Towards Noninvasive Biomarkers of Non-Alcoholic Steatohepatitis

I. R. Corbin[1], E. Furth[2], S. Pickup[1], E. J. Delikatny[1]

[1]Radiology, University of Pennsylvania, Philadelphia, Pennsylvania, United States, [2]Pathology and Lab Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States

Background. Hepatic steatosis (the accumulation of lipid droplets within hepatocytes) is increasingly common condition in the US due to its close association with obesity and diabetes [1] Within recent years the diagnosis of this condition has become important as it is now recognized that in some individuals, non-alcoholic steatosis can follow a degenerative course characterized by inflammatory infiltration, hepatocellular necrosis, liver fibrosis and eventual end stage liver disease.[2] This progressive form of fatty liver disease is known as non-alcoholic steatohepatitis (NASH). To date the liver biopsy is the only means with which to diagnose and assess NASH. However, this invasive procedure is associated with a number of inherent problems which limits its utility. We have previously demonstrated that localized *in vivo* proton magnetic resonance spectroscopy (^{1}H MRS) can provide quantitative and compositional information about the fat content within the liver. Here we describe the utility of this technique to distinguish steatohepatitis from benign fatty liver in two murine models of non-alcoholic fatty liver disease.

Methods: *Animals: Model 1*. Baseline MRS examinations were performed on nine-week-old male ob/ob C57BL/6J mice that were permitted *ad libitum* access pellet chow and water. Acute steatohepatitis was induced in ob/ob mice via intraperitoneal administration of lipopolysaccharide (LPS) (2.0 µg/g body weight). Hepatic MRS exams were repeated 24 hrs following LPS injection. *Model 2*. Nine-week old male C57BL/6J mice were placed on a diet deficient in methionine/choline (MCDD) for 10 weeks to induce chronic steatohepatitis. During this period MRS examinations were performed at 2 weeks and 10 weeks. *In vivo* MRS examinations were performed on a 4.7 T Varian Inova with a 40 cm horizontal bore magnet. Animals were anesthetized with isoflurane (1%). A 15 mm diameter receive surface coil / 60 mm diameter transmit birdcage volume coil system was used. Respiratory triggered multi-slice gradient echo images were acquired in the axial plane with a repetition time (TR)=300 ms, echo time (TE)= 5.0 ms, slice thickness of 1 mm, field of view (FOV) 5 cm x 5 cm and a matrix size of 256 x 128. Respiratory-gated localized ^{1}H spectroscopy was accomplished on a 8 µl voxel in the liver using a PRESS pulse sequence (TR=3 s, TE=12 ms, TE2=11 ms, NS=256, spectral width 2000 Hz, 2048 data points). Localized spectra were acquired with and without CHESS water suppression. Peak areas were calculated by Lorentzian/Gaussian curve-fitting using NUTS software. Unsaturation indices were calculated according to Zancanaro et al.[3]

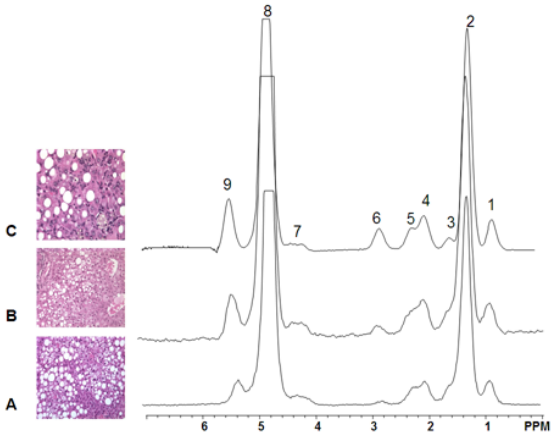

Figure 1. Representative histologic sections of liver and corresponding localized ^{1}H MRS from (A) Baseline ob/ob mouse; (B) ob/ob mouse 24 hrs following LPS injection (C) C57BL/6J mouse following 10 weeks MCDD. These spectra are scaled to set lipid -CH_2 as the largest peak. Neutral lipid resonances from fat are labeled: **1**: triglyceride terminal methyls; **2**: methylene $(CH_2)n$;**3**: C**H**$_2$CH_2CO; **4**: C**H**$_2$C=C; **5**: C**H**$_2$CO; **6**: C=CC**H**$_2$C=C; **7**: C**H**$_2$OCOR; **8**: Water; **9**: C**H**=C**H**.

Results and Discussion. Representative histologic sections from ob/ob and MCDD mice depict marked hepatic steatosis (Fig 1 left)**.** Localized *in vivo* MR spectroscopy (Fig 1 right) confirms this observation depicting a striking abundance of lipid resonances from steatotic liver. The major resonances observed arise from water (4.7 ppm) and from the saturated methylene chains of fatty acids in triglycerides (1.3 ppm). In addition, the a high degree of spectral resolution obtained in these studies allows the identification of resonances arising from saturated and unsaturated fatty acid moieties assigned in Fig. 1. From this, indices of fatty acyl chain saturation, average unsaturation and mono and polyunsaturation can be calculated (Table 1). Following the i.p. administration of LPS, ob/ob mice experience an acute inflammatory response within the liver characterized by neutophil infiltration. Localized hepatic ^{1}H spectra of LPS treated mice reveal that indices of fatty acid unsaturation (PUFA and MUFA) significantly increase with these acute events (Table 1 and Fig 1 peaks 6 and 9). For animals with chronic steatohepatitis induced by MCDD, a similar pattern emerges. Two weeks into the induction of NASH, mice histologically display zone 3 steatosis. Their hepatic spectral lipid profile is similar to baseline ob/ob mice except they have lower levels of total hepatic lipids and higher relative levels of PUFA (Table 1). By 10 weeks on a MCDD mice display numerous lesions within the liver that include panlobular steatosis, inflammation, hepatocyte ballooning and necrosis. Corresponding spectra depict an increase in the olefinic resonance and MUFA relative to mice at 2 weeks on a MCDD. Collectively these results suggest that the hepatic fatty acyl unsaturation indices as measured by *in vivo* ^{1}H MRS are sensitive biomarkers of necroinflammatory activity. This technique may provide a unique opportunity to non-invasively diagnose and delineate metabolic alterations in hepatic lipid metabolism during the progression of nonalcoholic fatty liver disease.

Table 1: Fatty Acid Composition in ob/ob and MCDD Treated Mice

Index	Fatty Acid Resonance Ratio	ob/ob baseline (n=6)	ob/ob LPS (n=6)	MCDD 2 wks (n=5)	MCDD 10 weeks (n=5)
Lipid to Water Ratio	$(CH_2)n$ /H_2O	0.48 ± 0.09	0.42 ± 0.03	0.06 ± 0.02	0.46 ± 0.13
Lipid CH_2/CH_3 Ratio	$(CH_2)n$ /CH_3	10.60 ± 1.00	11.30 ± 1.56	8.68 ± 0.63	9.22 ± 1.18
Mean Polyunsaturation (PUFA)	3(CH=CHCH_2CH=CH)/ 2(CH_3)	0.28 ± 0.08	0.50 ± 0.13	1.17 ± 0.13	1.5 ± 0.11
Mean PUFA and monounsaturation (MUFA)	3(CH_2CH=CHCH_2)/ 4(CH_3)	0.91 ± 0.17	1.35 ± 0.33	0.87 ± 0.08	1.3 ± 0.27
Mean Unsaturation	3(CH=CH) /2(CH_3)	1.77 ± 0.13	2.52 ± 0.35	1.77 ± 0.16	3.2 ± 0.35

References:1: Marchesini, G. Hepatology, 37, 917, 2003; 2:Brunt, EM, Semin Liver Dis, 21, 3, 2001; 3:Zancanaro, C., J. Lipid Res. 35, 2191, 1994.