repetition time = 2 x RR intervals = 2000 ms (assuming a heart rate of 60 beats/min), echo time (TE) = 23 ms, bandwidth = 2442 Hz/pixel, generalized autocalibrating partially parallel acquisitions (41) parallel imaging acceleration factor of 2, EPI echo train length = 16–20 readouts, depending on FOV, EPI readout duration = 10 ms, spatial resolution = $2.7 \times 2.7 \times 8$ mm³ interpolated to $1.35 \times 1.35 \times 8$ mm³, three slices, 4-mm slice gap, and eight averages. For diffusion encoding, the maximum available on axis gradient strength of 45 mT/m was used with a trapezoidal gradient pulse duration of 10 ms, leading to a diffusion sensitivity of $b = 350$ s/mm². The acquisition protocol consisted of two heartbeats (HBs) for external phase correction lines acquisition, two HBs for external parallel imaging reference lines acquisition, two HBs for b_0 , and two HBs for each of the six DWI images. No extra dummy scans were used. The acquisition duration per slice was therefore 18 heartbeats, which would result in 18 s for the MBH protocol, or the NAV protocol assuming 100% respiratory efficiency and a heart rate of 60 beats/min. To acquire three slices averaged eight times, the MBH protocol resulted in 24 breath-holds. The raw data were reconstructed using the product image reconstruction algorithms available on the scanner.

The signal-to-noise ratio (SNR) of both the MBH and NAV initial studies was measured in a region of interest (ROI) in the septal wall, using the multiple acquisition method described by Reeder et al. (42).

$$\text{SNR}(\text{ROI}) = \frac{\bar{x}(\text{ROI})}{\sigma(\text{ROI})} = \frac{\sqrt{N}}{N} \frac{\sum_{i=1}^N x_i(\text{ROI})}{\sqrt{\sum_{i=1}^N (x_i(\text{ROI}) - \bar{x}(\text{ROI}))^2}}$$

where $\bar{x}(\text{ROI})$ and $\sigma(\text{ROI})$ are the mean and standard deviation of the signal in the ROI over time respectively, and N is the number of averages.

Data Postprocessing

Images were averaged to create a single diffusion-encoded dataset at each of the three slice locations. In each voxel, the principal eigenvectors of the diffusion tensor were derived to calculate the FA, MD, and HA maps, as previously described (16,17). For quantification purposes, the LV was divided into four regions (anterior, lateral, inferior, and septum). With three acquired short axis slices (apical, mid, and basal), this resulted in measurements of each parameter from 12 regions per heart. FA and MD values were extracted from each of these 12 regions. Fiber HA was calculated in the same slices and regions of LV at four transmural locations (endocardial, midendocardial, midepicardial, and epicardial layers). Segmentation of the myocardium into these transmural zones was performed by calculating a distance transform map using the fast marching method (43–45), which propagates a planar wave front of constant unit speed from the endocardial surface as the starting and the epi-cardium as the ending surface. The propagation speed outside the myocardial tissue was set to zero, forcing the wave front to constrain itself within the myocardium. Subregion thickness was defined proportional to the arrival time of the front at the epicardium. Because the arrival times of the front are correlated with transmural depth, this technique enables the classification of the LV into four subregions based on their relative distance from the endocardium, while maintaining proportional subregion thickness between MBH and NAV. As the segmentation was performed once for the MBH dataset and then coregistered to the

NAV dataset, layers were spatially aligned and inaccuracies in the quantitative data minimized. In selected datasets, to demonstrate the potential of the acquired data to produce 3D tractography information, fiber tracts were constructed by integrating the primary eigenvector from the diffusion tensor field into streamlines using a fourth order Runge-Kutta approach (46–50) with a step length equal to one-fourth of the voxel size. An angle of 35° between adjacent vectors was used as the termination criterion. No further interpolations beyond the inherent interpolation mechanism of the fourth order Runge-Kutta approach were performed. Python, C++, and the Visualization Toolkit libraries were used to develop the framework used to process the DTI datasets (51).

Data Analysis

We hypothesized that no statistically significant differences in MD and FA values would be observed across the three slices (base to apex) and four regions (anterior, lateral, inferior, and septum) in the LV of the heart. This was tested by applying a linear mixed effects model with a variance component covariance structure to take into account the clustering of regions, slices, and volunteers. This test was applied both to the data acquired in the initial studies and to the data acquired in the repeat studies. The same test was applied to the HA data, further subdivided into endocardial, midendocardial, midepicardial, and epicardial layers. Analysis was undertaken using IBM SPSS Statistics (SPSS for Windows, Rel. 19.0.0. 2010. SPSS Inc.).

Mixed effects modeling confirmed our hypothesis that there were no statistically significant differences in FA, MD, and HA values across slices and regions in the heart. Therefore, for further analysis, individual regional FA, MD, and HA values were combined. Reproducibility of each parameter (FA, MD, and HA) from the MBH and NAV acquisitions, and comparison of MBH and NAV were assessed using a two-tailed paired t test, and by the Bland–Altman method (52) using Medcalc version 12.1.4.0 (Medcalc Software, Mariakerke, Belgium).

RESULTS

Scan duration with MBH was 14.4 ± 3.5 min compared with 18.1 ± 4.2 min for NAV. Figure 2 demonstrates the typical image quality of the b_0 and diffusion-encoded images (one average) for MBH and NAV techniques obtained throughout the study. Successful imaging was acquired in all cases. The mean SNR values over all volunteers was $\text{SNR}_{\text{MBH}b_0} = 29 \pm 14$, $\text{SNR}_{\text{MBH}DWI} = 20 \pm 7$ for MBH, and $\text{SNR}_{\text{NAV}b_0} = 20 \pm 9$, $\text{SNR}_{\text{NAV}DWI} = 14 \pm 7$ for NAV. While no statistically significant differences between the MBH and NAV techniques were seen for the SNR measurements of the b_0 images ($P = 0.30$), statistically significant differences were seen for the SNR measurements of the diffusion-weighted (DW) images ($P = 0.02$).

b_0 Images (eight averages) and derived FA, MD, and HA maps at three slice locations in a normal volunteer are shown in Figure 3, together with the segmentation scheme used to extract quantitative data from these maps. The MD and FA maps acquired with the MBH and NAV approaches are extremely similar and highly homogeneous. The transmural progression of HA can be clearly observed in all slices with both techniques.

Fractional Anisotropy

Initial and repeat studies were performed 8 ± 6 days apart. FA reproducibility data, comparing the initial and repeat studies for each technique, are provided in Table 1 and the associated plots are shown in Figure 4b,c. No statistically significant differences were found

between initial and repeat studies (MBH: bias = -0.009 ± 0.029 , $P = 0.35$; NAV: bias = -0.011 ± 0.026 , $P = 0.22$).

A comparison of FA values in the baseline MBH and NAV studies is provided in Table 1 and the associated plots in Figure 4d. The mean FA value for the initial studies was 0.60 ± 0.04 for MBH and 0.60 ± 0.03 for NAV, consistent with prior values in the literature (12). No statistically significant differences between the MBH and NAV techniques were seen (bias = -0.005 ± 0.026 , $P = 0.57$).

Mean Diffusivity

MD reproducibility data, comparing the initial and repeat studies for each technique, are provided in Table 1 and Figure 5b,c. No statistically significant differences were found between initial and repeat studies (MBH: bias = $0.06 \pm 0.16 \times 10^{-3} \text{ mm}^2/\text{s}$, $P = 0.28$; NAV: bias = $0.12 \pm 0.24 \times 10^{-3} \text{ mm}^2/\text{s}$, $P = 0.15$).

A comparison of MD values in the baseline MBH and NAV studies is provided in Table 1 and Figure 5d. The mean MD value for the initial studies was $0.8 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$ for MBH and $0.9 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$ for NAV, in accordance with prior values in the literature (12). No statistically significant differences (bias = $-0.08 \pm 0.13 \times 10^{-3} \text{ mm}^2/\text{s}$, $P = 0.07$) were seen between the MBH and NAV techniques.

Helix Angle

HA reproducibility data, comparing the initial and repeat studies for each technique, are provided in Table 2. The associated plots are shown in Figure 6a,b, and in Supporting Information Figures 9–12. No statistically significant differences were found between the HA values in initial and repeat studies for either technique (MBH: $P_{\text{Endo}} = 0.25$, $P_{\text{Mid-Endo}} = 0.99$, $P_{\text{Mid-Epi}} = 0.80$, $P_{\text{Epi}} = 0.73$; NAV: $P_{\text{Endo}} = 0.50$, $P_{\text{Mid-Endo}} = 0.51$, $P_{\text{Mid-Epi}} = 0.28$, $P_{\text{Epi}} = 0.38$).

A comparison of HA values in the baseline MBH and NAV studies is provided in Table 2, and the associated plots are shown in Figure 6 and in Supporting Information Figures 9–12. The mean HA values for all MBH studies range from $23 \pm 9^\circ$ in the endocardial layer, through $20 \pm 6^\circ$ in the midendocardial layer and $-1 \pm 7^\circ$ in the midepicardial layer, to $-17 \pm 7^\circ$ in the epicardial layer, which is a narrower HA range compared with the previously reported values in the literature (12). The mean HA values for all NAV studies range from $8 \pm 8^\circ$ in the endocardial layer, through $14 \pm 9^\circ$ in the midendocardial layer and $-1 \pm 8^\circ$ in the midepicardial layer, to $-13 \pm 6^\circ$ in the epicardial layer. From this, it can be observed that the endocardial layer HA values are lower for NAV compared with MBH. Consequently, statistically significant differences were seen between the MBH and NAV techniques for HA in the endocardial layer ($P = 0.002$) and the midendocardial layer ($P = 0.03$).

DTI and Tractography

Three-dimensional analysis of the DTI data is shown in Figure 7a for MBH and Figure 7b for NAV, by displaying the tensor fields in ROIs (red regions) located in the lateral wall of three contiguous slices with superquadric glyphs (22). The color and orientation of the glyphs are determined by the HA and primary eigenvector, respectively, and their shapes are parameterized by the eigenvalues and eigenvectors. The glyphs in all three levels are oriented similarly, showing consistent findings across a 3D volume.

Three-dimensional views of typical fiber tractograms derived from both MBH and NAV datasets are shown in Figure 7c,d, respectively. Despite the lower resolution in the slice

direction, the helical structure of the LV is clearly depicted and the dispersion in HA over the papillary muscles can be clearly observed.

DISCUSSION AND CONCLUSIONS

In vivo DTI of the heart is challenging to perform due to cardiac motion superimposed on intrinsically low SNR data acquisition. Although studies from several groups show that in vivo DTI in humans is possible (9–13), its accuracy and reproducibility remain unknown. We show here for the first time that DTI of the human heart in vivo can be performed with a high degree of reproducibility. Moreover, we show for the first time that cardiac DTI can be performed with a biofeedback NAV-based approach, which also provides highly reproducible measures of FA and MD. The reproducibility of the techniques described here lay the foundations for future use of cardiac DTI in the diagnosis, risk stratification, and evaluation of the treatment of human cardiac diseases.

Several techniques can theoretically enable DTI of the entire heart to be performed within a clinically feasible scan duration. In the current study, three contiguous slices at the midventricular level were acquired in 12–20 min using both the MBH and NAV approaches. All the scans were successful and reproducible. The EPI images in our study did not suffer from distortion artifacts due to the short EPI readout achieved with parallel imaging, zonal excitation, and a modest matrix size. The b value of 350 s/mm² was selected based on previous literature suggesting that b values of 300–350 s/mm² are sufficient to reveal the diffusion anisotropy in the myocardium (4,9,12,13). Larger b values require a longer TE and decrease SNR. Because of this low b value, the signal decrease is not apparent in the DW images. It is however sufficient to extract the diffusion information needed to calculate FA, MD, and HA, and even generate the glyphs and tractograms. However, a systematic study to define the optimum b value and number of averages needed to provide robust in vivo DTI data is required and will be the subject of future work. Comparison of the FA and MD values acquired in the initial and repeat scans showed good reproducibility for both MBH and NAV techniques. Furthermore, both MBH and NAV techniques showed no statistically significant differences in the FA and MD values. The MD maps in Figure 3 were scaled from the minimum to the maximum MD value found in the map. This scaling will make any heterogeneity appear more pronounced. The actual differences between the minimum and maximum MD values, however, show that the variation is not large. While it is possible to observe lower FA values in the left ventricle (LV)–right ventricle (RV) junction, the FA maps appear homogeneous. In this study, the transmural variations of FA and MD were not investigated. Several indices of diffusivity [trace apparent diffusion coefficient, principal eigenvalue λ_1 , MD] have been reported in the literature in humans in vivo, together with a range of values. In healthy volunteers, Reese et al. reported an MD = $0.9 \pm 0.3 \times 10^{-3}$ mm²/s (4), Dou et al. reported a $\lambda_1 = 0.9 \times 10^{-3}$ mm²/s (7), Gamper et al. reported values of λ_1 ranging from 1.8×10^{-3} to 2.3×10^{-3} mm²/s (9), Wu et al. reported trace apparent diffusion coefficient = $0.63 \pm 0.02 \times 10^{-3}$ mm²/s (13), and Rapacchi et al. reported trace apparent diffusion coefficient $\sim 7 \times 10^{-3}$ mm²/s (53). The quantitative MD values measured in this article (MD_{MBHday1} = $0.8 \pm 0.2 \times 10^{-3}$ mm²/s, MD_{NAVday1} = $0.9 \pm 0.2 \times 10^{-3}$ mm²/s) are in good agreement with the same measures by Reese et al. (4). However, further studies will be needed to compare measures, standardize and optimize the method. It should be noted that the quantitative results in this study are related to acquisitions at end-systolic phase. We may therefore expect differences between in vivo and ex vivo data, between fresh and fixed ex vivo data, and data acquired at different cardiac phases.

Statistically significant differences were found between MBH and NAV techniques for HA values. Further interrogation of the data suggested that the inconsistent HA patterns were found in NAV datasets in which a small fraction (up to 10%) of the diffusion-weighted

frames had signal voids in some part of the LV, as shown in Supporting Information Figure 13. This result correlates with the findings of the SNR calculation, which demonstrated that the MBH and NAV techniques produce b0 images with similar SNR, while significant differences between MBH and NAV were observed for the DW images. Inspection of the NAV plots showed that the frames with signal voids were correlated with those frames that were accepted immediately after deep inspiration (Supporting Information Fig. 13). To overcome this, it may be possible to modify the acceptance algorithm to reject a certain amount of data acquired immediately after a deep inspiration. Alternatively, as this portion always represents <10% of the data, a postprocessing step could edit out these frames before further DTI analysis. Future investigations into the effect of different breathing patterns and potential accept/reject algorithms are currently ongoing but are beyond the scope of the current paper. Nonetheless, these issues are highly addressable and our preliminary NAV results demonstrate the potential of this technique to be as robust as the MBH approach. We consider therefore that this approach establishes the right paradigm for routine clinical cardiac DTI.

Nonetheless, several limitations of the approaches used here merit discussion. First, the STEAM technique works well in healthy volunteers with stable heart rates but would perform less well in patients with highly variable heart rates. To overcome this, arrhythmia rejection algorithms will need to be implemented, which in turn will extend the acquisition time. The NAV approach is better suited to the incorporation of arrhythmia rejection algorithms because it can cope with extended acquisition times much better than the MBH technique. Alternatively, robustness against arrhythmia might be addressed using a spin-echo approach (9), which runs over only one HB, or the recently described PCATMIP method (53). Secondly, the HA range seen in our study was narrower (approximately 40° to -32°) compared with prior ex vivo and histological studies (16,23,24,31,54). The HA range can be influenced by many factors, including in vivo versus ex vivo imaging, motion artifacts, spatial resolution and the segmentation technique used. The study by Lombaert et al. of healthy ex vivo human hearts showed ranges of HA variations from $-41^{\circ} \pm 26^{\circ}$ on the epicardium to $+66^{\circ} \pm 15^{\circ}$ on the endocardium (54). The histological study of postmortem human hearts by Greenbaum et al. showed a distribution of HAs from -40° on the epicardium to about $+40^{\circ}$ on the endocardium (55). The narrower HA range of this study may be accounted for by the modest in-plane resolution of 2.7 mm, which rendered the transmural HA values susceptible to volume averaging. The segmentation algorithm used to extract the HA values imposes further averaging, and might also account for the narrower range seen in HA. This is particularly likely in the subepicardial layer, which was thicker than the other layers after segmentation. The papillary muscles, where HA is highly variable, were included in the subendocardial layer and might account for the lower endocardial HA values. However, since the same segmentation algorithm was used in all cases, these issues do not impact the MBH versus NAV method comparison and reproducibility study. The segmentation method used was an operator independent, automated algorithm, which behaved identically in both breath-hold and NAV datasets. Although this approach was adequate for the purposes of this study, which were to test the reproducibility of breath-hold and NAV cardiac DTI techniques and also to compare them to each other, the limitations mentioned before and the fact that fast marching method algorithms have rarely been used to segment the myocardium (45), make it unsuitable for immediate application to clinical studies. Further work will be required to compare this segmentation method to other techniques, such as manual/expert segmentation, in terms of reproducibility and diagnostically meaningful results. Designing, implementing, optimizing, testing, and comparing segmentation algorithms to extract robust and meaningful quantitative HA data will be a very important task for the translation of cardiac DTI into clinical routine, and is the subject of ongoing research in our group. The image reconstruction interpolation step used to increase the spatial resolution of the b0 and DW

images was based on zero-filling and should not influence the ROI-based quantitative analysis performed. For the quantitative analysis, the segmentation mask created to extract quantitative data from each ROI had the same resolution as the DWI images. Third, an average myocardial blood volume of 5–15% (56–59) has been reported in dogs. Therefore, blood pseudo diffusion might interfere with the quantitation of true diffusion (53,60). However, since intramyocardial capillaries are closely aligned with the muscle fibers, the directions derived from these data should still be valid. Moreover, these measurements were performed at end systole, when intramyocardial blood flow is significantly reduced. Finally, it should be noted that not all patients are able to adjust to a biofeedback based respiratory NAV, and some may prefer more conventional NAV techniques. These limitations, however, are all highly addressable, and the subject of ongoing work.

At present, the current MBH approach appears most suitable in normal volunteers. However, while the NAV technique still needs some bulk motion sensitivity issues to be tackled, these preliminary experiments show its feasibility and potential. The ability to perform navigator-gated DTI will be useful in normal volunteers but critical if the use of DTI is to be extended to patients with cardiovascular disease and limited breath-hold capacity. Furthermore, NAV DTI will be key not only in terms of patient comfort and compliance but also as a necessary step toward high-resolution in vivo 3D DTI tractography of the heart (10,61,62). Inspection of the superquadric glyph continuum in our study (Fig. 7a,b) showed that with both the MBH and NAV acquisitions, the principal glyph orientation in all three slices remained consistent with a coherent helical myofiber pattern. These results encouraged us to pursue application of postprocessing methods to provide preliminary in vivo cardiac 3D tractograms (Fig. 7c,d). Although only three slices were acquired, Figure 7c,d demonstrate that it is possible to create 3D fiber tractography maps with data acquired using both MBH and NAV techniques, with clear depiction of the HA structure of the LV. A detailed description of these tractography algorithms and quantitative validation of the tractograms together with an analysis of the robustness/error level of the tractograms and the relationship between the number of slices and the error level will be important to determine the amount of data needed for robust in vivo cardiac tractography data, but it is beyond the scope of this paper and the subject of future work, which will also focus on extending coverage and shortening scan duration.

In conclusion, we have implemented a robust reproducible quantitative in vivo cardiac DTI technique that has the potential to enhance our understanding of in vivo structure–function relationships of the normal heart and furthermore be applied to the study of human cardiovascular disease. We show here for the first time that DTI of the human heart in vivo can be performed with a high degree of reproducibility. In addition, the feasibility of biofeedback NAV-based DTI of the heart is shown. While further optimization will be needed to improve resolution, extend coverage, and reduce scan duration, the foundation for high-resolution in vivo DTI of the human heart has been laid. This constitutes an important step forward towards the clinical use of cardiac DTI.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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FIG. 1.

ECG-gated diffusion-weighted STEAM sequence diagram with navigators. This sequence runs over two heartbeats and assumes that the heart is in the same position at both diffusion-encoding times (end systole) on consecutive cardiac cycles. To minimize the length of the single-shot EPI readout, parallel imaging with external reference lines and zonal excitation were implemented. The prospective navigators implemented were based on spin echoes with crossed $90^\circ/180^\circ$ slices and were applied before and after the STEAM module to guarantee that the first and second halves of the STEAM module were in the same breathing position. TD, delay time; TM, mixing or diffusion evolution time.

FIG. 2.

Example b0 and diffusion-encoded images for the MBH and NAV techniques (one average). This data demonstrates the typical image quality obtained throughout the study. The EPI images do not suffer from distortion artifacts due to the short EPI readout (16–20 readouts) achieved with parallel imaging and zonal excitation.

FIG. 3.

Example b_0 images (eight averages) and derived FA, MD, and HA maps for MBH and NAV techniques at three contiguous slice locations in a normal volunteer, together with the segmentation scheme used to extract quantitative data from these maps. The MD and FA maps acquired with the MBH and NAV approaches are extremely similar and highly homogeneous. The transmural evolution in HA can be robustly seen in all slices with both techniques. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIG. 4.

Fractional anisotropy (FA) in LV: Reproducibility and comparison of MBH versus NAV. **a**: Plot of the mean $\pm$ SD fractional anisotropy values for MBH and NAV, initial (day 1) and repeat (day 2) studies. **b** and **c**: Bland–Altman plots and line plots showing the interstudy reproducibility of the MBH (**b**) and NAV (**c**) methods. No statistically significant differences were found between initial and repeat studies. The mean fractional anisotropy value for the initial studies was 0.60 ± 0.04 for MBH and 0.60 ± 0.03 for NAV. **d**: Bland–Altman plot and line plot showing the MBH versus NAV method comparison. No statistically significant differences were seen between the MBH and NAV techniques.

FIG. 5.

Mean diffusivity (MD) in LV: Reproducibility and comparison of MBH versus NAV. **a:** Plot of the mean $\pm$ SD MD values for MBH and NAV, initial (day 1) and repeat (day 2) studies. **b** and **c:** Bland–Altman plots and line plots showing the interstudy reproducibility of the MBH (b) and NAV (c) methods. No statistically significant differences were found between initial and repeat studies. The mean MD value for the initial studies was $0.8 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$ for MBH and $0.9 \pm 0.2 \times 10^{-3} \text{ mm}^2/\text{s}$ for NAV. **d:** Bland–Altman plot and line plot showing the MBH versus NAV method comparison. No statistically significant differences were seen between the MBH and NAV techniques.

FIG. 6.

Helix angle (HA) in LV: Reproducibility and comparison of MBH versus NAV. **a:** Plot of the HA values for every volunteer and for MBH and NAV, initial (day 1) and repeat (day 2) studies. No statistically significant differences were found between the HA values in initial and repeat studies for either technique (MBH: $P = 0.25$, NAV: $P = 0.28$). **b:** Diagrams of the mean $\pm$ SD of the HA values over all volunteers. The diagrams depict the anterior, lateral, inferior, and septal regions further subdivided in the endocardial, midendocardial, midepicardial, and epicardial layers, as defined in the segmentation scheme in Figure 3. From this, it can be observed that the endocar-dial layer HA values are lower for NAV

compared with MBH. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FIG. 7.

Three-dimensional visualization of tensor fields using superquadric glyphs (22) and 3D tractograms resulting from MBH and NAV acquisition techniques. **a** and **b**: Superquadric glyph fields within ROIs (red) located in the lateral wall of three contiguous short axis slices using MBH and NAV, respectively. The helical myofiber pattern is clearly depicted by the principal orientation of the superquadric glyphs. Variations in the glyph sizes and roundness (e.g., subendocardium) indicate that MBH and NAV are subject to different noise sensitivities. **c** and **d**: Overall perspective of 3D fiber tractograms using MBH and NAV, respectively. Both the MBH and NAV acquisitions depicted accurate transmural helical

angles, which demonstrates the reproducibility of the NAV technique despite a relative higher noise level (as shown by the stretched fibers in the subepicardial layer).

Table 1

Reproducibility (Day 1 versus Day 2) of FA and MD for MBH and NAV Techniques and Comparison Between MBH and NAV Techniques

	MBH	NAV	Comparison MBH/NAV	
			Bias	P
FA				
Day 1	0.60 ± 0.04	0.60 ± 0.03	-0.005 ± 0.026	0.57
Day 2	0.60 ± 0.02	0.61 ± 0.02		
Reproducibility	-0.009 ± 0.029	-0.011 ± 0.026		
	Bias			
	P	0.22		
MD (10 ⁻³ mm ² /s)				
Day 1	0.8 ± 0.2	0.9 ± 0.2	-0.08 ± 0.13	0.07
Day 2	0.8 ± 0.2	0.9 ± 0.2		
Reproducibility	0.03 ± 0.09	0.07 ± 0.14		
	Bias			
	P	0.28	0.15	

Table 2
Reproducibility of HA for MBH and NAV Techniques and Comparison Between BH and NAV Techniques

HA (°)		Endo	Midendo	Midepi	Epi
Reproducibility: Day 1 versus Day 2					
MBH	Day1	22 ± 10	20 ± 6	-1 ± 6	-17 ± 6
	Day2	24 ± 7	20 ± 7	0 ± 8	-18 ± 7
	Bias	-2.95 ± 7.53	0.03 ± 5.67	-0.53 ± 6.33	0.63 ± 5.53
	<i>P</i>	0.25	0.99	0.80	0.73
NAV	Day1	7 ± 7	13 ± 8	-2 ± 7	-14 ± 6
	Day2	10 ± 10	15 ± 10	0 ± 9	-12 ± 6
	Bias	-3.04 ± 13.45	-2.25 ± 10.32	-2.33 ± 6.40	-2.16 ± 7.35
	<i>P</i>	0.50	0.51	0.28	0.38
Comparison MBH versus NAV					
Day1	Bias	15.27 ± 10.75	6.94 ± 8.52	1.12 ± 4.68	-3.43 ± 5.63
	<i>P</i>	0.002	0.03	0.42	0.09