Insight Into Myocardial Microstructure of Athletes and Hypertrophic Cardiomyopathy Patients Using Diffusion Tensor Imaging

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Background: Hypertrophic cardiomyopathy (HCM) remains the commonest cause of sudden cardiac death among young athletes. Differentiating between physiologically adaptive left ventricular (LV) hypertrophy observed in athletes' hearts and pathological HCM remains challenging. By quantifying the diffusion of water molecules, diffusion tensor imaging (DTI) MRI allows voxelwise characterization of myocardial microstructure.
Purpose: To explore microstructural differences between healthy volunteers, athletes, and HCM patients using DTI.
Study Type: Prospective cohort.
Population: Twenty healthy volunteers, 20 athletes, and 20 HCM patients.
Field Strength/Sequence: 3T/DTI spin echo.
Assessment: In-house MatLab software was used to derive mean diffusivity (MD) and fractional anisotropy (FA) as markers of amplitude and anisotropy of the diffusion of water molecules, and secondary eigenvector angles (E2A)—reflecting the orientations of laminar sheetlets.
Statistical Tests: Independent samples *t*-tests were used to detect statistical significance between any two cohorts. Analysis of variance was utilized for detecting the statistical difference between the three cohorts. Statistical tests were two-tailed. A result was considered statistically significant at $P \leq 0.05$.
Results: DTI markers were significantly different between HCM, athletes, and volunteers. HCM patients had significantly higher global MD and E2A, and significantly lower FA than athletes and volunteers. ($MD_{HCM} = 1.52 \pm 0.06 \times 10^{-3}$ mm^2/s, $MD_{Athletes} = 1.49 \pm 0.03 \times 10^{-3}$ mm^2/s, $MD_{volunteers} = 1.47 \pm 0.02 \times 10^{-3}$ mm^2/s, $P < 0.05$; $E2A_{HCM} = 58.8 \pm 4°$, $E2A_{athletes} = 47 \pm 5°$, $E2A_{volunteers} = 38.5 \pm 7°$, $P < 0.05$; $FA_{HCM} = 0.30 \pm 0.02$, $FA_{Athletes} = 0.35 \pm 0.02$, $FA_{volunteers} = 0.36 \pm 0.03$, $P < 0.05$). HCM patients had significantly higher E2A in their thickest segments compared to the remote ($E2A_{thickest} = 66.8 \pm 7$, $E2A_{remote} = 51.2 \pm 9$, $P < 0.05$).
Data Conclusion: DTI depicts an increase in amplitude and isotropy of diffusion in the myocardium of HCM compared to athletes and volunteers as reflected by increased MD and decreased FA values. While significantly higher E2A values in HCM and athletes reflect steeper configurations of the myocardial sheetlets than in volunteers, HCM patients

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demonstrated an eccentric rise in E2A in their thickest segments, while athletes demonstrated a concentric rise. Further studies are required to determine the diagnostic capabilities of DTI.
Evidence Level: 1
Technical Efficacy Stage: 2

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INTENSE PHYSICAL EXERCISE over a sustained period of time leads to distinct structural and functional cardiac remodeling, a phenotype commonly described as athlete's heart.[1] In clinical practice, the physiologically adaptive left ventricular (LV) hypertrophy observed in athletes' hearts can be challenging to differentiate from pathological LV hypertrophy seen in patients with hypertrophic cardiomyopathy (HCM). This is particularly relevant, as HCM is one of the commonest causes of sudden unexpected cardiac death among young athletes.[2,3] On the one hand, a false-positive diagnosis of HCM may cause premature discontinuation of a professional sporting career, while the underdiagnosis of HCM may place athletes at an unduly increased risk of sudden cardiovascular death.

While transthoracic echocardiography remains the first-line imaging modality for imaging HCM patients, magnetic resonance imaging (MRI) has gradually established itself as a potent complementary tool thanks to its distinctive advantage of tomographic imaging allowing for tissue characterization and accurate measurement of wall thickness.[4] Postcontrast late gadolinium enhancement (LGE) techniques can depict areas of scarring in HCM patients.[4] However, in order to fully elucidate the different mechanisms underpinning physiological vs. pathological hypertrophy, an imaging tool capable of exploring the microstructural properties of myocardium is required. Diffusion tensor imaging (DTI) is an emerging MRI method that allows noninvasive in vivo characterization of myocardial microstructure.[5,6] DTI is based on the underlying principle that water diffusion is greatest in the direction of the cardiomyocytes. By deriving mean diffusivity (MD) of the water molecules and fractional anisotropy (FA) as a measure of the directional variability of the water molecules, information on the arrangement of the myocardium can be obtained.[7] By measuring the eigenvectors of the diffusion tensor, DTI provides helix angle (HA) maps[8] and secondary eigenvector angle (E2A) maps.[9] HA maps provide a voxelwise representation of local cardiomyocyte orientation, while E2A reflects the average orientations of laminar sheetlets, which depict local states of contraction.

Using DTI, recently published data demonstrate the myocardium of HCM patients to adopt a "fixed hyper-contractile" state with globally increased E2A values throughout the cardiac cycle[9,10] and increased MD in areas of fibrosis in hypertrophied segments.[11] In ventricular arrhythmia, globally decreased FA values reflect underlying cardiomyocyte disarray.[12] Swoboda et al demonstrated HCM patients to have higher extracellular volume (ECV) than athletes,[13] but thus far DTI has not been performed on athletes. The purpose of our study was to perform DTI on healthy volunteers, athletes, and HCM patients in order to explore the differences in the myocardial microstructure between these groups.

Materials and Methods

The regional research committee approved this study and written informed consent was obtained from all subjects.

Subject Recruitment

Athletes were contacted through advertisement at local competitive cycling clubs and were included if they had performed cycling-based exercise for over 6 hours/week, with no history of cardiovascular disease. Basic demographic information was gathered using a questionnaire, which included the frequency and duration of cycling per week as well as the number of years they had been training. Athletes had a cardiopulmonary exercise test (CPEX), after abstaining from intense physical exercise for 24 hours. Patients with HCM were recruited from referrals to the clinical MRI service. The diagnosis of HCM was made independently by clinicians in keeping with current guidelines and based on imaging including MRI, electrocardiogram (ECG), exercise testing, family history, and genetic testing, if possible.[14] Inclusion criteria were: left ventricular wall thickness (LVWT) of ≥15 mm in at least one myocardial segment on MRI. Patients with contraindications for MRI, significant valve disease, previous myocardial infarction, or coronary intervention were excluded. Healthy volunteers were enrolled from among students, staff, and alumni of the local university. They had no existing medical conditions and were not taking any regular medication. Blood samples were drawn from athletes and HCM patients in order to obtain the hematocrit concentration immediately prior to the MRI scan.

Imaging Protocol

MRI was performed using a 3T Philips Achieva TX system (Philips, Best, The Netherlands). Cine imaging used a balanced steady-state free precessional (bSSFP) pulse sequence (echo time [TE] / repetition time [TR] / flip angle 1.3/2.6 msec/40°, spatial resolution $1.6 \times 2.0 \times 10$ mm, typical temporal resolution 25 msec). Basal, mid and apical precontrast (native) short axis T_1 maps were generated using a validated modified Look–Locker inversion (MOLLI) protocol with the following parameters: 5/3/0 acquisition, TE/TR 2.1/0.82 msec, flip angle = 20°, spatial resolution $0.91 \times 0.91 \times 8$ mm, sensitivity encoding (SENSE) 2 acceleration, cardiac delay time 777 msec. Repeat MOLLI T_1 mapping was performed at 15 minutes postcontrast (4/3/2 acquisition, TE/TR 2.1/0.82 msec, flip angle 35°, spatial resolution $0.91 \times 0.91 \times 8$ mm, SENSE 2 acceleration, cardiac delay time 728 msec), and LGE imaging at 16–20 minutes postcontrast (inversion recovery-prepared

T_1-weighted gradient echo, inversion time according to Look–Locker scout, spatial resolution 0.91 × 0.91 × 8 mm, TR/TE/flip angle 3.7/2.0 msec/25°). To ensure consistent slice positioning, image acquisitions were performed in three matching short-axis positions by acquiring the central three slices of five parallel short-axis slices spaced equally from the mitral annulus to the LV apical cap.

DTI data were obtained using a second-order motion-compensated single-shot spin echo planar imaging sequence with SENSE acceleration; acquisition parameters were: TE/TR = 89 msec/3RR intervals, flip angle = 90°, field of view (FOV) = 238 × 238 mm, matrix size = 108 × 105, in-plane resolution 2.3 × 2.3 mm, reconstructed voxel size = 1.7 × 1.7 × 8 mm, SENSE acceleration = 1.8. Slice acquisition was planned by initially obtaining diffusion-weighted imaging (DWI) data in three directions to ensure data quality. Each dataset constituted 18 diffusion encoded acquisitions with b-values of 100 s/mm^2 (×3), 200 s/mm^2 (×3), and 500 s/mm^2 (×12) and up 12 repetitions available per direction.

Data Analysis

MRI data other than DTI maps were analyzed using cvi42 software v. 5.6 (Circle Cardiovascular Imaging, Calgary, Alberta, Canada). LV mass, end-diastolic volumes (EDV), end systolic volume (ESV) and LV ejection fraction (EF) were measured from short axis cine images excluding papillary muscles and trabeculations. Segment thickness was measured for each of 16 segments of the American Heart Association (AHA) model from the end-diastolic bSSFP cine image.[15] Quantitative assessment of LGE images was performed using a threshold of >5 standard deviations above remote normal myocardium for the HCM participants. Remote segments in athletes and HCM patients were identified as directly opposite to the thickest segment. Native and postcontrast T_1 relaxation time of myocardium were measured from the scanner-generated T_1 maps by contouring a region of interest in each AHA segment, in line with T_1 mapping consensus guidelines.[16] Blood pool intensity values were obtained by contouring a region of interest in order to permit the calculation of ECV.[17]

DTI DATA ANALYSIS. Data processing, including coregistration of diffusion-weighed images and myocardial delineation was performed using MatLab software (MathWorks, Natick, MA). Quality control was undertaken by visual assessment of individual diffusion-weighted images prior to averaging, and any that were corrupted by motion or artifact or that failed the registration, were omitted from further processing. Averaged magnitude images were generated from the registered data, from which diffusion tensors were calculated. Tensor eigenvalues, MD, FA, HA, and E2A maps were calculated as previously defined in the literature both globally and on a segmental basis.[9]

CARDIOPULMONARY EXERCISE TEST PROTOCOL. Ramp incremental spiroergometry was performed with the subject on a cycle ergometer (Excalibur Sport, Lode, Groningen, the Netherlands). Athletes were required to abstain from intense exercise in the 24 hours preceding the exercise test. Subjects were encouraged to exercise to exhaustion and a respiratory exchange ratio (VCO_2/

TABLE 1. Baseline Demographics

	HCM (n = 20)	Athletes (n = 20)	Volunteers (n = 20)	ANOVA/K-W test	HCM vs. athletes	HCM vs. volunteers	Athletes vs. volunteers
Age (years)	55 ± 9	41 ± 8	27 ± 8	<0.05	<0.05	<0.05	<0.05
Sex (Male %)	70%	95%	45%	—	—	—	—
SBP (mmHg)	128 ± 16	126 ± 10	—	—	0.2	—	—
DBP (mmHg)	74 ± 5	73 ± 8	—	—	0.5	—	—
BMI (kg/m^2)	28 ± 4	24 ± 3	25 ± 6	<0.05	<0.05	0.14	0.4

Values are displayed as mean ± standard deviation for continuous variables.
HCM = hypertrophic cardiomyopathy, ANOVA = analysis of variance, K-W = Kruskal–Wallis, SBP = systolic blood pressure, DBP = diastolic blood pressure, BMI = body mass index.

VO_2) > 1.1 was taken to suggest maximal effort. Metabolic gas exchange analysis on the expired air was continuously performed (Vmax29, Sensormedics, Yorba Linda, CA) and the peak pulmonary oxygen uptake (VO_2 peak) was obtained.

STATISTICAL ANALYSIS. Statistical analysis was performed using the commercially available software Statistical Package for the Social Sciences (SPSS) Statistics 22.0 (IBM, Armonk, NY). Normality was assessed through the Shapiro–Wilk test and variance was assessed by the Levene's test for equality of variance. Continuous variables are reported as mean ± standard deviation. Normally distributed data were compared using Student's *t*-test or analysis of variance (ANOVA), and nonnormally distributed data by Mann–Whitney *U*-test or Kruskal–Wallis test. ANOVA was utilized for distributed data, a whereas Kruskal–Wallis test was used for nonnormally distributed data to assess the presence of a statistical difference between all three cohorts. Pearson correlation analysis was used to calculate the correlations between independent variables. Statistical tests were two-tailed and $P \leq 0.05$ was considered statistically significant.

Results

Twenty athletes, 20 HCM patients, and 20 volunteers were included in this study. The baseline demographics are displayed in Table 1. Athletes on average performed 9.4 ± 3 hours of exercise per week, for an average of 7.7 ± 5 years and had significantly lower mean body mass than HCM patients ($P < 0.05$). Seventeen athletes completed the exercise test with a mean VO_{2peak} of 58 ± 6 ml/kg/min. There was no significant difference in systolic or diastolic blood pressure between the two cohorts.

The global MRI characteristics are shown in Table 2. Athletes had significantly higher mean body surface area (BSA)-indexed LV-end-diastolic-volume and stroke volume than HCM patients and volunteers ($P < 0.05$). Three athletes had evidence of LGE. Nineteen of the HCM patients were LGE-positive and overall had significantly higher global LGE ($P < 0.05$), native T_1 ($P < 0.05$), and ECV ($P < 0.05$) values than athletes.

Diffusion Tensor Imaging

DTI acquisition was successful in all subjects. Figure 1 shows representative LGE and DTI maps for a healthy volunteer, athlete, and HCM patient. Global DTI measurements are reported in Table 2. There was no significant difference in global MD between athletes and volunteers ($MD_{Volunteers}$ = $1.47 \pm 0.02 \times 10^{-3}$ mm^2/s, $MD_{Athletes}$ = $1.49 \pm 0.03 \times 10^{-3}$ mm^2/s, $P = 0.16$); however, HCM patients had significantly higher global MD than athletes (MD_{HCM} = $1.52 \pm 0.06 \times 10^{-3}$ mm^2/s, $P < 0.05$). There was no significant difference in global FA between athletes and volunteers ($FA_{Volunteers}$ = 0.36 ± 0.03, $FA_{Athletes}$ = 0.35 ± 0.02, $P = 0.5$); however, HCM patients had significantly lower global FA than athletes (FA_{HCM} = 0.30 ± 0.02, $P < 0.05$). Healthy volunteers had steeper HA gradient progression from endocardium to epicardium per mm of LV wall thickness compared to athletes, who had steeper progressions than HCM patients (HA $gradient_{Volunteers}$ = −8.26 ± 1.48 °/mm, HA $gradient_{Athletes}$ = −6.81 ± 1.17 °/mm, HA $gradient_{HCM}$ = −5.4 ± 0.86 °/mm, ANOVA $P < 0.05$). No significant differences were found when looking at the HA gradient progression per percentage of LV wall. Athletes had significantly higher global absolute E2A than volunteers ($E2A_{volunteers}$ = 38.5 ± 7°, $E2A_{athletes}$ = 47 ± 5°, $P < 0.05$), and HCM patients had significantly higher absolute E2A than athletes ($E2A_{HCM}$ = 58.8 ± 4°, $P < 0.05$), reflecting the increased contractile configuration in mid-systole.

Analysis of Thickest Segments

Given the eccentric nature of hypertrophy and pathology usually seen in HCM patients, additional focused analysis of the thickest myocardial segments was undertaken for each cohort. The thickest segments of HCM patients had significantly higher mean thickness ($P < 0.05$), more LGE ($P < 0.05$), higher native T_1 ($P < 0.05$), and ECV ($P < 0.05$) values than the thickest segments of athletes. DTI analysis of the thickest segments reflected similar patterns to those shown in global analysis, with HCM patients having significantly higher MD ($P < 0.05$), lower FA ($P < 0.05$, Fig. 2a), and higher absolute E2A ($P < 0.05$, Fig. 2b) than athletes. In line with previously published data, MD values of thickest segments correlated with the LGE% in the corresponding segment ($P < 0.05$, Fig. 2c). Athletes with LGE in their thickest segments ($n = 3$) had significantly higher MD values than athletes without LGE ($P < 0.05$, Fig. 2d).

Figure 3a demonstrates the relationship between the wall thickness and corresponding segmental ECV in the thickest segments of athletes and HCM patients. In the thickest segments of HCM patients, higher ECV values correlated with lower FA ($P < 0.05$, Fig. 3b) and higher MD ($P < 0.05$, Fig. 3c). Lower FA also correlated with higher absolute E2A values in the thickest segments of HCM patients ($P < 0.05$, Fig. 3d). HCM patients had significantly higher absolute E2A values in their thickest segments in comparison to the remote segments ($P < 0.05$, Table 2), while in athletes there was no significant difference in absolute E2A between thickest and remote segments ($P = 0.70$).

Discussion

This study directly compared DTI parameters between normal volunteers, athletes, and HCM patients. Our results demonstrate: 1) in mid-systole, the myocardial sheetlets of HCM patients and athletes adopt steeper configurations than volunteers, as reflected by the significantly higher global absolute E2A values. In HCM patients, this pattern is of an eccentric nature, with the highest values in their thickest segments, while athletes demonstrate a concentric rise in absolute E2A across the whole myocardium; 2) a significant

TABLE 2. MRI Characteristics

	HCM (n = 20)	Athletes (n = 20)	Volunteers (n = 20)	ANOVA/ K-W test	HCM vs. athletes (P value)	HCM vs. volunteers (P value)	Athletes vs. volunteers (P value)
LVEDV (mL)	152 ± 30	225 ± 27	165 ± 41	<0.05*	<0.05	0.3	<0.05
LVEDVi (mL/m^2)	76 ± 13	115 ± 12	90 ± 21	<0.05	<0.05	0.8	<0.05
LVSV (mL	93 ± 16	127 ± 15	104 ± 23	<0.05	<0.05	0.8	0.05
LVSVi (mL/m^2)	29 ± 7	50 ± 6	33 ± 12	<0.05	<0.05	0.42	<0.05
LVEF (%)	61 ± 7	56 ± 4	64 ± 6	<0.05	<0.05	0.4	0.05
LV Mass (g)	123 ± 27	110 ± 21	96 ± 31	0.03	0.09	<0.05	0.2
LV Mass index (g/m^2)	65 ± 18	56 ± 11	52.6 ± 17	0.09	0.10	0.42	0.95
LGE (% of LV)	6.5 ± 4	0.75 ± 0.1	—	—	<0.001	—	—
Native T_1 (msec)	1277 ± 77	1221 ± 38	—	—	<0.01	—	—
ECV (%)	27.8 ± 4	25.6 ± 3	—	—	0.05	—	—
Global DTI values							
E2A (°)	58.8 ± 4	47.0 ± 5	38.5 ± 7	<0.00001	<0.05	<0.05	<0.05
MD (x10^{-3} mm^2/s)	1.52 ± 0.06	1.49 ± 0.03	1.47 ± 0.02	0.0004	<0.05	<0.05	0.16
FA	0.30 ± 0.02	0.35 ± 0.02	0.36 ± 0.03	<0.00001	<0.05	<0.05	0.5
HA gradient °/mm	−5.4 ± 0.86	−6.81 ± 1.17	−8.26 ± 1.48	<0.001	<0.05	<0.05	<0.05
HA gradient °/%	−0.54 ± 0.10	−0.50 ± 0.10	−0.51 ± 0.08		0.22	0.28	0.78
Thickest segments							
Mean thickness (mm)	18.5	10.4	7.5	<0.001	<0.05	<0.05	<0.05
LGE (% of LV)	15.0 ± 4	0.6 ± 0.3	—	—	<0.05	—	—
Native T_1 (msec)	1296 ± 85	1251 ± 48	—	—	<0.05	—	—
ECV (%)	29.4 ± 5	26.0 ± 3	—	—	0.05	—	—
E2A (°)	66.8 ± 7	49.1 ± 12	38.9 ± 12	<0.001	<0.05	<0.05	<0.05
MD (x10^{-3} mm^2/s)	1.54 ± 0.09	1.49 ± 0.09	1.47 ± 0.08	0.02*	<0.05	<0.05	0.12
FA	0.26 ± 0.04	0.35 ± 0.04	0.38 ± 0.03	<0.001*	<0.05	<0.05	0.1

TABLE 2. Continued

	HCM (n = 20)	Athletes (n = 20)	Volunteers (n = 20)	ANOVA/ K-W test	HCM vs. athletes (P value)	HCM vs. volunteers (P value)	Athletes vs. volunteers (P value)
HA gradient °/mm	−4.8 ± 1.30	−6.75 ± 1.20	−8.10 ± 1.48	0.02	<0.05	<0.05	<0.05
HA gradient °/%	−0.57 ± 0.19	−0.57 ± 0.16	−0.56 ± 0.14		0.85	0.83	0.91
Remote segments							
Mean thickness (mm)	6.6 ± 2**	7.5 ± 1.8**	—	—	0.15	—	—
LGE (% of LV)	3.8 ± 7.1**	0.9 ± 1.3	—	—	0.07	—	—
Native T_1 (msec)	1244 ± 75	1251 ± 48	—	—	0.15	—	—
ECV (%)	26.2 ± 2.3**	25.2 ± 2	—	—	0.22	—	—
E2A (°)	51.2 ± 9**	48.7 ± 11	—	—	0.14	—	—
MD ($x10^{-3}$ mm^2/s)	1.50 ± 0.1	1.50 ± 0.10	—	—	0.99	—	—
FA	0.32 ± 0.05**	0.36 ± 0.03	—	—	<0.05	—	—
HA gradient °/mm	−5.62 ± 2.81	−7.05 ± 2.98	—	—	0.14	—	—
HA gradient °/%	−0.46 ± 0.21	−0.50 ± 0.21	—	—	0.49	—	—

Values are displayed as mean ± standard deviation for continuous variables.
HCM = hypertrophic cardiomyopathy, ANOVA = analysis of variance, K-W = Kruskal–Wallis, LVEDV = left ventricular end diastolic volume, LVEDVi = left ventricular end diastolic volume indexed for body surface area, LVSV = left ventricular stroke volume, LVSVi = left ventricular stroke volume indexed for body surface area, LVEF = left ventricular ejection fraction, LV = left ventricle, LGE = late gadolinium enhancement, ECV = extracellular volume, MD = mean diffusivity, FA = fractional anisotropy, HA = helix angle.
*Indicates the use of the K-W test.
**Indicates a significant difference ($P < 0.05$) in comparison to thickest segment.

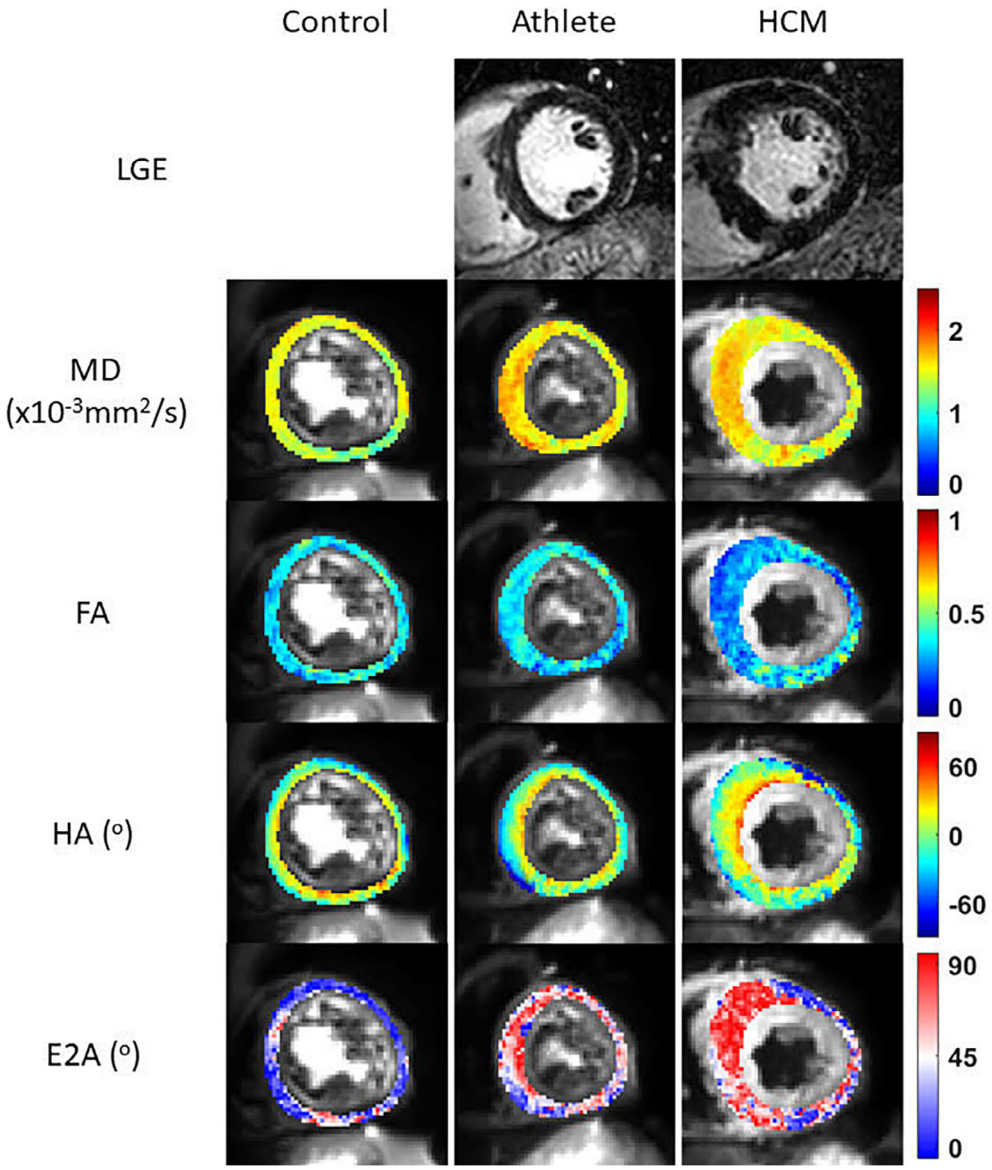

FIGURE 1: First column displays a typical basal left ventricular (LV) slice obtained in a healthy volunteer showing a normal mean diffusivity (MD), fractional anisotropy (FA), secondary eigenvector angle (E2A), and helix angle (HA) maps. Second column demonstrates a basal LV slice in an athlete with diffuse fibrosis in the septal segments, with an eccentric increase in MD and a concentric increase in E2A throughout the myocardium. Third column demonstrates a basal LV slice in a hypertrophic cardiomyopathy (HCM) patient with an eccentric increase in MD and E2A, and reduction in FA in the hypertrophied segment.

increase in the global amplitude and anisotropic diffusion in the myocardium of HCM patients in comparison to athletes and volunteers, as reflected by the increased MD and decreased FA values; and 3) furthermore, in the thickest segments of HCM patients a rise in LGE and ECV directly correlate with increased MD and decreased FA, while no significant associations between ECV and DTI parameters were seen in the thickest segments of athletes. These results provide valuable insights into the underlying myocardial microstructural differences between volunteers, athletes, and HCM patients.

Contractile Configurations Based on DTI

In agreement with previously published studies, we demonstrated HCM patients to have significantly higher absolute E2A values than volunteers.[9,10] This is attributable to the pathologically hypercontracted myocardial sheetlet configurations in HCM patients. Our results demonstrate that athletes have significantly higher absolute E2A values than volunteers, suggesting their laminar sheetlets are in a more hypercontracted state, possibly as a result of athletic remodeling; however, not to the same extent as that seen in HCM patients. In the thickest segments of HCM patients, higher

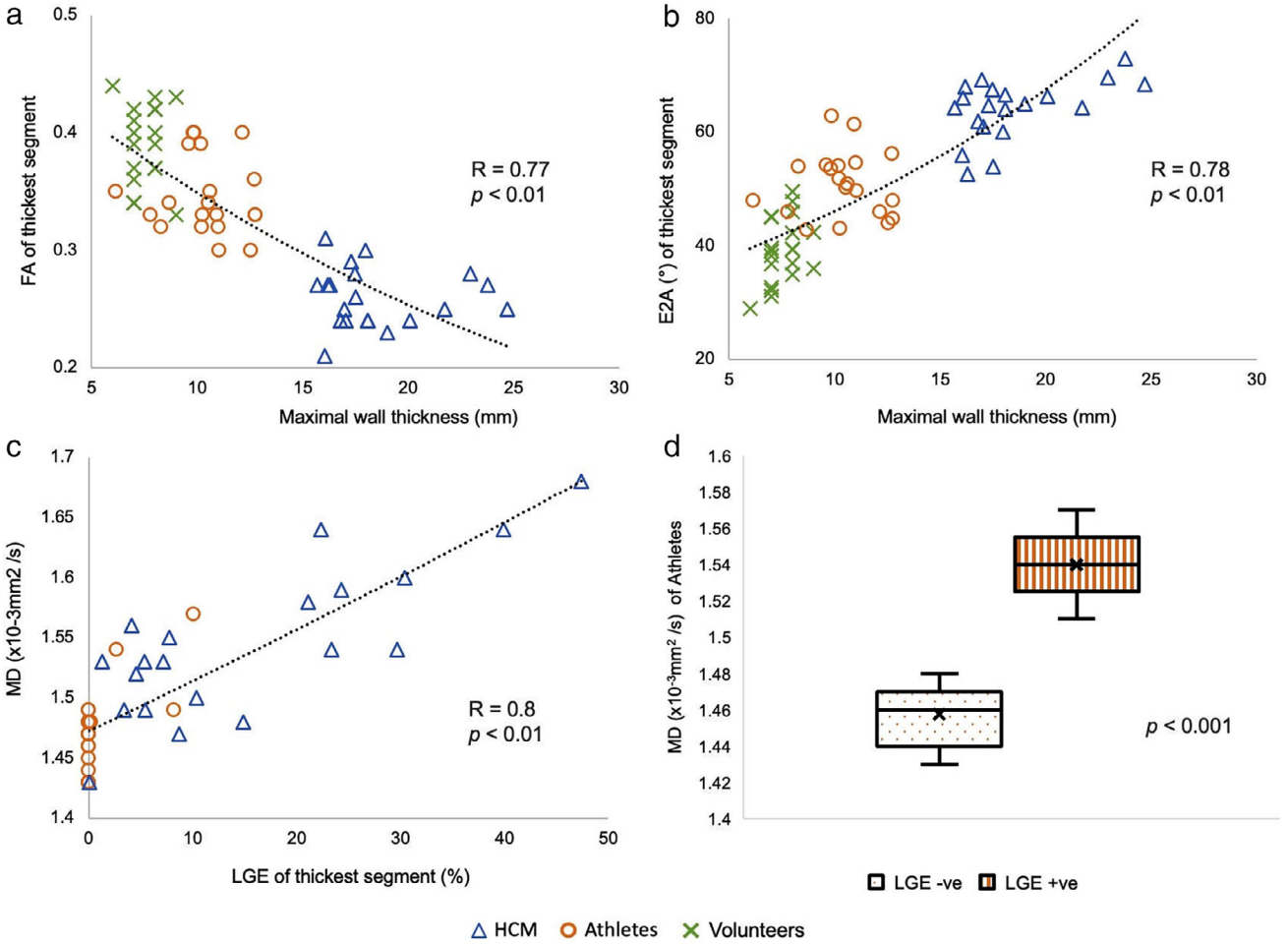

FIGURE 2: Wall thickness of the thickest segments across all cohorts correlated with their segmental fractional anisotropy (FA) (a) and secondary eigenvector angle (E2A) (b) values. Among athletes, late gadolinium enhancement (LGE)% correlated with segmental mean diffusivity (MD) values (c), and athletes with LGE had significantly higher MD than athletes without LGE in their thickest segments (d).

ECV correlated with lower FA and higher absolute E2A, suggesting that the expansion of extracellular space coupled with cardiomyocyte disarray results in the concomitant segment of myocardium adopting a more hypercontracted state in systole. Interestingly, in athletes there was no significant difference in absolute E2A between their thickest and remote segments, indicating that the hypercontraction appears to be uniformly spread in concentric fashion across the entire myocardium as opposed to being exaggerated in the thickest segment, as seen in HCM patients.

The cardiomyocytes in the myocardial mid-wall are made up of circumferentially oriented cardiomyocytes.[18] The mid-walls of HCM patients undergo disarray and fibrosis, particularly at the interventricular junctions and hypertrophied segments[19]; however, given that the pattern of infiltration is diffuse, the distribution of circumferential mid-wall cardiomyocytes remains relatively unchanged overall. Hence, the mean HA gradient progression from HCM and athletes is not expected to differ significantly from volunteers. The steeper HA gradient progression per mm of LV wall observed in HCM patients may be a reflection of the relatively increased wall thickness, rather than a marker of pathology, as no significant differences were found in the HA progression per percentage of LV wall between the three cohorts. These findings warrant further investigations.

Fibrosis and Cardiomyocyte Disarray

MD is a measure of how freely water molecules can diffuse, and as biophysiological barriers such as cell membranes break down, MD is expected to increase. In keeping with previous DTI studies on HCM patients,[11] our results show a direct correlation between segmental MD and LGE values in HCM patients, who had significantly higher MD and LGE than athletes and volunteers. Histological analysis of healthy athlete hearts is lacking; however, Swoboda et al[13] demonstrated athletes to have significantly lower ECV than HCM patients and postulated that the increase in LV mass in athletes is mediated by cellular hypertrophy, whereas in HCM it is driven by cellular disarray and extracellular matrix expansion. Cell membranes remain intact during cellular hypertrophy;

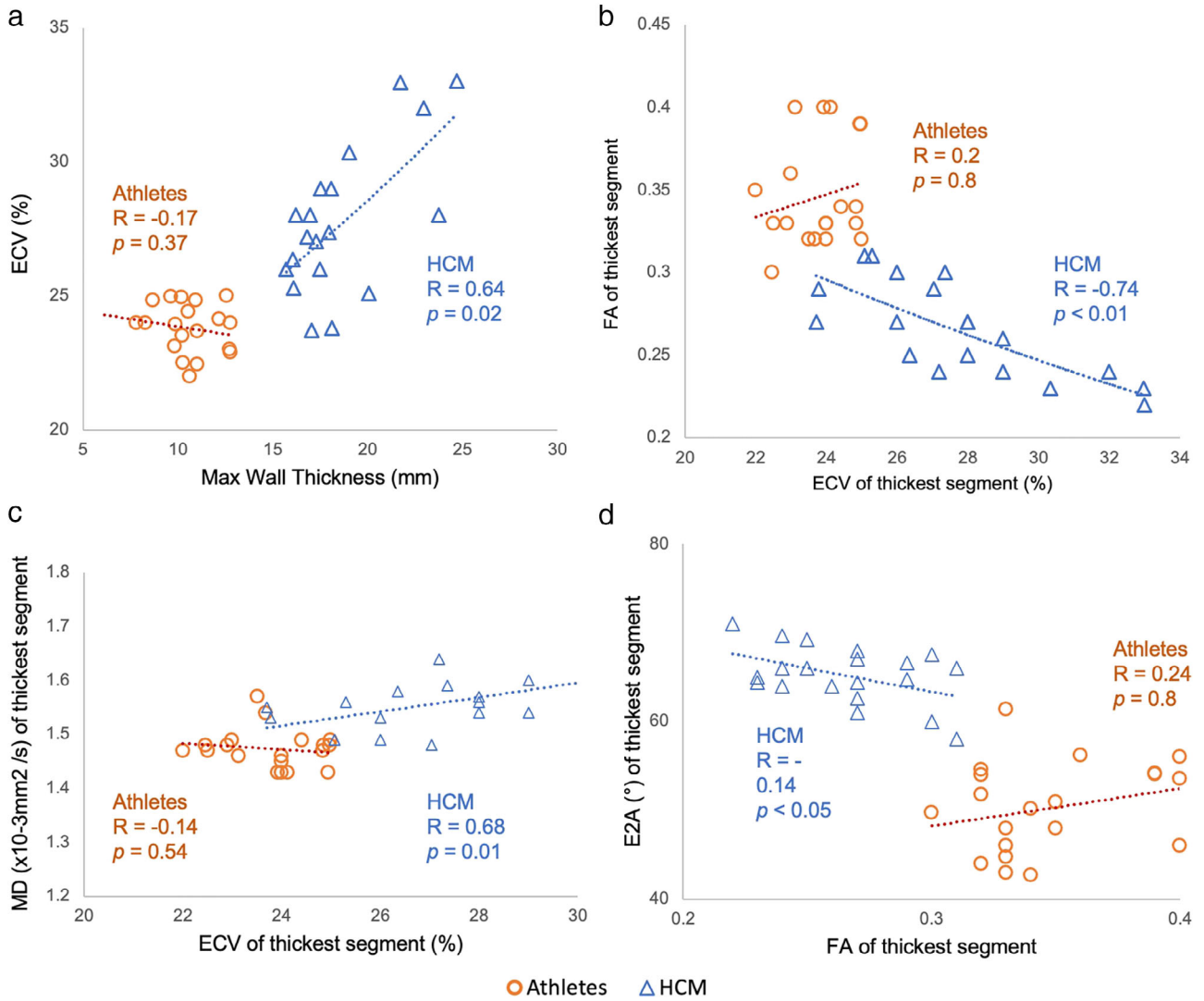

FIGURE 3: In the thickest segments of hypertrophic cardiomyopathy (HCM) patients, wall thickness correlated with extracellular volume (ECV) in the corresponding segment (a). ECV also correlated with fractional anisotropy (FA) (b) and mean diffusivity (MD) (c). Lower FA correlated with higher secondary eigenvector angle (E2A) (d). Athletes meanwhile did not show any significant correlations between ECV and DTI parameters in their thickest segments.

hence, changes in MD are likely to be less pronounced than in fibrosis. This likely explains how, despite having significantly thicker walls, athletes in our studies had comparable MD values to volunteers, but significantly lower than HCM patients. Meanwhile, athletes with evidence of LGE, ie, fibrosis, had significantly higher MD levels in their corresponding segments.

Histological analysis of myocardium resected from HCM patients undergoing surgical myectomy has demonstrated specimens to have disorganized matrix connective tissue and increased collagen content in comparison to volunteers.[20] In such areas of cardiomyocyte disarray, the diffusion of water molecules is expected to be more isotropic; hence, FA is expected to be low. Previous DTI studies of ex vivo human hearts have also demonstrated FA correlates negatively with histological measurements of collagen.[21] Therefore, the significantly lower FA values in HCM patients in this study compared with both volunteers and athletes is in agreement with findings from previous similar studies,[12] and likely reflects a composite measure of both cardiomyocyte disarray and fibrosis. As discussed previously, athletes in our study had significantly thicker LV walls than volunteers, likely in response to athletic remodeling, but despite having significantly higher absolute E2A values than volunteers, FA values did not differ significantly between the two cohorts. This suggests athletic remodeling may result in the myocardial sheetlets adopting steeper configurations in systole, but does not lead to the disarray of cardiomyocytes as seen in HCM patients.

Limitations

This is an exploratory study including only a limited number of subjects who were not age- and sex-matched; HCM patients were older than athletes and volunteers and there was

a preponderance of male participants in the HCM and athlete cohorts, whereas the volunteers consisted of more females. However, it is yet to be established whether DTI parameters change with age or gender. The ECV of healthy volunteers was not obtained, as it was not felt ethically justifiable to administer contrast in this cohort. Our study also lacked athletes and HCM patients with "borderline" wall thickness between the 12- and 15-mm range; however, the intention of this study was to observe the microstructural differences between the different cohorts rather than assess the diagnostic capabilities of DTI. Future studies could look to target patients with borderline wall thickness (12–15 mm) to evaluate if DTI can differentiate HCM patients from athletes.

Conclusion

Athletic training is associated with a spectrum of morphological and functional changes in the myocardium. Through the use of DTI, our study derived possible preliminary insights into some of these changes in myocardial microstructures in vivo. Future DTI studies should target larger cohorts of athletes with greater LV mass and wall thickness, in order to validate the clinical applicability and accuracy of these DTI parameters in differentiating athlete's heart from HCM.

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References

1. Pelliccia A, Maron MS, Maron BJ. Assessment of left ventricular hypertrophy in a trained athlete: Differential diagnosis of physiologic athlete's heart from pathologic hypertrophy. Prog Cardiovasc Dis 2012;54: 387-396.
2. Maron BJ. Sudden death in young athletes. N Engl J Med 2003;349: 1064-1075.
3. Maron BJ, Haas TS, Ahluwalia A, Murphy CJ, Garberich RF. Demographics and epidemiology of sudden deaths in young competitive athletes: From the United States National Registry. Am J Med 2016; 129:1170-1177.
4. Parisi R. Multimodality imaging in apical hypertrophic cardiomyopathy. World J Cardiol 2014;6:916-923.
5. Basser PJ. Inferring microstructural features and the physiological state of tissues from diffusion-weighted images. NMR Biomed 1995;8: 333-344.
6. Stoeck CT, Von Deuster C, GeneT M, Atkinson D, Kozerke S. Second-order motion-compensated spin echo diffusion tensor imaging of the human heart. Magn Reson Med 2016;75:1669-1676.
7. Scollan DF, Holmes A, Winslow R, Forder J. Histological validation of myocardial microstructure obtained from diffusion tensor magnetic resonance imaging. Am J Physiol — Hear Circ Physiol 1998;275:H2308-H2318.
8. Teh I, McClymont D, Burton RAB, et al. Resolving fine cardiac structures in rats with high-resolution diffusion tensor imaging. Sci Rep 2016;6:30573. https://doi.org/10.1038/srep30573.
9. Ferreira PF, Kilner PJ, Mcgill L-A, et al. In vivo cardiovascular magnetic resonance diffusion tensor imaging shows evidence of abnormal myocardial laminar orientations and mobility in hypertrophic cardiomyopathy. J Cardiovasc Magn Reson 2014;16–87. https://doi.org/10.1186/s12968-014-0087-8.
10. Nielles-Vallespin S, Khalique Z, Ferreira PF, et al. Assessment of myocardial microstructural dynamics by in vivo diffusion tensor cardiac magnetic resonance. J Am Coll Cardiol 2017;69:661-676.
11. Wu R, An DA, Shi RY, et al. Myocardial fibrosis evaluated by diffusion-weighted imaging and its relationship to 3D contractile function in patients with hypertrophic cardiomyopathy. J Magn Reson Imaging 2018;48:1139-1146.
12. Ariga R, Tunnicliffe EM, Manohar SG, et al. Identification of myocardial disarray in patients with hypertrophic cardiomyopathy and ventricular arrhythmias. J Am Coll Cardiol 2019;73:2493-2502.
13. Swoboda PP, McDiarmid AK, Erhayiem B, et al. Assessing myocardial extracellular volume by T1 mapping to distinguish hypertrophic cardiomyopathy from athlete's heart. J Am Coll Cardiol 2016;67:2189-2190.
14. Elliott PM. ESC guidelines on diagnosis and management of hypertrophic cardiomyopathy. Russ J Cardiol 2014;35:2733-2779. https://doi.org/10.1093/eurheartj/ehu284.
15. Cerqueira MD, Weissman NJ, Dilsizian V, et al. Standardized myocardial segmentation and nomenclature for tomographic imaging of the heart. Circulation 2002;105:539-542.
16. Messroghli DR, Moon JC, Ferreira VM, et al. Clinical recommendations for cardiovascular magnetic resonance mapping of T1, T2, T2 and extracellular volume: A consensus statement by the Society for Cardiovascular Magnetic Resonance (SCMR) endorsed by the European Association for Cardiovascular Imagin. J Cardiovasc Magn Reson 2017; 19:75.
17. Haaf P, Garg P, Messroghli DR, Broadbent DA, Greenwood JP, Plein S. Cardiac T1 mapping and extracellular volume (ECV) in clinical practice: A comprehensive review. J Cardiovasc Magn Reson 2016;18:89.
18. Streeter DD, Spotnitz HM, Patel DP, Ross J, Sonnenblick EH. Fiber orientation in the canine left ventricle during diastole and systole. Circ Res 1969;24:339-347.
19. Kuribayashi T, Roberts WC. Myocardial disarray at junction of ventricular septum and left and right ventricular free walls in hypertrophic cardiomyopathy. Am J Cardiol 1992;70:1333-1340.
20. Factor SM, Butany J, Sole MJ, Wigle ED, Williams WC, Rojkind M. Pathologic fibrosis and matrix connective tissue in the subaortic myocardium of patients with hypertrophic cardiomyopathy. J Am Coll Cardiol 1991;17(6):1343-1351.
21. Abdullah OM, Drakos SG, Diakos NA, et al. Characterization of diffuse fibrosis in the failing human heart via diffusion tensor imaging and quantitative histological validation NIH public access. NMR Biomed 2014;3100(1):1378-1386.