In Vivo Diffusion Assessment of Intramyocellular Lipid Droplet Size Changes Associated with High-fat Diet Induced Obesity and Streptozotocin Induced Diabetes

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INTRODUCTION: Intramyocellular lipid (IMCL) serves as the principal reservoir for storing cellular energy in muscles. Mounting evidence indicates that elevated IMCL in skeletal muscle is closely associated with insulin resistance and type 2 diabetes [1-3]. One biopsy study observed that the size of IMCL droplets was significantly smaller after weight loss by 16-wk exercise training and caloric restriction, and the improvement in insulin sensitivity was correlated with this change [4]. By using diffusion weighted MRS (DW-MRS), we examined the IMCL droplet size dynamics during high-fat diet induced obesity and streptozotocin (STZ) induced diabetes. These two animal models are both known to increase IMCL content but with different mechanisms [5-7]. In this study, we aimed to examine whether the IMCL apparent diffusion coefficient (ADC) measurement could differentiate these two models and to document their IMCL droplet sizes.

MATERIALS AND METHODS: Animal Preparation: Fifteen adult male Sprague-Dawley (SD) rats were divided into three groups. The control group (n=6) were examined at body weight of 400 to 450 g under normal feeding condition. The obese group (n=4) were given high-fat diet, starting from an averaged body weight of 300 g, for 3 weeks before examination to be age-matched with the control rats with an average body weight of 500 g. For diabetic group, 6 animals were injected intravenously with streptozotocin (100 mg/kg) at 7 weeks prior to the time of the MR experiment with an average body weight of 410 g [5]. During MR experiments, animals were anesthetized with a mixture of air and 1-1.5% isoflurane under mechanical ventilation and positioned by an in-house hindlimb fixation device. In addition, muscle paralyzer (bromide pancrunomium, 1 mg/kg/hr, intraperitoneal infusion) was administered to further reduce small physiological motions. **MRI Protocols:** All MRI measurements were made using a 7T Bruker scanner equipped with a 370mT/m gradient system along each axis. For diffusion-weighted 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 the two TE/2 intervals. DW proton spectra were acquired with δ/Δ=30/80 ms, 12 b-values (0 to 5.0×10^5 s/mm^2), TR/TE=1500/80 ms, NEX=64, and voxel size=8×8×8 mm^3. **Histological experiments:** To demonstrate the IMCL droplet size, excised gastrocnemius red muscle samples from three groups were examined under a TEM system (Philips EM208s). For each TEM sample, uniformly 15 to 20 positions covering various regions and fiber types were imaged with a magnification of 3400. **Data Analysis:** Spectral analysis was performed using the JMRUI and TOPSPIN software package. 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. IMCL ADC was computed by fitting the b-value dependent IMCL signals to a monoexponential model. All measurements were expressed as mean ± standard deviation. Two-tailed unpaired student's t-test was employed to examine ADC difference between different animal groups. Results were considered significant with $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns: not significant).

RESULTS: As seen in Fig. 1, IMCL level increased substantially in obese and diabetic groups. More importantly, IMCL ADC was significantly smaller in diabetic group (Figs. 1 and 2), i.e., $(0.64\pm0.10)\times10^{-6}$ mm^2/s vs. $(0.98\pm0.14)\times10^{-6}$ mm^2/s ($p<0.01$) for diabetic and control groups. Meanwhile, IMCL ADC in obese group, i.e., $(1.09\pm0.29)\times10^{-6}$ mm^2/s, was slightly larger than control group but the increase was not significant ($p>0.05$) (Figs. 2). Fig.3 shows the typical TEM images of the excised gastrocnemius red muscle samples from three groups. The IMCL level increased in obese and diabetic samples. Estimated IMCL droplet diameters based on these TEM images were 0.34±0.10 μm, 0.36±0.06 μm, and 0.27±0.06 μm for muscle samples from control, obese, and diabetic rats. In the diabetic sample, the IMCL droplet size was much smaller than in obese and control samples.

DISCUSSION: The increase of IMCL level in obese and STZ-diabetic groups was expected, since IMCL levels are known to alter in high-fat diet induced obesity and STZ induced diabetes [5-7]. The measured small IMCL ADC in diabetic group was consistent with the IMCL droplet histology (shown in Fig. 3), as smaller droplet size would lead to more restricted diffusion (i.e., reduced ADC). In previous studies, a biophysical model was suggested to explain the relationship between IMCL droplet size and insulin resistance. Assuming proteins controlling droplet size and lipase accessibility are located at the phospholipid monolayer surrounding the IMCL droplet, the phospholipid monolayer has been suggested to be active in modulating the accessibility of lipase to lipid content within the droplets [4, 8]. Interestingly, this model also revealed the biophysical meaning of the IMCL ADC, as the phospholipid monolayer not only restricts the accessibility of lipase to lipid content, but also restricts the lipid molecule diffusion. Therefore, the IMCL ADC measurements in these three groups may provide insights into their different lipid metabolic status.

CONCLUSION: Our experimental results demonstrated that in vivo IMCL ADC measurements can differentiate high-fat diet induced obesity and STZ induced diabetes. In obese model, the IMCL level increased but the droplet size and IMCL ADC didn't change. In diabetic model, IMCL level increased but droplet size and IMCL ADC were both smaller. Diffusion measurements were largely in agreement with histological observation. Such an MR approach may provide a new dimension in the study of intracellular lipogenesis and lipolysis, and lead to improved understanding and diagnosis in treatment and management of several prevalent metabolic disorders such as obesity and diabetes in both basic and clinical sciences.

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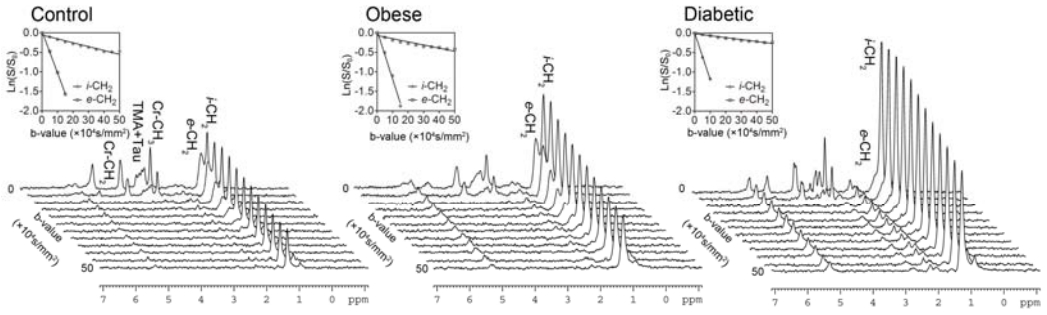

Fig. 1 Typical in vivo diffusion weighted spectra observed in hindlimb skeletal muscles from obese, diabetic, and control rats. IMCL level increased substantially in obese and diabetic rats. The IMCL (i-CH_2) ADC of diabetic rat, was smaller (i.e., the diffusion decay was slower) than obese and control rats.

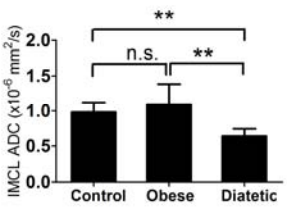

Fig. 2 The IMCL ADC of diabetic group was smaller than obese group, suggesting the IMCL ADC can differentiate two models.

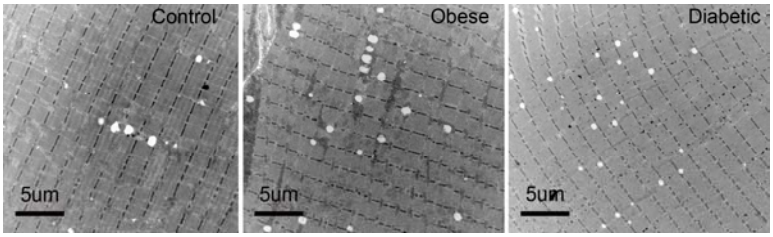

Fig. 3 The IMCL droplet electron microscopy observed in gastrocnemius red muscle samples from obese, diabetic, and control rats. IMCL droplets were marked by the bright spheres. The IMCL content increased in obese and diabetic samples. Droplet size in diabetic sample was smaller than in control and obese samples.