MR Diffusion Estimation of Intramyocellular Lipid Droplets in Myocardium

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Introduction:

In heart muscle, lipid serves as an important source of cellular energy and it is mostly stored as intramyocellular lipid (IMCL) droplets inside myocardium (1). IMCl droplet is composed of a relatively homogeneous lipid ester core and a phospholipid monolayer. Previous morphometric study demonstrated that the IMCL droplet size in heart increased during acute cerebral ischemia and cardio lipotoxicity (2, 3), implying that lipid droplet size may serve as a useful biomarker for cardiac diseases. Those studies were based on the method of transmission electron microscopy (TEM), which is laborious, invasive and can only cover very small portion of sample during quantitative analysis. Our recent MR studies ,have demonstrated that, by taking advantage of highly restricted diffusion property of IMCL droplet, it might be possible to employ diffusion-weighted MRS (DW-MRS) to probe the microstructure of lipid droplet (4, 5). In this study, we first investigated the distribution of IMCL droplet size by measuring apparent diffusion coefficient (ADC) in different parts of heart and different layers of myocardium. Then we estimated the droplet size from the MR diffusion signal decays acquired at two diffusion times.

Methods:

Sample preparation: Fresh muscle samples from normal adult pig hearts were examined. Five heart samples were exercised from five positions of heart (shown in Fig.1(a)) and scanned. Samples from lateral wall were scanned at four adjacent locations to measure $IMCL_{ADC}$ within different layers. Six samples from septum were scanned using two diffusion times (80ms and 220ms), and signal decays were used to estimate lipid droplet size of heart. ***MRI Protocol:*** MRI protocols were detailed in our previous study (5). In the current study, 12 b-values ranging from 0 to $4.0\times10^5 mm^2/s$ were used. In order to test ADC_{IMCL} in different layers of myocardium, voxel was adjusted to $5\times10\times10mm^3$. ***Data Analysis:*** All spectra were analyzed using JMRUI and Bruker TOPSPIN software package. The b-value dependent IMCL spectra were quantified by fitting to a Gaussian line shape using AMARES algorithm in JMRUI. Due to the contamination of other small molecules (e.g. lactate) at 1.28ppm, ADC_{IMCL} was determined by fitting the spectra decay to a bi-exponential decay. Analysis of variance (ANOVA) was performed for ADC_{IMCL} calculation in different parts of heart and different layers of myocardium. To visualize the lipid droplet structure and droplet distribution, heart samples were examined histologically using TEM. Lipid droplets were identified and their sizes were measured using ImageJ software.

Size estimation: The root mean square displacement (λ) was calculated by $\lambda = \sqrt{2Dt_{diff}}$, where $t_{diff} = \Delta - \delta/3$ is the effective diffusion time (6) at two diffusion times, and then they were averaged together. Assuming a spherical geometry for the compartment, the diameter is given by $\phi = \sqrt{10}\lambda$. If a nonuniform diameter distribution is taken into consider, diameter measurement represents the characteristic diameter $\phi_c = \sqrt{\sum_i \xi(\phi_i)\phi_i^2}$ which is the diameter weighted by its volume function (7): $\xi(\phi_i) = n(\phi_i){\phi_i}^3 / \sum_i n(\phi_i){\phi_i}^3$.

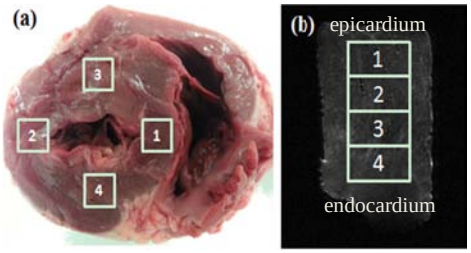

Fig.1 (a) Representative voxel placement in different parts of heart: (1) septum, (2) lateral wall, (3) posterior wall,(4) anterior wall, (5) apex (not shown). **(b)** Voxel location in different layers of myocardium.

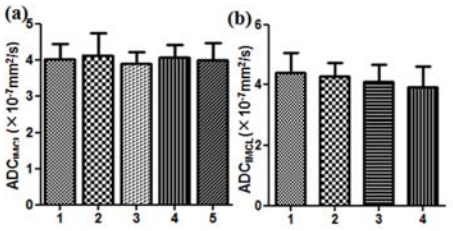

Fig.3 ADC_{IMCL} in different parts of heart **(a)** and different layers of myocardium**(b)**.

	$ADC_{IMCL}(mm^2/s)$		ϕ_c(m)
	Δ=80ms	Δ=220ms	
#1	4.14E-07	8.70E-08	6.77E-07
#2	4.02E-07	9.96E-08	6.93E-07
#3	4.91E-07	1.47E-07	8.01E-07
#4	5.41E-07	1.49E-07	8.24E-07
#5	3.24E-07	6.90E-08	6.01E-07
#6	4.43E-07	1.06E-07	7.21E-07
mean	4.36E-07	1.10E-07	7.19E-07

Table 1 ADC_{IMCL} measured using short and long diffusion times and estimated characteristic IMCL droplet diameter in six heart samples.

Results and Discussion:

Fig.2 illustrates the typical DW spectra and diffusion decays from heart muscle at two diffusion times. The $(-CH_2-)_n$ peak at 1.28ppm was observed to decay bi-exponentially. The ADC_{IMCL} in different parts of heart and different layers of myocardium are shown in Fig.3(a) and Fig.3(b), respectively. Statistical analysis results (p = 0.99 and 0.95 respectively) show that there was no significant difference in ADC_{IMCL} within each group, indicating that IMCL droplet size was relatively the same within different parts of heart and different layers of myocardium. In addition, TEM observation (Fig.4) revealed that IMCL droplets were seen to be scattered swithin almost all muscle cells in myocardium. This observation may be related to the fact that synchronized contraction of the myocardium likely demands energy supply and storage in a spatially homogeneous manner. The mean diameter of IMCL droplet measured from TEM images was 0.52±0.17um and their characteristic diameter was 0.65um. Table 1 shows the ADC_{IMCL} measured with short and long diffusion times, and the estimated results in 6 samples. When diffusion time Δ increased from 80ms to 220ms, ADC_{IMCL} exhibited significant decrease, i.e., from $4.36\times10^{-7}mm^2/s$ to $1.10\times10^{-7}mm^2/s$, indicating the spatially restrictive nature of small IMCL droplets. From MR results, the characteristic diameter of lipid droplet was estimated to be 0.72±0.08um, which was comparable with the TEM measurement. The good agreement between these MR and TEM results supported the use of DW-MRS approach for IMCL droplet size estimation in myocardium.

Conclusion:

In this study, we investigated the lipid droplet size distribution in different parts of heart and different layers of myocardium by measuring their ADC_{IMCL}. ADC_{IMCL} was documented to be relatively homogeneous within myocardium, indicating homogeneous IMCL droplet size distribution within heart. Lipid droplet size was estimated from MR diffusion decays and result was in good agreement with the TEM measurement, demonstrating DW-MRS as a non-destructive and efficient method to quantify IMCL droplet size and provide insights into IMCL droplet dynamics in heart muscle.

References:

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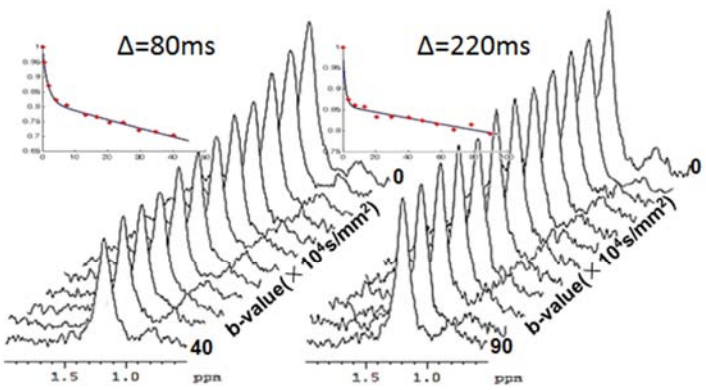

Fig.2 Typical ex vivo DW spectra

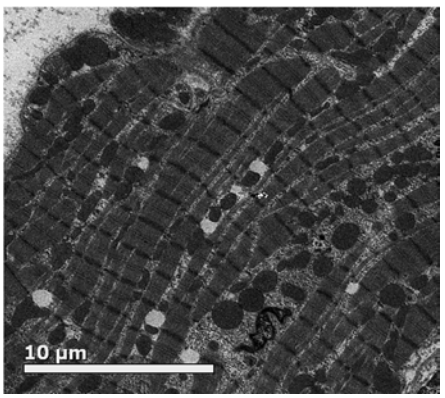

Fig.4 TEM images of myocardium.