Reproducibility of Measurements of Intramyocellular Lipid Levels by Single- and Multi-voxel Proton MRS in Overweight and Lean Subjects During Controlled Dietary Fat Intake

W. Shen[1], X. Mao[2], C. Wolper[1], S. Dashnaw[3], J. Hirsch[3], S. Heshka[1], S. B. Heymsfield[1], D. C. Shungu[2]

[1]Obesity Research Center, St. Luke's-Roosevelt Hospital and Institute of Human Nutrition, Columbia University, New York, NY, United States, [2]Radiology, Weill Medical College of Cornell University, New York, NY, United States, [3]fMRI Research Center, Radiology, Columbia University, New York, NY, United States

Background

Intramyocellular lipid (IMCL) provides an important source of cellular energy for skeletal muscle that can be metabolized under conditions of increased energy demand. Investigation of the interplay between IMCL and insulin resistance is providing better understanding of the etiology of obesity and diabetes mellitus[1,2]. Most previous attempts to establish correlations between IMCL and insulin resistance in obesity and diabetes mellitus were based on analysis of muscle biopsy samples. However, measures of IMCL derived in this manner may be of limited value due to the possibility of extramyocellular lipid (EMCL) contamination. Also, IMCL and EMCL are not easily separated on biopsy specimens. Recently, it was demonstrated that magnetic resonance spectroscopy (MRS) is a convenient noninvasive technique that allows discrimination and measurement of IMCL and EMCL pools *in vivo*[3-5]. In this study, the reliability of making repeated measurements of IMCL by single-[3] and multi-voxel MRS[4,5] under conditions of controlled caloric and dietary fat intake is investigated.

Methods

In the aggregate, 10 healthy female subjects, 25 to 45 years of age, were recruited for the study. Of these, 5 were obese as assessed by body mass index (BMI) [age, (Mean±SD), 32.5±5.7 yrs;BMI, 33.5±4.2 kg/m^2] and 5 were considered lean (age, 32.3±8.7 yrs; BMI, 20.4±0.9 kg/m^2). Starting on the 5th day of the menstrual cycle, subjects maintained a diet of stable calorie and fat intake (±5%) for one week. On day 4 and day 7 of the diet regimen, single-voxel and multi-voxel ^{1}H MRS scans were performed on each subject, using the standard PRESS sequence with a transmit/receive quadrature knee coil on a 1.5 T GE Signa "LX" MR system.

Single-voxel: A 1.5x1.5x1 cm^3 voxel was positioned in the left calf tibialis anterior (TA) muscle and the data were acquired in 3.2 min with the PRESS sequence using TR/TE, 1500/35 ms,128 signal averages, 2048 time-domain points, and a 1-kHz spectral width.

Multi-voxel: Multi-voxel MRS was prescribed on an oblique localizer traversing the same TA muscle as in the single-voxel scan, and then data were recorded with the PRESS technique using TR/TE 1500/35 ms, 24x24 phase encoding steps over a 16-cm FOV, 1 excitation per phase-encoding step, a 5-mm slice thickness and 2048 time-domain points. This yielded voxels with a nominal size of 0.22 cm^3 after zero-filling to 32x32 before 3-dimensionl Fourier transformation.

Data Analysis: The resulting single- and multi-voxel data were processed using an IDL-based MRS data analysis software developed in-house by two of the investigators (XM, DCS). IMCL levels were expressed as peak area ratios relative to total muscle creatine (IMCL/Cr). Since the single-voxel PRESS sequence supplied by General Electric automatically records a spectrum of the unsuppressed water resonance from the voxel of interest, IMCL levels for single-voxel were also expressed as peak area ratios relative to internal tissue water (IMCL/W). The IMCL/Cr values reported for multi-voxel MRS were obtained by computing the mean values for all TA voxels that exhibited adequately resolved IMCL and EMCL resonances. Poor EMCL and IMCL separation was a criterion for data rejection for both methods.

Results

Spectral data: **Figure 1** shows a representative spectrum from a voxel in the TA muscle of a lean 29-year old subject. Clearly resolved resonances for EMCL and IMCL can be seen. In addition, the spectrum reveals resonances for total muscle creatine (Cr) and choline (Cho). These readily detectable total Cr, Cho, EMCL and IMCL resonances were fitted in the time-domain as shown in Fig 1, with the area of total Cr serving as an internal intensity reference to quantify IMCL.

Reproducibility: IMCL levels from the duplicate MRS measurements obtained from each subject were compared. For the single voxel measurements, these duplicate measures of IMCL, recorded 3 days apart, were highly correlated, whether expressed relative to Cr ($r = 0.80$, $P < 0.05$) or relative to tissue water ($r = 0.83$, $P<0.05$). Comparison of the duplicate measures of IMCL obtained by multi-voxel MRS for each subject (**Figure 2)** showed a significant correlation ($r = 0.95$, $P <0.05$) that was higher than that for single-voxel. The mean of the duplicate measures of IMCL/Cr by single- and multi-voxel MRS correlated with each other ($r = 0.89$, $P<0.05$).

IMCL in Lean and obese subjects: Values of IMCL/Cr obtained by multi-voxel MRS were significantly higher ($P<0.05$) in obese subjects (8.3±3.8) than in lean subjects (4.3±2.4). On the other hand, the difference between group means from the single-voxel MRS measures of IMCL/Cr and IMCL/W did not reach statistical significance (IMCL/Cr, obese, 6.7±2.8 vs. lean, 4.5±1.9, $P = 0.15$;IMCL/W, obese, 0.043±0.016 vs. lean, 0.031±0.017, $P =0.18$). IMCL/Cr level approached positive correlation with BMI for multi-voxel MRS ($r = 0.592$, $P = 0.07$), but not for single-voxel MRS ($r= 0.267$, $P=0.455$).

Discussion

This study has demonstrated that reproducible single- and multi-voxel MRS measurements of IMCL can be obtained from the human TA muscle at 1.5 T under conditions of controlled caloric and dietary fat intake. Although daily dietary variability can be a confounding factor in the measurement of IMCL by MRS in longitudinal studies, here we have demonstrated that it is possible to obtain subject compliance for at least 1 week. This has contributed to enhancing the reliability and applicability of MRS in the present study. For the limited sample size of this study, we have demonstrated that multi-voxel MRS was able to detect a significant difference in IMCL levels between obese and lean subjects, whereas single voxel failed to detect such a difference. This is likely due to the following two important advantages of multi-voxel over single-voxel MRS for *in vivo* detection of IMCL, as we previously reported[5]: (a) higher spatial resolution, and (b) increased flexibility in selecting the voxels of interest after acquisition. The higher spatial resolution of multi-voxel MRS yields IMCL spectra with minimal EMCL contamination from adipose tissue, while its greater flexibility in voxel selection also allows voxels with minimal adipose tissue contamination to be selected after data acquisition. Together, these advantages lead to greatly improved spectral purity. On the other hand, single-voxel is easy to implement and is readily available even on clinical instruments without extensive research capability, which might make it an attractive alternative in some instances.

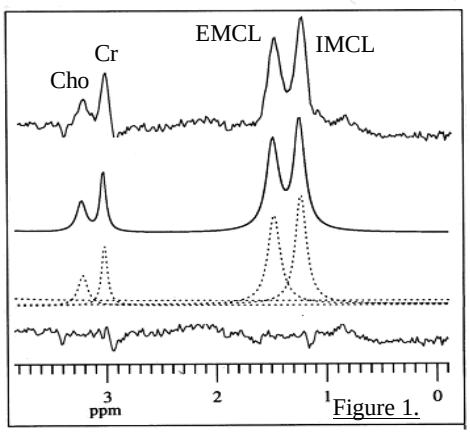

Figure 1.

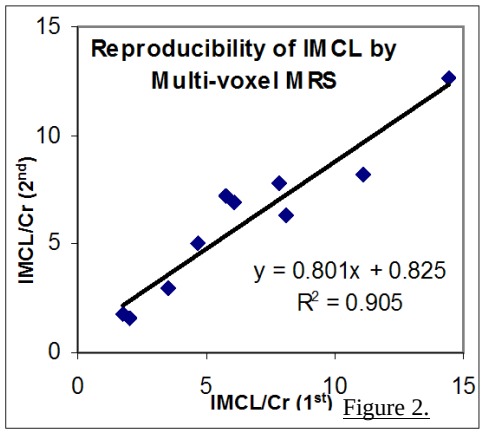

Figure 2.

Conclusion

This study has assessed the reliability of making repeated measurements of IMCL by single- and multi-voxel MRS techniques on a clinical 1.5 T MR system. To minimize variations in IMCL due to daily fluctuations in dietary fat and caloric intake we made an effort in this study to control for these two variables. Under these conditions, we have demonstrated that reproducible repeated measurements of IMCL can be made by either single-voxel or multi-voxel MRS. On the other hand, we have found that in addition to yielding more reproducible results, multi-voxel MRS was more sensitive to differences in IMCL levels between obese and lean subjects, suggesting that this technique may offer more flexibility and reliability for *in vivo* measurements of IMCL in a variety of disorders of lipid metabolism.

References

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