Dynamic 1H-MRS Revealed Muscle Type Dependent IMCL Storage At Resting State

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INTRODUCTION: The relationship between accumulation of intramyocellular lipid (IMCL) in skeletal muscle and insulin resistance is not well understood at present time [1]. IMCL storage in skeletal muscles depends on lipid oxidative capacity and fatty acid flux rate [2]. Several ex vivo studies demonstrated that the slow-oxidative soleus (SOL) muscle exhibits higher triglyceride synthesis rate than the fast-oxidative glycolytic plantaris (PL) muscle [3-4]. However, IMCL storage rate in individual skeletal muscle has not investigated in vivo. In this study, we explore the IMCL storage in SOL and PL muscles at resting state in SD rats under normal feeding (postprandial) and 24h fasting by use of dynamic proton MR spectroscopy. We hypothesize that the capability of IMCL storage depends on muscle types.

MATERIALS AND METHODS: **Animal Preparation:** Healthy adult SD rats (N=7, 380-420g) were fed ad libitum and housed at 22±0.5°C with a 12h/12h day/night cycle. **MRS Protocol:** All MRS experiments were conducted on a 7T Bruker scanner using a receive-only surface coil and rats were anesthetized with a mixture of air and 1-1.5% isoflurane, fixed by an in-house hindlimb fixation device. For each animal, 3h dynamic ^{1}H-MRS was performed first in the morning (postprandial), and immediately after 24h water-only fasting. Single-voxel ^{1}H-MRS was acquired with PRESS and water suppression using VAPOR sequence, TR/TE= 1200/20ms, spectral width=4000Hz, number of data point=2048, number of averages=512 (10 min per measurement). The voxel was 4×4×2 mm^3 and localized in SOL and PL muscles (Fig.1). During 3 hours, four measurements were made in each muscle in an interleaving manner. **Data Analysis:** All spectra were analyzed using jMRUI 3.0. The chemical shift of all metabolites was referenced to that of creatine at 3.02 ppm. IMCL (1.28 ppm) signal intensity was quantified by fitting the spectrum to a Gaussian-shaped line using AMARES method. Measurement errors were assessed by Cramér-Rao lower bounds (<15%). IMCL values were calculated as the ratio of IMCL and total creatine signal integral [5]. Dynamic IMCL changes were characterized by both percentage increase (ΔIMCL%) and absolute increase (ΔIMCL) from the baseline values at 0h. The results were presented as mean ± SEM. IMCL changes were compared using two-tailed paired t-tests. The difference was considered significant when *p<0.05 and **p<0.01.

RESULTS: The baseline IMCL values at postprandial was significantly higher in SOL (1.14±0.12) than in PL (0.57±0.09) (p<0.001). Compared to postprandial, IMCL baseline in SOL significantly decreased (-43±13%) after 24 fasting (0.58±0.10, p=0.033) as expected from short term fasting [6-8], but did not significantly decreased (-8±16%) in PL (0.48±0.08, p=0.427). During 3h resting, IMCL increase was more pronounced in SOL than in PL (Fig.2). Percentage increase ΔIMCL% at 3h was significantly higher in SOL than that in PL at postprandial (47.0±9.2% in SOL vs. 23.5±8.3% in PL, p=0.001) and after 24h fasting (111.6±18.1% in SOL vs. 56.8±11.8% in PL, p=0.028) (Fig.3). Significantly higher ΔIMCL% changes were observed after 24h fasting in both SOL (p= 0.030) and PL (p=0.042). In terms of absolute increase, ΔIMCL was significantly higher in SOL than that in PL (Fig. 4). Mean rate of absolute increase (ΔIMCL/h) in SOL was higher than that in PL at postprandial (0.178±0.036 in SOL vs. 0.044±0.017 in PL, p=0.003), as well as after 24h fasting (0.179±0.019 in SOL vs. 0.072±0.007 in PL, p=0.004). However, ΔIMCL changes during 3h resting were not significantly different at postprandial and 24h fasting in both SOL (p=1.000) and PL (p=0.311) (Fig. 4). Note that the average coefficient of variation (CV) of creatine signals in each 3h dynamic measurement series was 2.75±0.35%.

DISCUSSIONS AND CONCLUSION: Using dynamic ^{1}H-MRS, we demonstrated the IMCL accumulation or storage in skeletal muscles at resting state. More importantly, IMCL increase at resting state was found to be more rapid in SOL than in PL. Such IMCL storage in skeletal muscle occurs at resting state likely because the available fatty acid supply exceeds the lipid oxidation demand [9-10]. A more/faster IMCL storage in SOL at rest should be attributed to a higher muscle triglyceride synthesis [3-4]. IMCL is known to change dynamically. However, it is difficult to use conventional biopsy method to quantify such IMCL storage. With dynamic ^{1}H-MRS and creatine as the internal reference, a muscle type dependent IMCL storage at resting state was observed and quantified in this study. Such functional IMCL storage quantified by dynamic ^{1}H-MRS could be used as an indicator for studying and probing the dynamic balance between IMCL synthesis and utilization during lipid metabolism in both animal models and humans in vivo.

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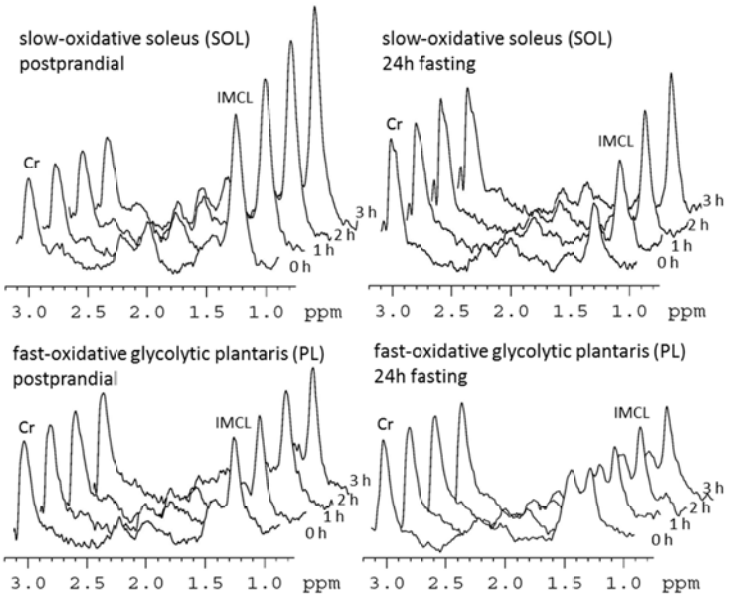

Fig.2 Typical IMCL (at 1.28ppm) changes during 3hr resting in SOL and PL muscles at postprandial and 24h fasting while creatine signals were unchanged.

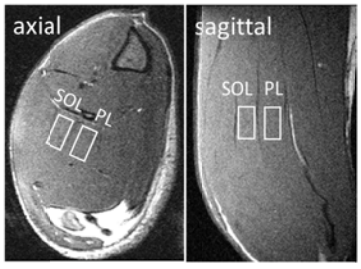

Fig.1 Typical MRS voxel locations for SOL and PL muscles in a rat hindlimb.

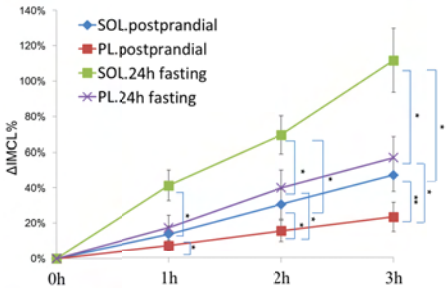

Fig.3 IMCL percentage increase (ΔIMCL%) in SOL and PL muscles at postprandial and 24h fasting.

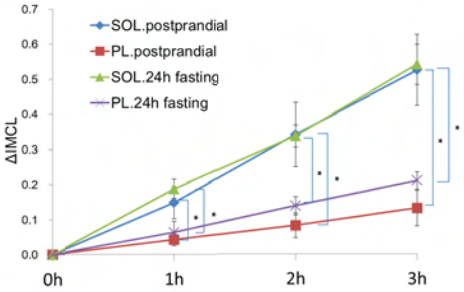

Fig.4 IMCL absolute increase (ΔIMCL) in SOL and PL muscles at postprandial and 24h fasting.