Metabolite Quantitation in Muscles using ^{1}H MR Spectroscopic Imaging and Validation by Single Voxel MR Spectroscopy.

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Synopsis
A method for quantitation of intra-myocellular lipids (IMCL) in human muscle measured by MR spectroscopic imaging (MRSI) is presented. The method employs the signal from bone marrow lipids as internal reference for quantitation. IMCL concentrations in tibialis anterior (TA) obtained by this method were compared with concentrations obtained in the same session by single-voxel measurements. The results of the two methods are significantly correlated (R=0.90), demonstrating that MRSI measurements of IMCL are feasible in human muscle, providing reliable quantitative results. IMCL concentrations were significantly different between muscles, with low values in TA and three times higher values in soleus.

Introduction
Although recently several studies have investigated intra-myocellular lipids (IMCL) using ^{1}H MRS [1-8], all studies have used either single voxel spectroscopy (SVS), or analyzed few selected voxels from MR spectroscopic imaging (MRSI) data [7,8]. Few studies have reported IMCL concentrations from different muscles [3,6,7]. These studies used water as internal reference. However, for MRSI studies this method requires an additional time consuming measurement, or, if acquired with reduced matrix, poses the problem of different signal contributions due to signal bleeding. In this study we extend an alternative method originally presented by Larson-Meyer et al. [8] for m. soleus: We used bone marrow lipids as internal reference to obtain IMCL concentrations in different muscles employing all acquired voxels within muscle tissue and assigned to different muscles. The main goal was to compare and validate this method with IMCL concentrations obtained by SVS in the same session using water as reference [3].

Methods
Measurements: 22 measurements on the right and/or left calf of five subjects at different stages of diet and exercise were performed on a 1.5T system (SIGNA, General Electric, Milwaukee). A 2D MRSI sequence with PRESS volume pre-selection was used. The slice (thickness 15mm) was selected in transverse orientation. Position and size of the PRESS box with outer volume suppression was adjusted to partly exclude subcutaneous fat without excluding muscle tissue. Two additional pairs of saturation bands were used to reduce strong lipid signals from bone and subcutaneous fat. Spectra were acquired with TR 1200 ms, TE 35 ms, water presaturation, Matrix: 36×36 over a FOV of 20 cm resulting in a scan time of 26 min. For quantitation a single voxel measurement was performed in bone marrow (voxel size: 8.2×8.2×15mm^3). A single voxel measurement was performed in Tibialis anterior (TA) as previously described [3] (Voxel size: 12×11×18 mm^3, TR=3000 ms, TE=20ms, 128 acq) for comparison with MRSI data.
Processing: Postprocessing of the MRSI data included spatial zerofilling to 64x64, and moderate spatial apodization. Lipid extrapolation was performed to reduce contamination from extramyocellular lipids (EMCL) as described in Ref. [9]. The spectra were fitted using an iterative non-linear least squares fitting algorithm ("TDFDFIT" [10]), using prior knowledge for EMCL and IMCL. IMCL frequency and linewidth was fixed with respect to creatine (Cr). The calf was segmented into 7 muscles using a co-registered gradient-echo MRI. The segmented images were used to assign the metabolites of each voxel to a muscle. Approx. 200 voxels were assigned to different muscles for each measurement. For each muscle an average IMCL signal area was calculated from all voxels located within this muscle and the concentration was calculated using the lipid signal from the bone marrow spectrum as internal reference. A calibration factor between MRSI and SVS voxel signal contribution was determined in phantom measurements. Bone marrow fat content of 1.07 mol / kg wet wt was confirmed in an additional subject scan. IMCL concentration from the SVS measurement was obtained blinded to the MRSI results using the unsuppressed water signal as internal reference [3].

Results
The IMCL concentration in TA obtained by MRSI measurements was significantly correlated to IMCL concentration obtained by SVS (R=0.90, Fig.1). Furthermore, the data are close to the identity line. MRSI processing yielded an average IMCL concentration in TA of 2.55 mmol / kg wet wt, compared to 2.50 mmol / kg wet wt by SVS.
For seven different muscles in the calf an average IMCL concentration was calculated (Table 1). While IMCL was significantly different between muscles, IMCL distribution over different muscles was similar for all subjects. Highest IMCL was found in m. soleus (SL and SM), while it was much lower in TA or m. gastrocnemius (GL and GM).

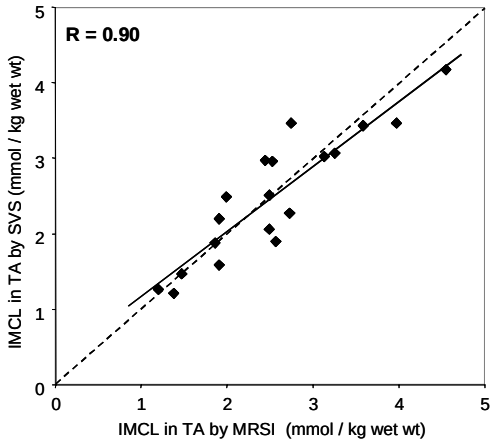

Figure 1: Correlation between IMCL concentration in TA obtained by MRSI and by SVS

Discussion
MRSI and SVS measurements, employing completely different quantitation strategies, yielded similar results for IMCL concentration in TA. This result clearly shows that absolute quantitation of IMCL by MRSI measurements is feasible, providing reliable results. The remaining differences between MRSI and SVS results may be due to the measurement or postprocessing or may represent real differences, possibly due to different voxel sizes of the two measurements.
IMCL in TA was found to be three times lower than in m. soleus. This is in agreement with a recent study at 4T on selected voxels using water as reference [7]. The high variance of IMCL concentration between subjects (Table 1) was expected and is due to inter-individual differences.

TA	SL	SM	GL	GM	ED	PB
2.5±0.9	5.8±1.6	7.9±3.0	2.1±1.0	2.3±0.9	2.6±1.3	1.7±1.1

Table 1: Average IMCL (mmol / kg wet wt) of 22 measurements in seven different muscles (TA = Tibialis anterior; SL = Soleus lateral part; SM = Soleus medial part; GL = Gastrocn lateralis; GM = Gastrocn medialis; ED = Extensor digitorum; PB = Peroneus brevis).

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