Diffusion Magnetic Resonance Monitors Intramyocellular Lipid Droplet Size *In Vivo*

Peng Cao[1,2], Shu-Juan J. Fan[1,2], Anna M. Wang[1,2], Victor B. Xie[1,2], Zhongwei Qiao[1,2], Gary M. Brittenham[3], and Ed X. Wu[1,2]

[1]*Laboratory of Biomedical Imaging and Signal Processing, The University of Hong Kong, Pokfulam, Hong Kong, China,* [2]*Department of Electrical and Electronic Engineering, The University of Hong Kong, Pokfulam, Hong Kong, China,* [3]*Departments of Pediatrics and Medicine, Columbia University College of Physicians and Surgeons, New York, NY, United States*

INTRODUCTION: Intramyocellular lipid (IMCL) droplets, principal repositories of the intramuscular triglyceride used as metabolic fuel during exercise, are dynamic complex organelles with vital roles in regulating the synthesis, utilization, and trafficking of lipids [1-4]. The involvement of IMCL droplets in the pathogenesis of insulin resistance in obesity and in type 2 diabetes is an area of intense investigation [5, 6]. The IMCL droplet is composed of a relatively homogeneous lipid ester core and a phospholipid monolayer [1, 7, 8], presenting a spatially restricted environment for lipid molecular diffusion (Fig.1). MR diffusion studies offer a potential means of probing lipid droplet microstructures *in vivo* by characterizing the diffusion behavior of lipid protons. In this study, the restricted diffusion behavior of IMCL was characterized by examining the IMCL apparent diffusion coefficients (ADC) with DW-MRS sensitized to the very slow diffusion of lipid molecules. MR results were compared with histological analyses of lipid droplet size using transmission electron microscopy (TEM). To determine the sensitivity of IMCL ADC to lipid droplet size changes *in vivo*, IMCL ADC were examined in rodent hindlimb muscles under normal feeding condition, after 60-hr fasting, with streptozotocin (STZ)-induced diabetes, and with high-fat-diet (HFD)-induced obesity.

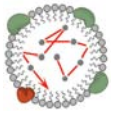

Fig. 1 Illustration of lipid diffusion restricted by IMCL droplet microstructure.

METHODS: Animal Preparation: Adult SD rats were divided into four groups. The control group (n=14) were examined under normal feeding condition. The fasting group (n=6) were examined after 60-hr food deprivation. The obese group (n=11) were first given high-fat diet starting from postnatal day 25 and then examined at 8 weeks later. The diabetic group (n=6) were first injected intravenously with streptozotocin (100mg/kg) and then examined at 7 weeks later. During MR experiments, animals were positioned by an in-house hindlimb fixation device, anesthetized with 1-1.5% isoflurane, paralyzed with bromide pancrunomium (1mg/kg/hr IP), and mechanically ventilated. **MR Protocols:** All MR measurements were made using a 7T Bruker scanner with 370mT/m gradient along each axis. For diffusion-weighted (DW) MRS, a STEAM based single-voxel MRS sequence was implemented by adding a pair of unipolar diffusion gradients along the x-axis during the two TE/2 intervals. DW proton spectra were acquired with δ=30 ms, Δ=80 and 220ms, 12 b-values, TR/TE = 1500/100ms, and voxel size=8×8×8mm^3. For Δ=80ms, b-value=0-5.0 ×10^5 s/mm^2 and NEX =64. For Δ=220ms, b-value=0-1.45 ×10^{6}s/mm^2 and NEX=128 were used. **Histological experiments:** To quantify the IMCL droplet size, excised soleus and plantaris muscle samples from four groups (n=1 for each group) were examined by TEM at ×1450 to ×3400. **Data Analysis:** Each FID was stored individually, and automatic line-to-line FID phase correction was applied to mitigate motion-induced ADC overestimation. IMCL (i-CH_2) and EMCL (e-CH_2) signals were quantified by fitting the spectrum to a Gaussian line shape using the AMARES algorithm in JMRUI software. IMCL ADC was computed by fitting the b-value dependent IMCL signals to a monoexponential model. Droplet diameter was estimated from ADC measurement using a spherically bounded diffusion model [9]. Two-tailed unpaired student's t-test with Welch's correction was performed for comparisons (*p<0.05, **p<0.01 & ***p<0.001).

RESULTS AND DISCUSSION: As shown in Fig.2, monoexponential diffusion decays were observed for IMCL. More importantly, IMCL ADCs decreased markedly with diffusion time, confirming the spatially restricted diffusion of lipid molecules within IMCL droplets. Furthermore, IMCL ADC was sensitive to metabolic alterations, decreasing in the 60-hr fasting and diabetic groups while increasing in the obese group. These findings indicated that the IMCL droplet size decreased following 60-hr fasting and in STZ-induced diabetes but increased in high-fat-diet-induced obesity. IMCL droplet size as determined by TEM was closely correlated with ADC and droplet diameters estimated from ADC (Figs. 3 and 4).

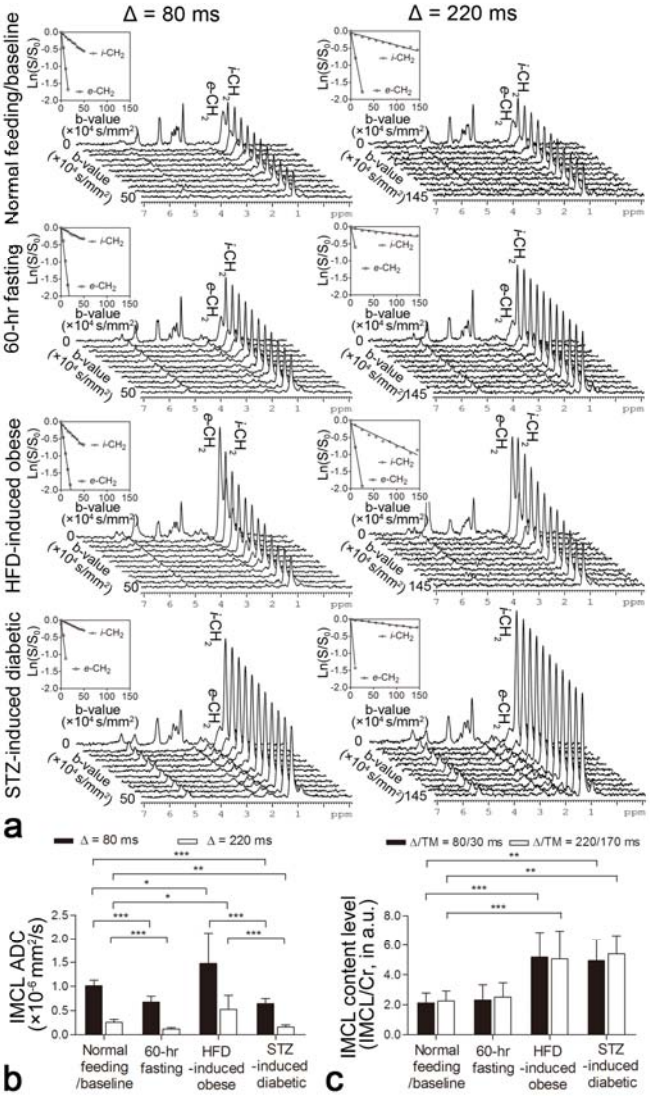

Fig. 2 (**a**) *In vivo* DW spectra observed in hindlimbs of control, fasted, obese, and diabetic rats. (**b**) The IMCL (i-CH_2) ADC (mean±SD) decreased with increase of diffusion time Δ. IMCL ADC was observed to decrease in fasted and diabetic groups but increase in obese group. (**c**) IMCL levels (mean±SD) increased substantially in obese and diabetic groups.

CONCLUSION: Our results clearly demonstrate the feasibility of MR diffusion characterization of IMCL droplet microstructure and provide evidence of the sensitivity of this method to metabolic alterations. The use of diffusion MR methodology *in vivo* promises to provide new biophysical insights in the investigation of droplet dynamics and lipid metabolism in both animal models and human subjects. The diffusion MR results may help contribute to an improved understanding and diagnosis of obesity, diabetes and other metabolic disorders.

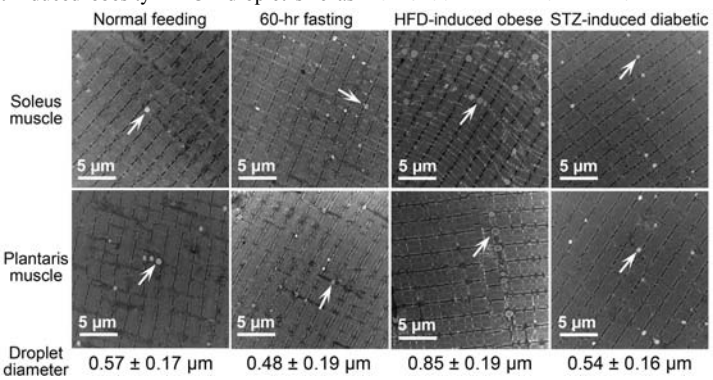

Fig. 3 IMCL droplets (arrows) shown in TEM images and their estimated average diameters (mean±SD) in muscle samples from control, fasted, obese, and diabetic rats (n=1).

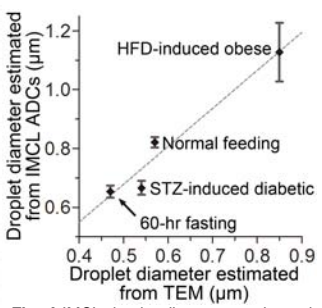

Fig. 4 IMCL droplet diameters estimated from ADC measurements (mean±SEM) exhibited a positive linear correlation (R^2=0.95) with the droplet diameters determined by TEM.

REFERENCES: 1. Martin S et al., Nat Rev Mol Cell Biol 2006; **2.** Boesch C, NMR Biomed 2006; **3.** Guo Y, Nature 2008; **4.** Coen P et al. Trends in endocrinology and metabolism 2012; **5.** Kelley DE, Annu Rev Nutr 2002; **6.** Greenberg AS, J Clin Invest 2011; **7.** Beller M, FEBS Lett 2010; **8.** Suzuki M, J Electron Microsc (Tokyo) 2011; **9.** Garasanin T, Langmuir 2002.