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

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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 mellitus1,2. 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, measurements 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 vivo3,4. In this study, the reliability of making repeated measurements of IMCL by single- and multi-voxel MRS5,6 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/m² and 5 were considered lean (age, 32.3±8.7 yrs; BMI, 20.4±0.9 kg/m²). Starting on the 5th day of the menstrual cycle, subjects maintained a diet of stable caloric and fat intake (+5%) for one week. On day 4 and day 7 of the diet regimen, single-voxel and multi-voxel 1H 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 Sigma LX® MR system.

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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 (Cr) and Cho (Cho) are seen in the spectrum. The ratio of the peak area of total Cr to EMCL and Cho (EMCL/Cho) was used as a measure of IMCL and EMCL levels.

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, with the standard deviation of the mean 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/Cho 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/Cho obtained by multi-voxel MRS were significantly higher (P<0.05) in obese subjects (8.3±3.8) than in lean subjects (4.3±2.2). On the other hand, the difference between group means from the single-voxel MRS measurements of IMCL/Cho and EMCL/W did not reach statistical significance (IMCL/Cho, obese, 6.7±2.8 vs. lean, 4.5±1.9, P = 0.15). IMCL/W, obese, 0.043±0.016 vs. lean 0.035±0.017, P = 0.18). IMCL/Cho level approached positive correlation with BMI for multi-voxel MRS (r = 0.592, P = 0.07), but not for single-voxel MRS (r = 0.259, P = 0.185).

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 measurements in this 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 important advantages of multi-voxel over single-voxel MRS for in vivo detection of IMCL, as we previously reported: (a) higher spatial resolution, (b) increased flexibility in selecting the voxels of interest after acquisition. The higher spatial resolution of multi-voxel MRS yield IMCL spectra with minimal EMCL contamination, 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.

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 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