Apparent Diffusion Coefficient of Intramyocelluler Lipid in Heart Muscle

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INTORDUCTION:

In heart muscle, fat is stored as intramyocellular lipid (IMCL) droplets within the cardiomyocytes and extramyocellular lipid (EMCL) within the relatively large adipocytes[1]. Recent study suggests that myocardial lipid deposition induce cardiovascular disease[2] and be related to insulin resistance status[3]. Proton magnetic resonance spectroscopy (^{1}H-MRS) offers a valuable in vivo tool to measure the intramyocardial lipid (IMCL) in humans[4]. However, the characteristics of IMCL droplets in heart muscle such as their droplet microstructure remain largely unknown though they may reflect the lipid metabolism in myocardium. Recent MR studies have demonstrated the use of diffusion weighted MRS (DWMRS) to characterize the restricted IMCL diffusion in skeletal muscles[5, 6]. In this study, we investigated the IMCL diffusion property in heart muscle samples. We hypothesized that IMCL in heart muscle would exhibit slow and isotropic diffusion due to the spatially restrictive nature of small IMCL droplet size.

MATERIALS AND METHODS:

Sample preparation: Seven fresh muscle samples from normal adult pig hearts were examined (N=7). Samples were excised from the interventricular septum with epicardium and endocardium carefully removed. For comparison, three fresh skeletal muscle samples from pig lower hindlimbs were also examined (N=3). **MRI Protocol:** All experiments were performed on a 7T horizontal-bore Bruker MRI scanner with max gradient 360mT/m along each direction. The pre-emphasis of gradient system was carefully adjusted, exhibiting minimum eddy current along x axis. For DW-MRS, a stimulated-echo (STEAM) based single-voxel MRS sequence was implemented by adding a pair of unipolar diffusion gradients along the x axis during two TE/2 intervals. To measure diffusion decays in heart samples, DW-MRS were performed with diffusion duration δ=30ms, diffusion time Δ=80ms, TR/TE=1500/80ms, voxel size=8×8×8 mm^3, 4 b-values ranging from 0 to 3.0×10^5mm^2/s with diffusion gradient (which was always applied along x-axis) parallel and perpendicular to the muscle fiber orientation by rotations along y-axis and z-axis. For skeletal muscle samples, long TE of 180ms was employed to provide more reliable separation between IMCL and EMCL resonances. **Data Analysis:** All spectra were analyzed using JMRUI and Bruker TOPSPIN software package. The b-value dependent IMCL signals were quantified by fitting to a Gaussian line shape using AMARES algorithm in JMRUI. The apparent diffusion coefficients (ADCs) were calculated with a monoexponential model. To visualize the lipid droplet structure and droplet distribution, both heart and skeletal muscle samples were examined histologically using Oil Red O immunohistochemical staining for lipid[7].

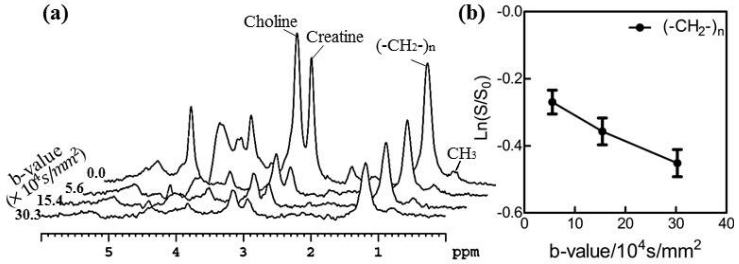

Fig. 1 (a) Typical ex vivo diffusion weighted (DW) spectra observed in a fresh muscle sample from a normal adult pig heart. **(b)** DW decay of IMCL signal $(-CH_2-)_n$ at 1.28 ppm observed in all seven samples studied.

IMCL ADC	Heart muscle	Skeletal muscle
(// muscle fiber)	0.76±0.20	
(⊥ muscle fiber)	0.74±0.20	
(⊥ muscle fiber)	0.77±0.21	1.75±0.61

Table 1 Ex vivo IMCL ADC in heart muscles (N=7) along three directions with respect to muscle fiber orientation. Measurements are expressed as mean ± standard deviation in unit of 10^{-6}mm^2/s. IMCL ADC in skeletal muscles (N=3) was also shown.

RESULTS AND DISCUSSION

Fig. 1 shows the typical DW spectra from heart muscle with 3 b-values of 0.56×10^5, 1.5×10^5 and 3.0×10^5s/mm^2. IMCL resonance $(-CH_2-)_n$ was observed at 1.28 ppm, which was independent of muscle fiber orientation. Table 1 shows that ADCs were similar along three directions, as intuitively expected from the isotropic droplet microstructure. Moreover, IMCL ADC was observed to be lower in heart muscle than in skeletal muscle. Fig. 2 shows the typical Oil Red O staining of the micron-sized IMCL droplets in heart and muscle samples. IMCL droplets were observed to exhibit generally smaller sizes in heart muscle than in skeletal muscle. This histological observation was consistent with the lower IMCL ADC observed in heart muscle, i.e., more restricted diffusion (within smaller droplets). In addition, IMCL droplets were seen to be scattered within almost all muscle cells in heart muscle, however, only within certain muscle cells (of oxidative type) in skeletal muscle. Note that, in present study, DW spectra were obtained from a relatively large voxel in myocardium septal wall within which the muscle fiber directions are known to diverge, this might lead to underestimation of the IMCL diffusion anisotropy, if any, in heart muscle. Also note that it was plausible that IMCL signals (Fig. 1a) could be partly contaminated by the EMCL signals whose resonance locations could spread due to the diverging fibers within the large voxel. Such contamination was likely present given that the signal decay occurred faster between b=0 and 0.56×10^5s/mm^2 (Fig. 1b), for which IMCL ADC was estimated only using b=0.56×10^5, 1.5×10^5 and 3.0×10^5s/mm^2 (excluding b=0) in this study.

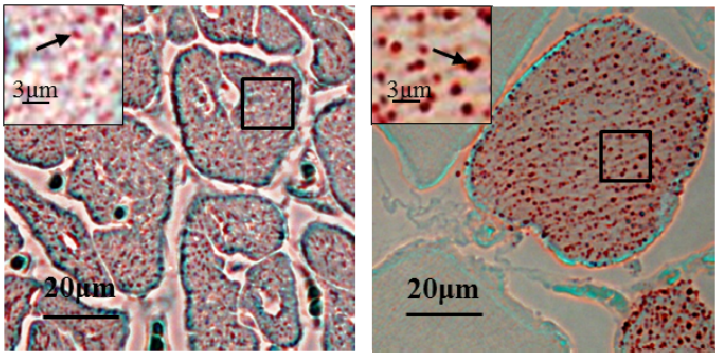

Fig. 2 Oil Red O staining of IMCL droplets (in red dots scattered within myocytes in heart muscle (left) and skeletal muscle (right).

CONCLUSION:

In this study, we investigated the IMCL diffusion property in heart muscle. IMCL ADC was documented in fresh heart muscle samples at 80ms diffusion time, exhibiting slow and largely isotropic diffusion. IMCL ADC was found to be lower than that in skeletal muscle, which likely resulted from the smaller IMCL droplet size, i.e., more restricted diffusion. Such diffusion characterization of IMCL heart muscle may provide insights in study of the IMCL droplet microstructure and lipid dynamics in heart muscle.

REFERENCE:

[1] B. Gaborit, et al., Int J Obes (Lond) (2011). [2] J. M. McGavock, et al., Annals of internal medicine 144 (7), 517-524 (2006). [3] A. R. Wende, et al., Biochimica et biophysica acta 1801 (3), 311-319 (2010). [4] L. S. Szczepaniak, et al., Magnetic resonance in medicine 49 (3), 417-423 (2003). [5] V. Brandejsky, et al., Magnetic resonance in medicine (2011). [6] L. Xiao, et al., Magnetic resonance in medicine 66 (4), 937-944 (2011). [7] E. E. Spangenburg, et al., Journal of biomedicine & biotechnology 2011, 598358 (2011).