Diffusion Investigation of Intramyocellular Lipid Droplet Changes in Skeletal Muscle under Fasting Condition

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INTRODUCTION: Intracellular lipids exist in the form of small droplets, and they are now increasingly recognized as dynamic functional organelles that play a critical role in many intracellular processes including lipid metabolism (1-3). In skeletal muscle, intramyocellular lipid (IMCL) serves as the principal reservoir for storing cellular energy. MRS offers a noninvasive method to study IMCL in vivo. A strong negative correlation between IMCL level and insulin sensitivity has been previously reported in insulin resistant human subjects (4). However, confounding findings also exist (5,6), indicating that determining IMCL level alone is insufficient. Other biophysical properties of IMCL such as droplet size may reflect the balance between droplet synthesis and degradation. IMCL droplet is typically micron-sized and often found next to mitochondria. It is composed of a relatively homogeneous lipid ester core and a phospholipid monolayer, thus presenting a highly restricted environment for lipid diffusion. Recently, the apparent diffusion coefficient (ADC) of IMCL protons has been reported using a diffusion weighted MRS (DW-MRS) approach in rat skeletal muscle (7). In this study, we hypothesized that diffusion characterization of IMCL protons could serve as a sensitive marker for monitoring IMCL droplet dynamics during metabolic intervention/abnormality. Specifically, we aimed to examine whether IMCL diffusion behavior alters in normal adult rats during 60-hr fasting.

MATERIALS AND METHODS: Animal Preparation: Normal adult male Sprague-Dawley (SD) rats (N=12; 2-mon old, 420-450g) were divided into two groups. For fasting group, 6 animals were examined after a 60-hr water-only fasting. For control group, 6 animals under normal feeding condition were examined. Animals were housed two per cage in a 22ºC room on a 12-hr light/dark cycle. During MR experiments, animals were anesthetized with a mixture of air and 1-1.5% isoflurane, positioned by an in-house hindlimb fixation device, and mechanically ventilated. In addition, muscle paralyzer (Pavulon 1mg/kg IP) was administered to further reduce the small physiological motions such as muscle tone related motion. **MRI Protocol:** All MRI measurements were made using a 7T Bruker scanner equipped with a 370mT/m gradient system along each 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 the two TE/2 intervals. For each animal, DW proton spectra were acquired with diffusion duration δ=30ms, 7 b-values (0 to 3.03×10^5 s/mm^2), and diffusion time Δ of 80 and 220ms. Other parameters were TR/TE=1500/80ms, NEX=64, spectral width=4kHz, and voxel size=8×8×8mm^3. **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. The quantification was considered to be relevant only when the corresponding Cramer-Rao lower bounds were below 25%. IMCL ADCs were 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 two animal groups and two diffusion times. Results were considered significant when $p<0.05$ (*$p<0.05$, **$p<0.01$, ***$p<0.001$, ns: not significant).

RESULTS AND DISCUSSIONS: As seen in Fig. 1, IMCL level was higher in fasting group, which was expected since fasting is known to alter lipid metabolism and increase IMCL level (8). When diffusion time Δ increased from 80ms to 220ms, IMCL ADCs decreased drastically in both normal and fasting groups (Figs. 1 and 2), indicating highly restricted diffusion due to the IMCL droplet microstructure. More importantly, IMCL ADCs decreased significantly in fasting group, i.e., from $(2.26\pm0.63)\times10^{-6}$ to $(1.65\pm0.49)\times10^{-6}$ mm^2/s and $(1.55\pm0.39)\times10^{-6}$ to $(0.74\pm0.32)\times10^{-6}$ mm^2/s for Δ=80ms and Δ=220ms, respectively (Figs. 1 and 2). Fig.3 shows the preliminary IMCL droplet Oil Red O staining observed in the excised muscle samples from two groups. It indicated that, after 60-hr fasting, the IMCL level increased, and this increase was manifested by the drastic increase in number of IMCL droplets, however, their sizes were generally much smaller. Note that small droplet size increases the surface-to-volume ratio of the spherical droplets, thus improving the lipases accessibility with the triacylglycerols within droplets improved (9). Improved lipases access may reflect the increase of lipid metabolism during 60-hr fasting. As smaller droplet sizes would lead to more restricted diffusion (i.e., reduced ADC), our diffusion measurements in Figs. 1 and 2 were consistent with the IMCL droplet histology shown in Fig. 3. More quantitative analysis of droplet numbers and sizes, including TEM examination of ultra-thin muscle samples, is currently underway.

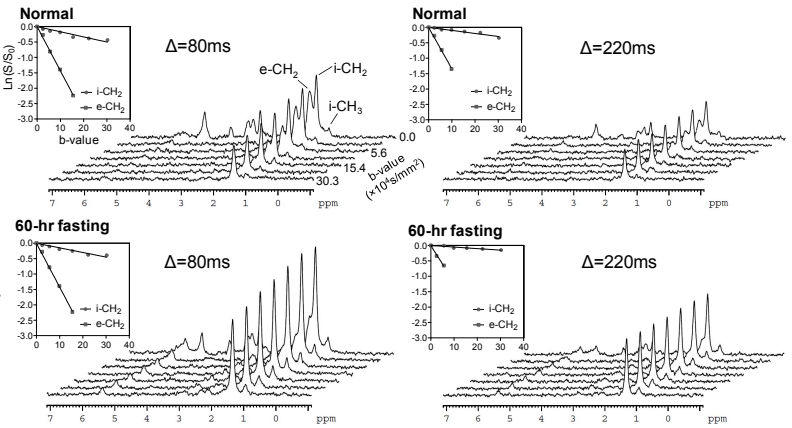

Fig. 1 Typical in vivo diffusion weighted spectra observed in hindlimb skeletal muscles from one normal adult SD rat (top row) and one fasted for 60 hrs (bottom row). Substantially reduced diffusion was observed with long diffusion time Δ=220ms (left vs. right column) in both normal and fasting groups, indicating highly restricted diffusion. More importantly, IMCL ADC decreased drastically after 60-hr fasting at both diffusion times.

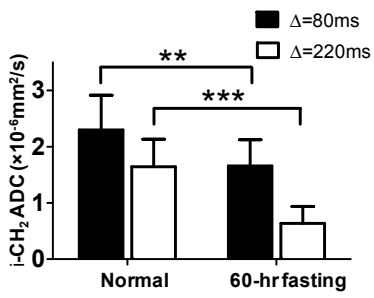

Fig. 2 Effect of 60-hr fasting on IMCL (i-CH_2) ADC. 60-hr fasting caused drastic decrease in IMCL ADC, suggesting the IMCL ADC is sensitive to lipid metabolic manipulations/interventions.

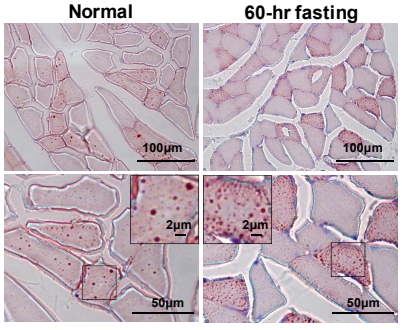

Fig. 3 Oil Red O staining of IMCL droplets (in red scattered dots within muscle cells) showed that, after 60-hr fasting, number of IMCL droplets increased, but their sizes were much smaller.

CONCLUSION: Our experimental results demonstrated that in vivo IMCL diffusion characteristics are sensitive to metabolic interventions such as fasting. During the 60-hr fasting, IMCL level increased but exhibited more restricted diffusion, largely in agreement with our histological observation of more droplets but of smaller sizes. IMCL diffusion characterization may serve as a sensitive marker to probe the IMCL droplet dynamics in vivo. 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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