High-Resolution Magic Angle Spinning 1H MRS Detects Lipid Biomarkers in Liver after High Fat Diet

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Introduction

Fatty liver is common in humans and may develop into steatohepatitis and in some cases into cirrhosis requiring liver transplantation [1]. Fat accumulation in the liver has also been associated with obesity, insulin resistance and type II diabetes although the mechanism underlying this relationship is not fully understood [2]. Until recently the only method for quantification of intrahepatocellular lipids was through liver biopsy techniques. Since this method has many drawbacks CT has been proposed. Due to the repeated CT scanning required and the exposure to radiation, *in vivo* ^{1}H MRS has been proposed as a fast, safe and non-invasive technique [2]. Animal models have been developed and are particularly useful because genetic manipulations are highly developed in inbred mice. Here, we present the findings from a murine model of a hepatic steatosis and the possible findings that ^{1}H MRS may reveal. We used high-resolution magic angle spinning (HRMAS) ^{1}H MRS because of the novel metabolites it might reveal.

Materials and Methods

Two cohorts of 6 week old C57BL6 male mice were placed on chow (17% calories from fat) or high fat diet (42% calories from milkfat) (Harlan Teklad, Madison, WI) and followed for 11 weeks. For tissue analysis, random-fed mice were anesthetized with pentobarbital (50 mg/kg); liver was removed and snap-frozen in liquid nitrogen.HRMAS ^{1}H MRS was performed on a Bruker Bio-Spin Avance NMR spectrometer (proton frequency at 600.13 MHz, 89 mm Vertical Bore) using a 4mm triple resonance (^{1}H, ^{13}C, ^{2}H) HRMAS probe (Bruker). The temperature was controlled at 4 °C by a BTO-2000 unit in combination with a MAS pneumatic unit (Bruker). The tissue was placed into 4mm zirconium oxide (Zirconia, Bruker) rotors with spherical inserts. 10 µl D2O containing 50 mM TSP (trimethylsilyl propionic-2,2,3,3-d4 acid, Mn=172, δ=0ppm) was added to the rotor with the sample to serve as the deuterium lock reference and external chemical shift reference respectively. The MAS speed was stabilized at 4.0 ± 0.001 kHz by a MAS speed controller. The one dimensional ^{1}H MRS spectra were acquired on all samples using a rotor synchronized Carr-Purcell-Meiboom-Gill (CPMG) spin echo pulse sequence, [90°-(τ-180°-τ)n-acquisition], which works as a T2 filter to remove the spectral broadening. The inter-pulse delay (τ) was synchronized to the MAS speed to 250µs. The value for n was 20 (2nτ = 10ms). The relaxation delay was set to 5s. The number of transients was 256 with 32,768 (32k) data points. A line-broadening apodization function of 1.0Hz was applied to all HRMAS ^{1}H FIDs prior to Fourier transformation. The metabolite concentrations [M] were calculated according to:

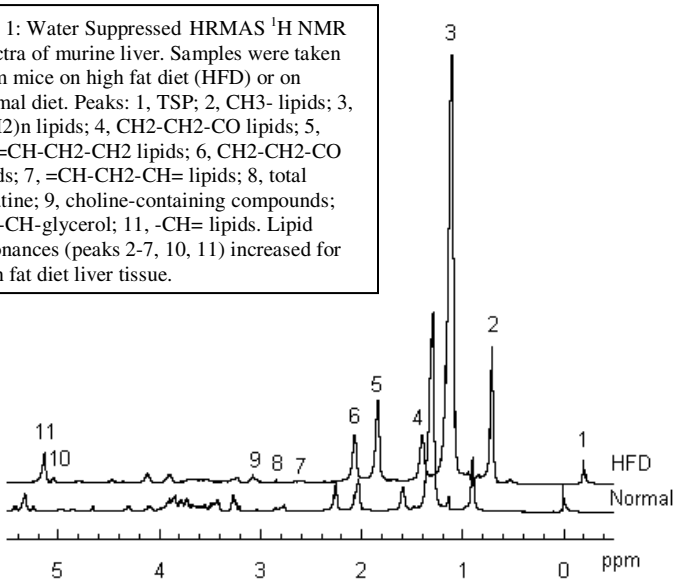
$$[M] = \frac{I_M / n}{I_{TSP} / 9} \times \frac{0.5 \times 10^3}{m_{sample}\,(mg)} (\mu mol / g)$$

where I_M and I_{TSP} are the measured intensities for metabolites and TSP respectively, and n is the number of protons giving rise to the resonance. TSP has 9 protons. m_{sample} is the weight of the measured sample in mg. The spectra were curve-fitted using Lorentzian and Gaussian functions before integration of resonance intensities of metabolites and TSP. 10 µl of 50mM TSP solution was put into the rotor as the external standard and the total amount of TSP was 0.5 µmol.

Results

Representative HRMAS ^{1}H NMR spectra of normal murine liver and liver from mice on high fat diet are shown in figure 1. The spectra were normalized to the intensity of TSP. Increased lipid peaks were observed in HFD versus normal. Peak 3 represents intrahepatocellular lipids. The concentration of selected metabolites are shown in the bargraph of figure 2. All of the lipid peaks including intrahepatocellular lipids are significantly increased (P<0.05).

Fig. 1: Water Suppressed HRMAS ^{1}H NMR spectra of murine liver. Samples were taken from mice on high fat diet (HFD) or on normal diet. Peaks: 1, TSP; 2, CH3- lipids; 3, (CH2)n lipids; 4, CH2-CH2-CO lipids; 5, CH=CH-CH2-CH2 lipids; 6, CH2-CH2-CO lipids; 7, =CH-CH2-CH= lipids; 8, total creatine; 9, choline-containing compounds; 10, -CH-glycerol; 11, -CH= lipids. Lipid resonances (peaks 2-7, 10, 11) increased for high fat diet liver tissue.



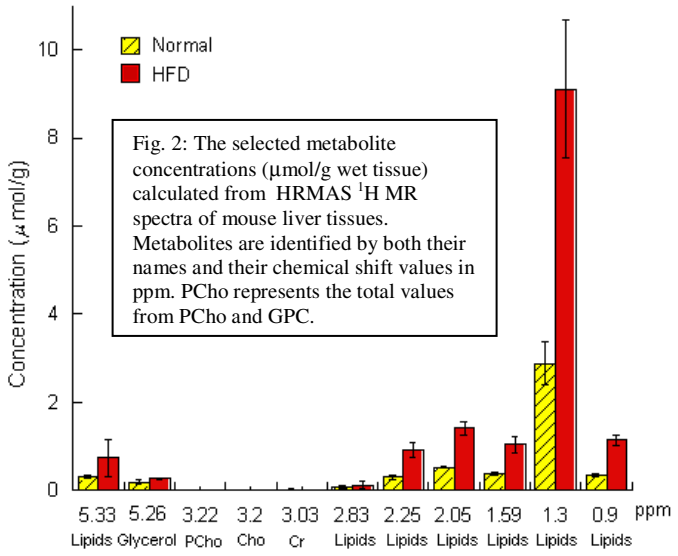


Fig. 2: The selected metabolite concentrations (µmol/g wet tissue) calculated from HRMAS ^{1}H MR spectra of mouse liver tissues. Metabolites are identified by both their names and their chemical shift values in ppm. PCho represents the total values from PCho and GPC.

Discussion

HRMAS ^{1}H NMR permits the identification of novel liver biomarkers. Other investigators using human volunteers have shown that intrahepatocellular lipids calculated from in vivo ^{1}H NMR spectra relate to body adiposity and especially central obesity [1]. In a previous animal study others have demonstrated that MRS can be useful for serial studies aimed at providing important insights into the genetic, environmental and dietary factors affecting fat deposition and accumulation within mouse livers [2]. Our findings extend these previous reports and demonstrate that a higher number of lipids may be revealed using HRMAS ^{1}H NMR. Ultimately, we expect HRMAS ^{1}H NMR to identify metabolic biomarkers of predictive value, and thus significantly shift and advance the existing paradigm for the management of pancreatic cancer patients, and permit tailored therapies for individual patients. Ultimately, we expect HRMAS ^{1}H NMR to identify metabolic biomarkers of predictive value, and thus significantly shift and advance the existing paradigm for the management of fatty liver, and permit tailored therapies for individual patients.

References

1. Thomas, EL, Hamilton, G, Patel, N, O'Dwyer, R, Dore, CJ, Goldi, RD, Bell, JD, Taylor-Robinson, SD. Gut, 54:122, 2005.
2. Garbow, JR, Lin, X, Sakata,, N, Chen, Z, Koh, D, Schonfeld, G. Journal of Lipid Research, 45:1364, 2004.