Liver Metabolites in Rat Model of Non-Alcoholic Fatty Liver Disease: Quantification of Choline-Containing Compounds and Lipid Content by Using In vivo Proton Magnetic Resonance Spectroscopy

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TARGET AUDIENCE: Magnetic resonance (MR) scientists, medical doctors, and clinicians interested in proton MR spectroscopy (^{1}H MRS) of the liver

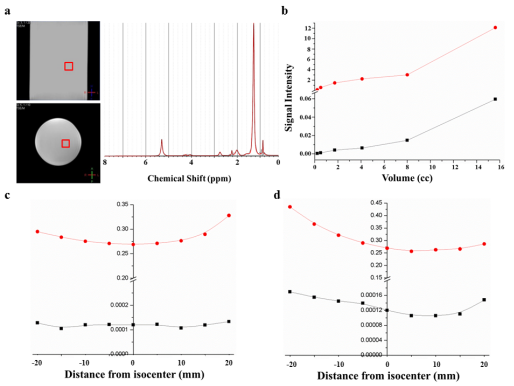

Fig. 1. (a) Sagittal image and axial image with a voxel position (red box) in lipid phantom and typical MRS spectrum from canola oil. (b) Linear relationship between voxel size and signal intensity from 0.6 cm^3 to 2.5 cm^3. Spatial variation in relative intensity measured within voxel size of 0.8 × 0.8 × 0.8 cm^3 right-to-left (c), anterior-posterior (d) with lipid (red line), Cho (black line).

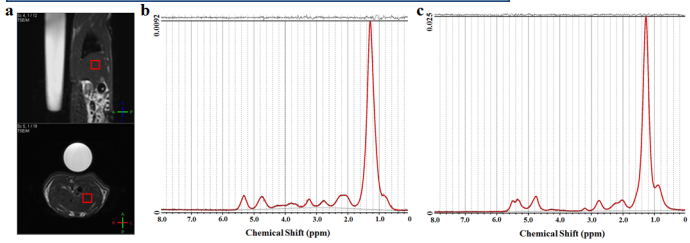

Fig. 2. (a) Sagittal image (top) and axial image (bottom) with the chosen VOI (red box) and corresponding in vivo liver spectrum with Cho and lipid region (0.90–5.30 ppm). Typical liver ^{1}H MRS spectrum showing various lipid and choline-containing compound peaks, with effectively suppressed water signals from NC diet rat (b), and HF diet rat (8 weeks) (c).

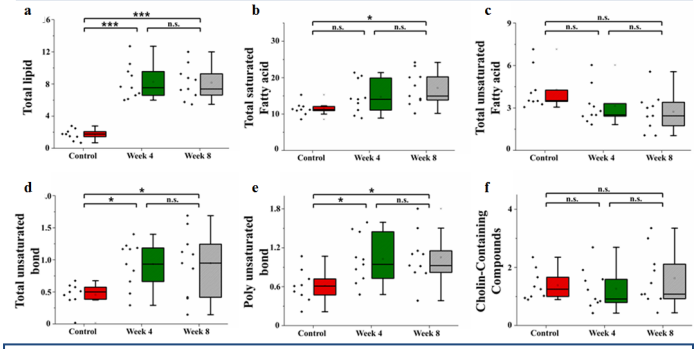

Fig. 3. Comparison of NC and HF diet rats by using one-way analysis of variance with the Turky multiple comparison test; (a) Total lipid, (b) total saturated fatty acid, (c) total unsaturated fatty acid, (d) total unsaturated bond, (e) poly unsaturated bond, and (f) choline-containing compounds in NC and HF diet rats. (***, $p < 0.001$; **, $p < 0.01$; *, $p < 0.05$; n.s, not significant)

PURPOSE: Disease progression can be prevented by early diagnosis, treatment, and characterization of fatty liver disease using computed tomography, ultrasonography, and magnetic resonance imaging techniques. ^{1}H MRS has also been used to monitor and characterize liver disease, including fatty liver.[1] Typically, the spectra obtained from in vivo ^{1}H MRS of liver tissues show various lipids peaks, as well as choline-containing compounds (CCC).[2] Changes of lipid content, namely saturated- and unsaturated-fatty acid, have also been associated with the pathogenesis of fatty liver.[2] Therefore, accurate measurement of lipid fraction composition is preferred over measurement of total lipids. The objective of this research is to characterize the metabolic changes in a fatty liver rat model induced by a high-fat (HF) diet.

MATERIALS AND METHODS: Phantoms were used for testing T_1 and T_2 estimation and coil performance for quantification of metabolites. To test coil performance, a lipid standard phantom containing canola oil, and a choline (Cho) standard phantom containing 6 mM phosphocholine chloride, copper sulfate ($CuSO_4$, 3 mM) and trimethylsilyl-propionic acid (TSP, 1 mM) in sodium chloride (NaCl, 34 mM) were prepared in a cylindrical bottle. These phantoms were used for measurement of T_1-, T_2-relaxation time. The calibration phantom contained an identical 6 mM phosphocholine chloride, $CuSO_4$, NaCl, and TSP solution, prepared in a conical tube. Male Sprague-Dawley rats (n = 12) fed the HF diet, with weight-matched normal-chow (NC) diet rats were housed with ad libitum access to water. The HF diet comprised pellets composed of 60% fat. Examinations were performed on a 3.0 T scanner (Achiva Tx 3.0 T; Philips Medical Systems, Netherlands) by using 4-channel animal coil. Localized point-resolved spectroscopy sequence was used for periodically acquiring liver spectrum at fortnightly intervals with the following parameters: repetition time (TR)/echo time (TE) = 1500/35 ms; the number of signal averages (NSA) = 64; the iterative volume of interest (VOI) shim and total scan was $\leq$10 minutes. The water signal of each VOI was suppressed by variable pulse power and optimized relaxation delays applied before the scan. Cho T_1 measurements (VOI, 0.8 × 0.8 × 0.8 cm^3; TE, 40 ms; TR, 600-1400 ms; 64 acquisitions) and Cho T_2 measurements (TR, 6000 ms; TE, 40-220) were obtained. For relative quantification, total lipid ($(-CH_2-)_n$ / noise), saturated fatty acid ($3(-CH_2-)$ / $2(-CH_3)$), total unsaturated fatty acid ($3(-CH_2-C=C-CH_2-)$ / $4(-CH_3)$), total unsaturated bond ($3(-CH=CH-)$ / $2(-CH_3)$), and polyunsaturated bond ($3(=C-CH_2-C=)$ / $2(-CH_3)$) were quantified.[2] Raw spectral data were analyzed by using a commercially available linear combination of model spectra (LCModel, version 6.3-1H, Stephen W. Provencher) software.

RESULTS: Fig. 1a shows the lipid standard phantom and a typical spectrum from the canola oil sample. Fig. 1b shows the linear relationship between signal intensity and volume, with respect to the VOI (Cho, R^2 = 0.9040; lipid, R^2 = 0.8897) located from the center of the coil. Fig. 1c and 1d show the signal intensity of the isocenter for the sensitivity distribution of the coil (Cho, x-axis: 7-8%, y-axis: 17-18%). Fig. 2b and 2c show the spectrum with voxel position (Fig. 2a) selected in anatomical image. The Cho concentration in HF diet rats was to be 6.0 ± 2.7 mM; the Cho concentration in NC rats was 5.4 ± 1.3 mM. The total lipid of HF diet rats at 4 weeks (8.3 ± 2.2 × 10^3) and 8 weeks (8.2 ± 2.1 × 10^3) was similar values. These values were higher than those of NC rats (1.7 ± 0.6 × 10^3). The total saturated fatty acid of HF diet rats at 4 weeks (14.7 ± 4.5) and 8 weeks (17.2 ± 4.7) were similar. The 8 weeks value of HF rats was higher than that of NC diet rats. HF diet rats had a higher total unsaturated bond and polyunsaturated bond at 4 weeks and 8 weeks, compared with NC diet rats.

DISCUSSION AND CONCLUSION: For metabolite quantification, the external method with high spectral resolution was conducted sufficient signal-to-noise and T_1 and T_2 relaxation times of major metabolite in fatty liver. In this study, we show the feasibility of accurately measuring hepatic lipids, with a correction of relaxation time, and a practical external standard method. The results are applicable to the study of liver disease in both human and animal models.

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