Evaluation of vastus lateralis muscle fat fraction measured by two-point Dixon water-fat Imaging and 1H-MRS
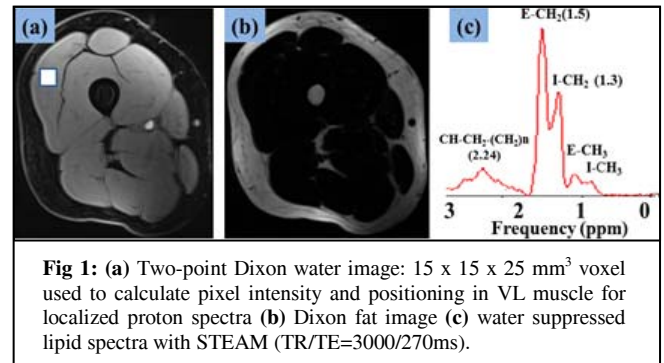
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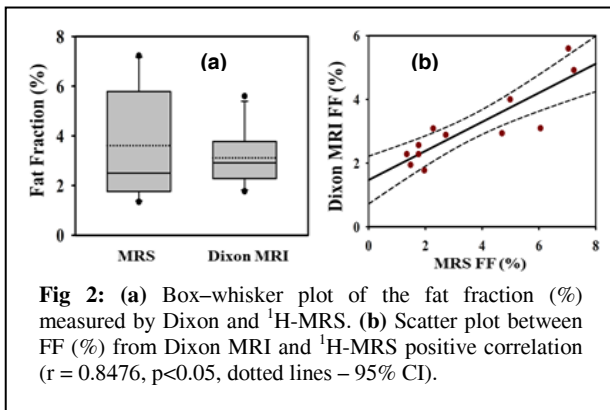
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Target Audience: MR scientists and researches involved in utilizing imaging and spectroscopic methods to evaluate skeletal muscle fat metabolism

Introduction: Magnetic resonance techniques provide for a noninvasive means of estimating fat content in vivo. Two-Point Dixon (Dixon MRI) imaging relies on a time dependent phase difference that evolves as a result of chemical shift difference between water and fat (1). It is reported that long TE single-voxel proton MR spectroscopy (^{1}H-MRS) improves separation of lipid peaks in skeletal muscle and allows for fat quantification with superior sensitivity and dynamic range over that of various MR imaging methods (2-3). Correctly applied, these techniques provide reliable fat fraction data, correlating well with findings in tissue biopsies, which is considered as the current standard. High correlations between the Dixon MRI and ^{1}H-MRS or histology have been reported in previous studies involving fat quantification in soleus (2) and tibialis anterior (6) and myocardium (5). The purpose of this study is to compare the fat fraction (%) in human vastus lateralis muscle (VL) using Dixon MRI and ^{1}H-MRS methods and evaluate their reliability. VL is of particular interest in physiological studies of diabetes and obesity, as biopsies are taken from it for lipid evaluation and a non-invasive validation is needed for better ways of clinical diagnosis.

Fig 1: (a) Two-point Dixon water image: 15 x 15 x 25 mm^3 voxel used to calculate pixel intensity and positioning in VL muscle for localized proton spectra **(b)** Dixon fat image **(c)** water suppressed lipid spectra with STEAM (TR/TE=3000/270ms).

Methods: ^{1}H-MRS and Dixon chemical shift water-fat imaging were performed on 12 healthy subjects (age: 28.17 ± 3.59 yo, BMI: 23.89 ± 3.14 kg/m^2) on Siemens 3T Magnetom Trio. Four channel flex receive-only extremity coil was positioned over the mid-section of right thigh of each subject. T_1-weighted localizer images were used for exact positioning of slices on the VL. Spoiled GRE two-point Dixon MRI with in-phase and opp-phase images (8 slices, TR = 18.8 ms, TE = 2.45 / 3.675 ms, α = 25°, FOV = 250 mm, matrix size = 256 x 256, slice thickness = 5 mm) was acquired from each subject. Automated reconstruction of fat and water images was performed. ^{1}H-MRS was acquired using stimulated acquisition mode (STEAM) sequence to minimize chemical shift displacement effect. Exact positioning of the voxel 15 x 15 x 25 mm^3 was done corresponding to the Dixon slice positions. For each voxel placement, manual shimming was performed and water linewidth was adjusted around 20-35Hz. Unsuppressed water spectra (NSA = 16) with TR/TE = 3000 / 30 ms, and water-suppressed spectra (NSA = 128) with TR = 3000 ms at TE = 270 ms, BW = 2000 Hz. Water pre-saturation was used for the lipid spectrum acquisitions, with fixed water suppression BW=50 Hz.

Fig 2: (a) Box–whisker plot of the fat fraction (%) measured by Dixon and ^{1}H-MRS. **(b)** Scatter plot between FF (%) from Dixon MRI and ^{1}H-MRS positive correlation (r = 0.8476, p<0.05, dotted lines – 95% CI).

Data Analysis: Dixon fat and water images were evaluated using Mango image analysis software. Signal intensities were recorded using an ROI (relative to voxel size) at the same location where ^{1}H-MRS voxel was positioned. Relative fat and water signal intensities were corrected for T_1, T_2* and α based on spoiled gradient echo. Fat fraction was calculated on a pixel-by-pixel basis from the ratio of separate water-only and fat-only images using the equation (100×F/(W+F)) and expressed as mean ± sd of multiple measurements. All ^{1}H-MRS data were processed by AMARES fit using jMRUI 5.0 software. First order phase correction was fixed to zero and zero-order phase correction was estimated. The methylene $(CH_2)_n$, methyl (CH_3) peaks of intramyocellular (I), extramyocellular (E) lipids and water peaks were resolved by Lorentzian lineshapes. Prior knowledge was developed considering the theoretical molar ratio ($-(CH_2)_n-/-CH_3 = 62/9$) to adjust the amplitude and linewidth of the methyl and methylene peaks, and a fixed frequency shift of 0.2 ppm with respect to methylene and methyl peaks, $CH_3 = CH_2 - 51.1Hz$, was applied(2). T_1 & T_2 relaxation correction was performed for water and fat resonances. Fat fraction is calculated by (100×F/(W+F)) and reported in percentages, where F includes the signal intensities of methylene $(CH_2)_n$, methyl (CH_3) peaks only. Linear regression analysis was used to examine the association between calculated fat fractions on the Dixon MRI and ^{1}H-MRS.

Results and Discussion: Mean fat fraction exhibited considerable inter-individual variability for ^{1}H-MRS FF (%) = 3.61 ± 2.25 (range: 1.34 – 7.23) and Dixon MRI FF (%) = 3.11 ± 1.17 (range: 1.77 – 5.60). ^{1}H-MRS obtained at long TE = 270ms enabled lower contamination of extramyocellular lipid and better separation of methylene (I-CH$_2$ and E-CH$_2$) peaks. Fig 2 (a) shows Box plots of fat fraction of VL muscle calculated from Dixon MRI and ^{1}H-MRS. Mean FF values (dotted black) are median (solid black) are shown as the horizontal lines in each box. Interquartile range (height of each box), limits of 25th and 75th percentiles (lower and upper ends of each box) and whiskers (vertical lines extending above and below each box end on data points farthest from mean but within distance of 1.5 times inter-percentile range from end of each box) have shown considerable variability in ^{1}H-MRS data compared to Dixon MRI. Fig 2(b) shows scatter plot with linear regression analysis between FF (%) at 95% CI have shown strong correlation (r = 0.8746). Limitations of this study include small sample size and recent studies (4) have shown that two-point Dixon MRI do not reach fat contents below 3% due to remaining relaxation between echoes and underestimate the chemical fat composition, therefore validation with varying fat composition phantoms or chemical analysis of triglycerides and fatty acid content from muscle biopsies is necessary. The methods used in this study can be combined with biochemical assays and could effectively provide insight on how excess skeletal muscle fat could interfere with insulin signaling in obese and diabetic states and potentially be used to establish phenotypic characteristics of subjects at risk for type 2 diabetes (T2DM) and give a reliable basis for longitudinal clinical and research studies.

Conclusion: This study investigated the use of chemical-shift based two-point Dixon water-fat imaging in vastus lateralis muscle of healthy volunteers and validated the quantitative measure of fat fraction by direct comparison with co-registered ^{1}H-MRS. Preliminary *in vivo* examinations have shown strong correlation between Dixon MRI and ^{1}H-MRS methods and future studies will involve studying large cohort of T2DM subjects for assessment of muscular fat comparing 2pt Dixon MRI, ^{1}H-MRS and triple-echo chemical shift gradient echo MRI.

References [1] Jingfei Ma et al. JMRI 2008; 28:543-558 [2] Ren et al. MRM 2010; 64:662-671 [3] Lindeboom et al. Proc. ISMRM 19: 2011 [4] Gloor et al. ISMRM 20: 2012 [5] Liu et al. MRM 2010 63:892-901 [6] Boesch et al. NMR in Biomedicine 2006 19:968-988 [7] Kovanlikaya et al Pediatr Radiol 2005; 35:601-607.