1H-MRS Investigation of IMCL Storage during Resting in Skeletal Muscle: Obese Versus Normal Rats

Zhongwei Qiao[1,2], Peng Cao[1,2], Anna M. Wang[1,2], Victor B. Xie[1,2], Shujuan Fan[1,2], and Ed X. Wu[1,2]

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

INTRODUCTION: Intramyocellular lipid (IMCL) accumulation in sedentary individuals is associated with reduced insulin sensitivity. However, in trained athletes, elevated IMCL storage is rather related to the available energy storage and increased insulin sensitivity, a phenomena called 'athletes paradox'[1]. A higher oxidative, lipolytic, and storage capacity in the muscle of trained athletes reflects a higher turnover rate [2]. In this study, we hypothesized that the gradual IMCL storage occurs in skeletal muscle during resting, and such storage at resting state would alter in obesity. Specifically, we investigated the IMCL storage at resting state in obese rats by quantifying the rates of IMCL accumulation using continuous and dynamic ^{1}H-MRS.

MATERIALS AND METHODS: **Animal Preparation:** Obese rats (N=4, 520-560g) were induced with high-fat diet (containing 20 g high-fat /100 g diet for 10 weeks [3]. Seven age-matched normal adult rats (N=7, 380-420g) were fed ad libitum as control group. **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 after rats normally feeding. Single-voxel ^{1}H-MRS was acquired with a PRESS sequence with VAPOR water suppression, TR/TE=1200/20ms, spectral width=4000 Hz, number of data point=2048 and number of averages=512 (10 min per measurement). The voxel was 4×4×2 mm^3 and carefully localized in slow-oxidative soleus (SOL) muscle and fast-oxidative glycolytic plantaris (PL) muscles. During each hour, two 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 [4]. IMCL signal intensity at 1.28 ppm (i-CH_2) 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 to total creatine signal integral [4]. Dynamic IMCL changes during 3h resting were quantified by both percentage increase (ΔIMCL%) and absolute increase (ΔIMCL) from the baseline values at 0h. IMCL changes were presented as mean ± SEM and compared using two-tailed t-tests. Difference was considered significant when $p<0.05$.

RESULTS AND CONCLUSION: The baseline IMCL values in both SOL and PL were significantly higher in obese rats than in control rats (Table 1 and Fig. 1). During 3h resting, the IMCL percentage increases (ΔIMCL%) in SOL and PL were higher in control rats (Figs. 1 and 2) while the absolute increases were higher in obese rats (Figs. 1 and 3). Table 1 shows the rates of percentage increase (ΔIMCL%/h) and absolute increase (ΔIMCL/h) in SOL and PL muscles observed during the 3h resting. These preliminary results demonstrated that, in obese rats, the rates of IMCL percentage increase during resting were significantly lower while the rates of absolute IMCL increases were higher when compared to normal controls. During resting, plasma fatty acids are trafficked largely to IMCL droplets before they enter long-chain acylcarnitines oxidative pools [5]. Thus, IMCL is an important pool that regulates the delivery of fatty acids to the intracellular environment. IMCL pool is highly dynamic and reflects the balance between lipolysis and lipid synthesis. This dynamic balance is influenced by mitochondrial fat oxidation and plasma free fatty acid availability. A high turnover rate of the IMCL pool has been considered as a means to reduce accumulation of lipotoxic intermediates. Conversely, reduced oxidative capacity and a mismatch between IMCL lipolysis and β-oxidation can be detrimental to insulin sensitivity by generating several lipotoxic intermediates in sedentary populations including obese/type 2 diabetic subjects [1]. Some studies ex vivo showed the increased FA uptake and altered FA disposal to storage may contribute to the development of muscle insulin resistance in obese rats [6-7]. Thus elevated IMCL in obesity may reflect a chronically elevated blood lipid profile, an alteration in triglyceride–fatty acid cycling within the muscle, and/or a chronically low muscle lipid oxidation in these individuals. Such lipid metabolism dysfunctions may cause a slow turnover of IMCL pool [1-2]. Biochemical and histological methods are not adequate for sensitive measurement of IMCL accumulation and turnover due to the labile nature of the IMCL pool. In this study, we demonstrated that dynamic 1H-MRS could quantify the IMCL accumulation at resting state in individual muscles. Our preliminary results revealed that the disorder of lipid metabolism in obesity is associated with not only an increased IMCL content, but also the alterations in the rates of IMCL storage at resting state in both slow-oxidative muscle and fast-oxidative glycolytic muscles.

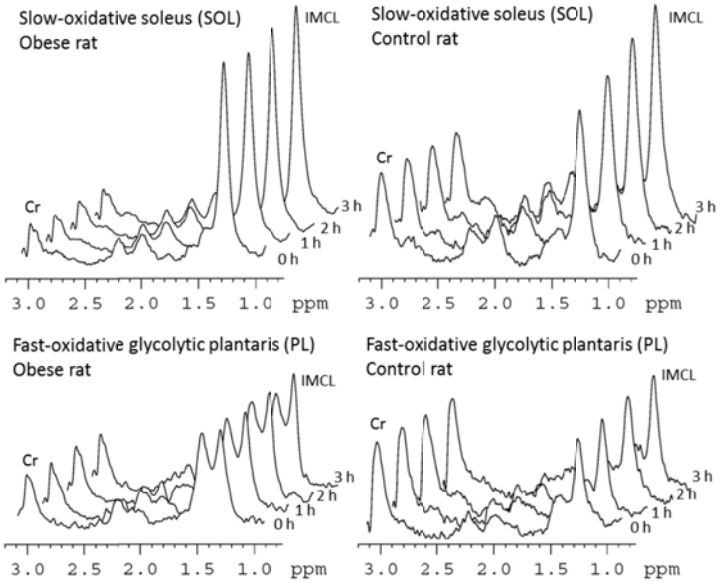

Fig.2 Typical IMCL (at 1.28ppm) changes during 3hr resting in SOL and PL muscles of obese and control normal rats while creatine signals were unchanged.

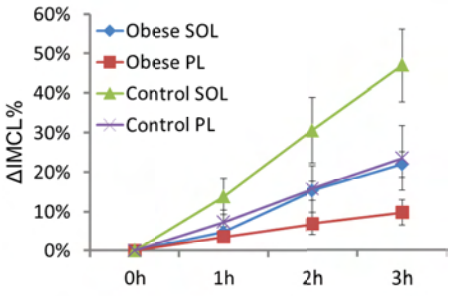

Fig.2 IMCL percentage increase (ΔIMCL%) in SOL and PL muscles of obese and control normal rats.

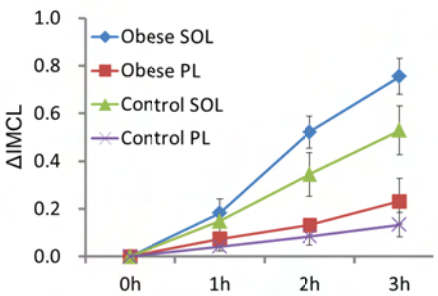

Fig.3 IMCL percentage increase (ΔIMCL%) in SOL and PL muscles of obese and normal rats.

Table 1 IMCL baseline and changes in SOL and PL of obese and control rats.

GROUP	IMCL in slow-oxidative soleus (SOL)			IMCL in fast-oxidative glycolytic plantaris (PL)		
	baseline	ΔIMCL%/h	ΔIMCL/h	baseline	ΔIMCL%/h	ΔIMCL/h
Obese rats	3.88±0.73*	7.60%±1.3%*	0.220±0.067	1.98±0.51*	4.26%±0.9%*	0.073±0.002
Control rats	1.14±0.30*	15.7%±3.1%*	0.178±0.036	0.57±0.23*	7.83%±2.8%*	0.044±0.017

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