Is intramyocellular lipid a diffusion-restricting factor in skeletal muscle cells?

Yoshikazu Okamoto[1], Shintaro Mori[1], Tomonori Isobe[1], Yuji Hirano[1], Hiroaki Suzuki[1], and Manabu Minami[1]

[1]*University of Tsukuba, Tsukuba, Ibaraki, Japan*

Introduction

We previously reported a change of the water diffusion in the skeletal muscle cell in lifestyle-related disease patients after six months of hybrid training (HYBT) (1). In that study, the water diffusion in the skeletal muscle increased after training despite an increase in muscle strength and no change in the cross-sectional area of the skeletal muscle on magnetic resonance imaging (MRI). Specifically, the apparent diffusion coefficient (ADC), and all three eigenvalues (λ1, 2, 3) were increased after training in this study.We hypothesized that this phenomenon might be induced by an enlargement of the intracellular space of the skeletal muscle cell induced by HYBT. There have been many publications reporting that water diffusion by diffusion tensor image (DTI) represents the intracellular water diffusion in skeletal muscle, and that the cell membrane was the main water diffusion restricting factor (2-6). However, after we presented this study at several conferences, some researchers pointed out that there might be another cause of the increase in eigenvalues after HYBT because the cross sectional area measured on MRI image was not increased in our study. Therefore, there was sufficient evidence of enlargement of the skeletal muscle cell by HYBT. Additionally, it was suggested that intramyocellular lipids (IMCL) could be another water diffusion-restricting factor because the subjects were all lifestyle-related disease patients. The systemic metabolic rate might be increased by HYBT resulting in reduced IMCL and an increase in the space for water diffusion in skeletal muscle cells.However, if this hypothesis were true, IMCL must be one of the water diffusion restricting factors. The purpose of the present study is to investigate whether IMCL is a diffusion-restricting factor in skeletal muscle cells. For this purpose, we tried to assess the relationship between the amount of IMCL and several focal diffusion parameters including ADC, fractional anisotropy (FA), and three eigenvalues.

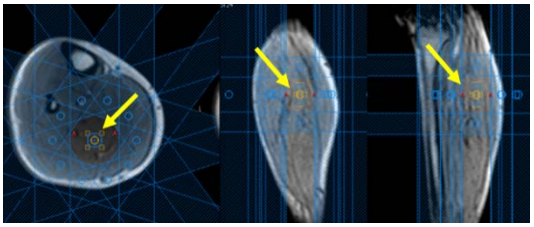

Figure 1 57-year-old male patient
Parallelepiped shaped orange colored VOI is indicated by yellow arrows. We measured the diffusion parameters in this VOI placing a sphenoid shaped ROI at 3 points and averaged.

Materials and Methods

Twenty-three lifestyle-related disease patients who were diagnosed by a specialist of internal medicine in endocrinology & metabolism at our institution participated in this study. The ethics committee of our institution approved this study and survey. We acquired written informed consent for research participation from all subjects. Subjects underwent 1H MR spectroscopy for measuring IMCL and DTI of the calf. 1. T1–fast-field echo (FFE) image: This acquisition was used as the anatomical image.2. 1H MRS: The subject was scanned in a supine feet-first position on a 3T clinical MR machine (Achieva, Novadual, Philips, Best, Netherlands). IMCL in the right soleus (SOL) muscle were measured. The volume of interest (VOI) was determined at the slice showing the largest area of the SOL. The VOI size was 12 mm x 12 mm x 35 mm. A screenshot of the placement of the VOI was obtained. A single voxel-localized 1H MRS acquisition was performed using a point-resolved spectroscopy (PRESS) sequence, both with and without water suppression, and was accomplished using three preceding chemical-shift–selective (CHESS) pulses (bandwidth, 140 Hz). Before the spectroscopic acquisition, the field homogeneity was optimized over the selected VOI by automatic shimming of the tissue water. The following PRESS parameters were used: TR = 3000 ms; TE = 40 ms; number of points sampled = 1024; spectral width = 2,000 Hz; number of signal acquisitions was 96 for metabolites and 16 for water. The scan time was 5 min 24 s for data acquisition, before which shimming took about 5 min. The fitting of the 1H MRS data was performed using the LCModel (LA Systems). Data for IMCL (1.3 ppm) and EMCL (1.5 ppm) methylene protons were used for quantification. The IMCL and EMCL estimates were automatically scaled to the unsuppressed water peak. 3. DTI: DTI of the right calf was scanned using a single-shot spin-echo echo planar imaging (EPI) sequence with the following parameters: b values of 0 and 500 s/mm2, field of view (FOV) 380 (mm), rectangular FOV 100%, matrix size 256 × 256, slice thickness 6 mm without gap, internal number of slices 12, TR = 4,000 ms, TE = 67 ms, SENSE factor 2, number of motion probing gradient directions 6, and number of excitations 6. Volume shims were set focusing on the calf. Spectral Attenuated Inversion Recovery was used for fat suppression. Effects on the DWIs from B0 and B1 inhomogeneities and inhomogeneity from fat suppression were minimal.4. Data were analyzed by dTV II software (University of Tokyo, diffusion TENSOR Visualizer, version 2) on the image with the b value of 0 s/mm2. A sphenoid shaped region of interest (ROI) was set at 3 points on the VOI referring screen shot (Figure 1). The values of the FA, ADC, and three eigenvalues were measured in each subject. The correlations between the ADC, FA, λ1, λ2, λ3 and IMCL values were analyzed by Pearson's correlation coefficient.

Results & Discussion

Table 1 shows the correlation coefficient table (R) between IMCL and the ADC, FA, and three eigenvalues. Figure 2 shows the scatter plot between IMCL (mm / mol) and λ3. FA showed a positive correlation. λ3 showed a negative correlation. ADC, λ1 and λ2 showed a weak negative correlation.

Previously, we compared the diffusion properties between athletes and non-athletes (7). We hypothesized that athletes would show higher values compared to non-athletes because the skeletal muscle cell might be larger in athletes versus non-athletes and the intracellular space might be wider than that of non-athletes. However, the results indicated the opposite was true. This finding might be due to the rich intracellular component in the athlete group, namely the dense myofilament lattice, which might be the main diffusion-restricting factor in the athletes. The cell membrane was not the main diffusion-restricting factor. As the results of this present study showed a negative correlation between the amount of IMCL and the diffusion properties (ADC and eigenvalues), it could be possible that IMCL is a diffusion-restricting factor, like the myofilament lattice. In particular, the water diffusion direction orthogonal to the long axis of the cell, namely the λ3 seemed to be restricted.The reason why this direction was especially restricted is not clear. However, several studies (8, 9) have reported that IMCL deposit droplets in the skeletal muscle cell. According to the literature, the droplets are located in the intermyofibrillar space with close proximity to mitochondria (9), possibly affecting the results of the present study. At any rate, our results suggest that IMCL might be one of the water diffusion-restricting factors. We also could not explain why FA showed a positive correlation. We hypothesized that the increase of the R value of the λ1 was much lower than that of the λ2 and λ3. Therefore, FA might have a positive correlation.

Table 1
The correlation coefficient table between IMCL and the diffusion parameters

IMCL vs ADC (10^{-3}mm2/sec), FA, and 3 eigenvalues

ADC	FA	λ1	λ2	λ3
-0.35	0.44	-0.21	-0.37	-0.43

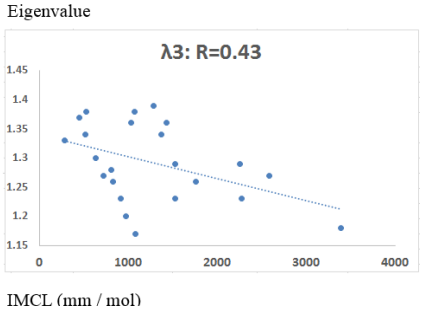

Figure 2 Figure 2 indicates the scatter plot between IMCL and λ3.

Conclusion Our results suggest that IMCL could be one of the water diffusion-restricting factors in patients with lifestyle-related disease.

References 1) Okamoto Y, et al. Magn Reson Imaging. 2014., 2) M. Hatakenaka, et al. J Magn Reson Imaging, 2008; 27: 932–937, 3) C.J. Galbán,et al. J Gerontol A Biol Sci Med Sci, 2007; 62 : 453–458, 4) C.J. Galbán, et al. Eur J Appl Physiol 2004; 93: 253–262, 5) N.F. Schwenzer, et al. NMR Biomed, 2009; 22: 1047–1053, 6) A.M. Heemskerk,et al. Magn Reson Med 2005;53: 1333–1340, 7) Okamoto Y, et al. MAGMA. 2012;25:277-84., 8) Ruth CR et al. Am J Physiol Regul Integr Comp Physiol. 2009;297:913-24., 9) Nielsen J, et al. Am J Physiol Endocrinol Metab. 2010;298: 6-13.