Assessment of Lipids in Skeletal Muscle: Comparison of the Water and Fat Referenced Spectroscopy

J. Weis[1], L. Johansson[2], F. Ortiz-Nieto[1], and H. Ahlström[1]

[1]Department of Radiology, Uppsala University Hospital, Uppsala, Sweden, [2]Astra Zeneca R & D, Uppsala University Hospital, Uppsala, Sweden

Introduction: The unsuppressed water line is almost exclusively used as a concentration reference in the processing of muscle spectra. In contrast, the acquisition of a water magnetic resonance spectroscopic imaging (MRSI) data set in addition to the water-suppressed is not always done since it can lengthen the study undesirably. In spectroscopy of the skeletal muscle, the necessity for relaxation correction of water reference is a fundamental disadvantage. This correction complicates the relative wide range of T_2 values (25 < T2 < 35 ms, 1.5 T) (1-3), which depend on the age, type and distribution of muscle fibbers. Alternatively, the fat signal can be used as the reference in quantitation of muscle lipids. Yellow bone marrow (4, 5) can serve as the internal standard since both extra- (EMCL) and intramyocellular (IMCL) lipids have essentially the same composition of fatty acid triglycerides with very close relaxation times T_1, T_2 (6, 7). The fat reference is extraordinarily suitable for the quantitation of muscular lipids because no relaxation corrections are needed. In this study we compare two methods for the determination of lipid content in human skeletal muscle: relaxation effects sensitive water referenced single-voxel ^{1}H MRS and relaxation effects robust high-spatial-resolution MRSI with fat (yellow bone marrow) as the internal or vegetable oil as the external standard.

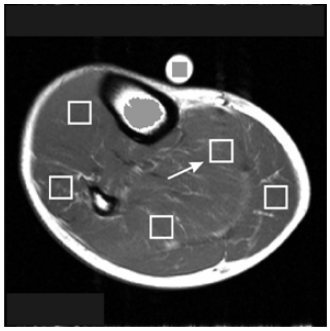

Fig. 1

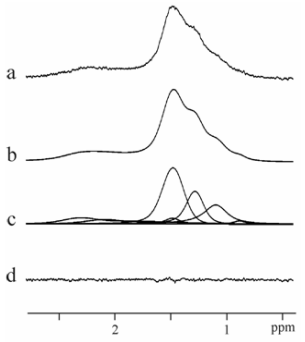

Fig. 2

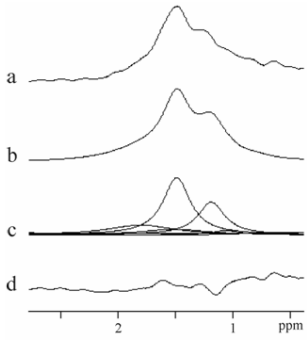

Fig. 3

Materials and Methods: Muscle spectra of five healthy male volunteers were measured with a mean age of 42.2±11.0 years (range 28-56 years), and mean body mass index of 29.2±5.4 kg/m^2 (range 24.2-36.8 kg/m^2). Measurements were performed on a 1.5 T Gyroscan Intera MR scanner (Philips). The external fat reference was fixed to the calf (Fig.1). The reference consisted of plastic probe (ϕ = 13 mm, 110 mm long) filled with vegetable oil (T_1/T_2 = 225/55 ms). The measurements started with the water-suppressed high-spatial-resolution MRSI sequence (8) in axial slice (Fig. 1). The slice thickness was 15 mm. Incrementing the echo time of the subsequent 48 images by ΔTE 2.6 ms (TE_1 = 8 ms) led to a spectral bandwidth of 6 ppm and spectral resolution 0.125 ppm. Image matrix 256x256, FOV 150 mm and 128 phase encoding steps led to resolution in the plane of 0.59x1.17 mm. The net measurement time was 15.3 minutes (1 scan, TR 150 ms). High-spatial-resolution MRSI was followed by the single-voxel spectroscopy (SV PRESS) (TR/TE 3000/26 ms, 64 scans, 1024 points, bandwidth 1000 Hz). The voxels (10x10x15 mm^3) were placed within the MRSI slice. Figure 1 shows voxel positions. The measurement sequence included 16 unsuppressed water reference acquisitions. Lipid content of single voxel spectra was quantified by LCModel. Concentrations of lipid's methylene groups $EMCL_{CH2}$ and $IMCL_{CH2}$ were computed as mM, and were corrected for T_1, T_2 relaxation effects of the water reference using LCModel's control parameter atth2o. This value was determined by the expression $\exp(-TE/T_2)[1-\exp(-TR/T_1)]$ assuming relaxation times T_1 = 1300 ms, T_2 = 28 ms (1). The concentration of lipid molecules was computed by the summation of $EMCL_{CH2}$ and $IMCL_{CH2}$ concentrations and division by factor 31. Value 31 is based on the assumption (1) that the average number of methylene protons is 62 per triacylglycerol (TG) molecule (equivalent to 31 CH_2 groups). The resulting concentration was then corrected for relaxation effects of methylene lines using the same expression as for water line and assuming T_1 = 340 ms, T_2 = 85 ms for the $EMCL_{CH2}$ and $IMCL_{CH2}$ lines (7). Division by the tissue density (1.05 kg/liter for normal muscle tissue) (9) was performed to convert mM to millimoles per kg wet weight (mmol/kg ww). High-spatial-resolution MRSI processing was described elsewhere (4, 8). The resulting spectra were computed by the summation of magnitude spectra. Volume of interest (VOI) was defined by the mask matrix (4) that represents voxel size and position in axial slice (Fig. 1). The magnitude spectra were processed by AMARES/MRUI (10). Prior knowledge was described elsewhere (4). The lipid content in volume % (LV) was computed using $EMCL_{CH2}$ intensity of the voxels with a 100% fat content. The spectrum of yellow bone marrow and vegetable oil were used as the internal and external reference, resp. VOIs show gray pixels in Fig. 1. No relaxation corrections were performed. In order to convert concentration based on volume % to mmol/kg ww, a specific density of muscle tissue 1.05 kg/liter, average density of lipids 0.918 kg/liter, and average molecular mass of triglyceride molecule 0.858 kg/mol was assumed (1). It can be shown that lipid content (mmol/kg ww) LC = 1069.9LV/(0.918LV+1.05LTV) where LTV = 100-LV represents lean tissue content (LTV) in vol. %.

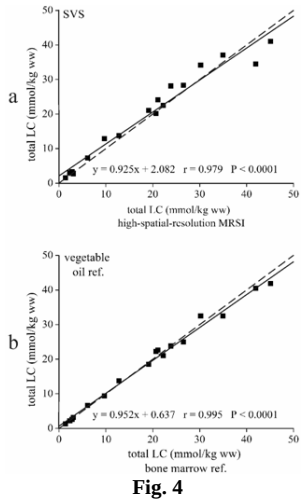

Fig. 4

Results: White squares (Fig. 1) show voxel positions in different muscles used in SV PRESS and high-spatial-resolution MRSI. The arrow shows voxel whose spectrum is shown in Fig. 2a (SV-PRESS) and Fig. 3a (MRSI, module spectrum). Figures 2b-d and 3b-d show fitting results and residue. Figure 4a shows the correlation (solid line) between total lipid content measured by water referenced SV PRESS and high-spatial-resolution MRSI with bone marrow as the internal standard. Dashed line represents identity. Mean ratios between SV PRESS and MRSI concentrations were 1.08±0.13. Correlation (solid line) between total lipid content estimated by high-spatial-resolution MRSI using internal (bone marrow) and external fat (vegetable oil) standard is shown in Fig. 4b. Dashed line represents identity.

Discussion and Conclusions: Correlation between concentrations determined by SV-PRESS and MRSI (Fig. 4a) is appealing taking into account different experimental approaches, spectrum processing and quantitation methods. Deviations can be explained by differences in prior knowledge, different baseline correction methods and by limited precision of the relaxation times T_1, T_2. Difficulties caused by relaxation corrections can be avoided by high-spatial-resolution MRSI which offers unique possibilities for VOI definition. VOI can be non-continuous and irregularly shaped. This feature enables VOI selection with 100% fat content, e.g. bone marrow. Since relaxation times T_1, T_2 of the yellow bone marrow and muscular fat (EMCL, IMCL) are almost identical (6,7) no relaxation corrections are needed in MRSI approach. Figure 4b reveals very good correlation between total lipid concentrations evaluated using bone marrow and vegetable oil as the fat reference. From this correlation, it follows that it is possible to use external fat standard (vegetable oil) in MRSI quantitation. However, (small) differences in relaxation times between muscle lipids and external fat standard have to be taken into account.

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