Diffusion weighted spectroscopy: a novel approach to investigate intramyocellular lipids

L. Xiao[1,2], and E. X. Wu[1,2]

[1]Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Hong Kong, Hong Kong SAR, China, People's Republic of, [2]Department of Electrical and Electronic Engineering, The University of Hong Kong, Hong Kong, Hong Kong SAR, China, People's Republic of

INTRODUCTION: Recognizing the correlation between insulin sensitivity and intramyocellular lipid (IMCL) levels, muscle ^{1}H MRS has been extensively pursued for assessing IMCLs and extramyocellular lipids (EMCLs) [1, 2]. One major technical challenge in ^{1}H MRS IMCL quantitation is the spectral overlap by the large, broad and muscle orientation (with respect to main B_0 field) dependent EMCL spectrum. Although several studies have demonstrated the improved IMCL resolvability by using long echo time [3, 4], it remains difficult to achieve robust separation in some muscles such as the vastus muscles which are typically used for biopsies. Moreover, in obese patients which are the targeted population in study of insulin sensitivity, presence of large amount of EMCLs in muscles and their contamination render the reliable IMCL separation and quantitation even more difficult. Given that EMCLs are from the fat cells in muscle tissue while IMCLs are constrained within the small fat droplets within muscle cells, we hypothesized that their proton diffusivities would significantly differ because of the more restrictive proton diffusion environment in IMCLs. This study aimed to investigate the proton diffusion properties in IMCLs and EMCLs in muscles ex vivo and in vivo, and the feasibility of suppressing EMCLs by diffusion weighted MRS for assessment of IMCLs.

MATERIALS AND METHODS: All ^{1}H MRS and diffusion weighted MRS (DW-MRS) experiments were performed on a 7T horizontal-bore Bruker MRI scanner (max gradient of 360mT/m). For ex vivo and in vivo experiments, fresh skeletal muscle samples (from pig lower hind limbs; N=5) and the hind limbs of adult Sprague-Dawley rats (200-250g; N=3) were examined using a surface receive coil and quadrature transmit/receive coil, respectively. The DW-MRS sequence was modified from the single voxel PRESS sequence with unipolar diffusion weighting gradients located on both side of the last 180 RF pulse (90-TE/4-180-TE/4-TE/4-180-TE/4). For ex vivo study of muscle samples, DW-MRS was performed with TR/TE=1500/250ms, voxel size=1.2ml, sample points=2048, spectral width=4kHz, δ/Δ=50/71ms, b-values ranging from 0 to 8.2×10^5 s/mm^2 with diffusion gradient direction perpendicular and parallel to muscle fiber direction, and scan time 2.5mins per b-value. For in vivo study of SD rat hind limbs, DW-MRS was performed using TR/TE=1500/180ms, δ/Δ=35/55ms, voxel size=0.5ml, b-values ranging 0 to 2.5×10^5s/mm^2, scan time 5mins per b-value, and other parameters same as those in ex vivo study. Spectra analysis was performed using QUEST in JMRUI. The apparent diffusion coefficients (ADCs) were measured with by monoexponential fitting of areas under peak using an in-house Matlab software.

RESULTS: Fig. 1 shows the typical diffusion weighted (DW) spectra and corresponding diffusion fitting curves for ex vivo muscle samples. The four resonance peaks in each spectrum correspond to EMCL(CH_2) (*e*-CH_2), IMCL(CH_2) (*i*-CH_2), EMCL(CH_3) (*e*-CH_3) and IMCL(CH_3) (*i*-CH_3), respectively. The diffusion gradient directions were along x, y and z for (a,d), (b,e) and (c,f), respectively. Note that the spectral broadening in Fig. 1c was caused by Gz eddy current in our system. Fig. 2 shows the in vivo results. As the *e*-CH_3 and *i*-CH_3 spectra were negligible at the long TE used, only *e*-CH_2 and *i*-CH_2 were observed and characterized. These ex vivo and in vivo findings indicated that the diffusion decays with respect to b-values are largely monoexponential for both IMCLs and EMCLs. More importantly, they demonstrated that EMCL peak(s) can be largely suppressed by diffusion weighting (DW) with certain large b-values while IMCL peak(s) remains visible. As shown in Table 1, IMCL ADCs were found to be substantially smaller than EMCL ADCs. Note these IMCL and EMCL ADCs were drastically smaller than those for protons in water.

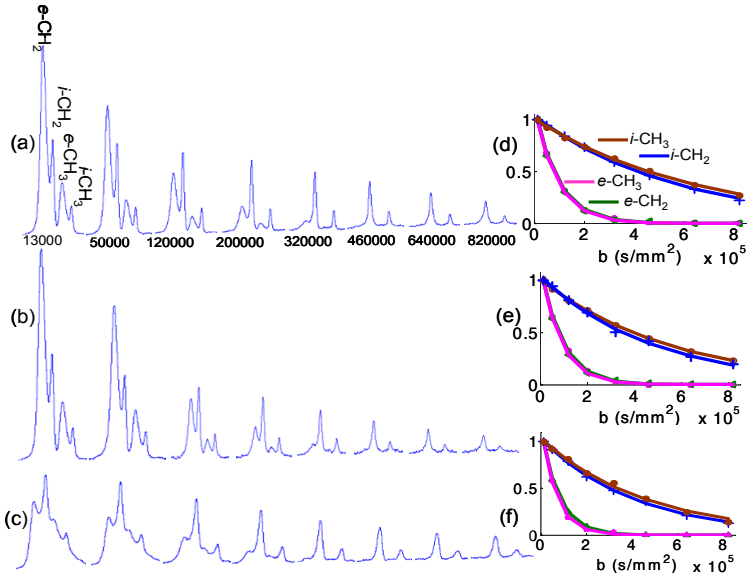

Fig. 1 Typical diffusion weighted MRS (DW-MRS) of pig skeletal muscle sample ex vivo. (**a-c**) DW spectra at various b-values in unit s/mm^2 with muscle fibers parallel to the main magnetic field Bo and the diffusion gradient along x, y and z, respectively; (**d-f**) Corresponding monoexponential fitting curves for ADC measurements.

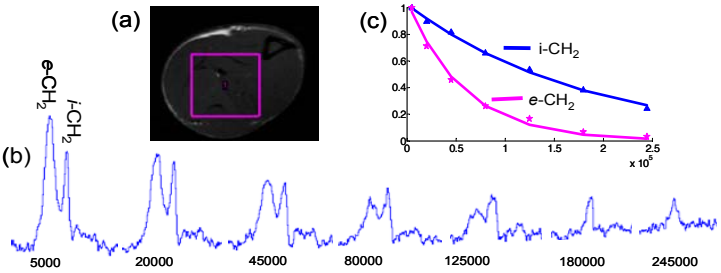

Fig. 2 Typical DW-MRS of adult rat hind limb muscles in vivo. (**a**) T1-weighted image showing MRS voxel size and location; (**b**) DW spectra at various b-values in unit s/mm^2 with diffusion gradient direction along x; (**c**) Corresponding DW monoexponential fitting for ADC measurement in EMCL and IMCL.

Direction of the diffusion gradients	ADC (Ex Vivo)				ADC (In Vivo)	
	e-CH_2	*e*-CH_3	*i*-CH_2	*i*-CH_3	*e*-CH_2	*i*-CH_2
x(⊥muscle fiber)	10.8±0.5	11.2±0.7	1.8±0.5	1.6±0.6	13.9±2.5	6.1±0.5
y(⊥muscle fiber)	11.1±0.5	11.4±0.5	2.0±0.6	1.7±0.7		
z(//muscle fiber)	14.2±0.7	15.8±0.8	2.4±0.7	2.1±0.6		

Table 1 Apparent diffusion coefficients (ADCs) of EMCLs and IMCLs ex vivo and in vivo. Measurements are expressed as mean ± SD in unit of 10^{-6}mm^2/s.

DISCUSSION AND CONCLUSIONS: It is well recognized that IMCLs and EMCLs exist in two different compartments in skeletal muscles. Our experimental findings supported our hypothesis that their diffusion properties substantially differ. Because EMCLs have significantly higher ADCs than IMCLs, DW-MRS can permit effective EMCL suppression and allow more accurate IMCL quantitation. Such DWMRS approach is advantageous over the existing spectral separation approach (which requires the alignment of muscle fiber in parallel with B_0). First, because EMCL suppression by DW is muscle fiber orientation independent, it can be used to examine various muscles including vastus muscle, or large muscle volume to achieve better SNR. Secondly, water suppression is not necessary. At the large b-value used to suppress EMCLs, water proton signal will be also drastically suppressed because of its much higher ADC. Thirdly, because only IMCLs remain after DW, the current approach could be potentially adopted for direct imaging of IMCLs in vivo. Note that technically eddy current can lead to inaccurate ADC estimation (see Fig .1c), but this can be mitigated for example by replacing the unipolar diffusion gradients with bipolar ones. Also note that long TE was used in the current study to accommodate the large b-value, this also provides further EMCL suppression as EMCLs have been shown to have shorter T2 values [5]. In conclusion, we demonstrated a novel diffusion weighted MRS approach for robust separation and characterization of IMCLs. This approach can be readily employed in study of human skeletal muscles in vivo, providing more reliable IMCL quantitation and metabolic information in MRS/MRI investigation of obesity and diabetes.

References: [1] F. Schick et al, Magn Reson Med 29 (1993), 158–167. [2] C. Boesch et al, MRM 37 (1997), 484–493. [3] J. M. Ren et al, MRM 64 (3) (2010), 662-671. [4] S. Ramadan et al, JMR 204 (2010), 91-98. [5] L. G. Wang et al, JMRI 29 (2009), 1457-1464.