In Vivo Measurement of Hepatic Triglyceride Composition in Murine Non-alcoholic Steatosis

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Background.

Hepatic steatosis (the accumulation of lipid droplets with in hepatocytes) has become an increasing common condition in the US due to its close association with obesity and metabolic syndrome (hypertriglyceridemia, low levels of high-density lipoproteins, hypertension and insulin resistance). [1] Within recent years the diagnosis of this condition has become important as it is now recognized that non-alcoholic steatosis can progress to end stage liver disease.[2] Current clinical methods of diagnosing this condition include the liver biopsy and abdominal imaging. The liver biopsy is the gold standard to accurately assess the severity of steatosis and provide information on secondary events such as tissue inflammation and degeneration. Subsequent *in vitro* biochemical analysis can be performed on the biopsy specimen. This invasive technique, however, has a number of inherent problems, foremost of which is its' limited utility in obese individuals. Steatosis can also be non-invasively diagnosed with CT or MR imaging. However, these modalities only provide an estimate on the quantity of fat infiltration in the liver. An alternative imaging modality, *in vivo* magnetic resonance spectroscopy (MRS) has the potential to provide information about both the quantity and composition of fat within the liver *in situ*. In this study we utilize localized *in vivo* ^{1}H MRS to assess the content and composition of hepatic triglycerides in a murine model of non-alcoholic steatosis.

Methods: *Animals*. Nine-week-old male ob/ob C57BL/6J mice were permitted *ad libitum* access pellet chow and water. *In vivo ^{1}H MRS.* 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%) and placed prone in a 50 mm diameter birdcage volume coil operating at 200.8 MHz. 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 1H spectroscopy was accomplished on a 32 µl voxel in the liver using a STEAM (stimulated echo acquisition mode) pulse sequence (TR=3 s, TE=7 ms, TM=30 ms, NS=256, spectral width 2500 Hz, 2048 data points) incorporating outer volume suppression. 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]

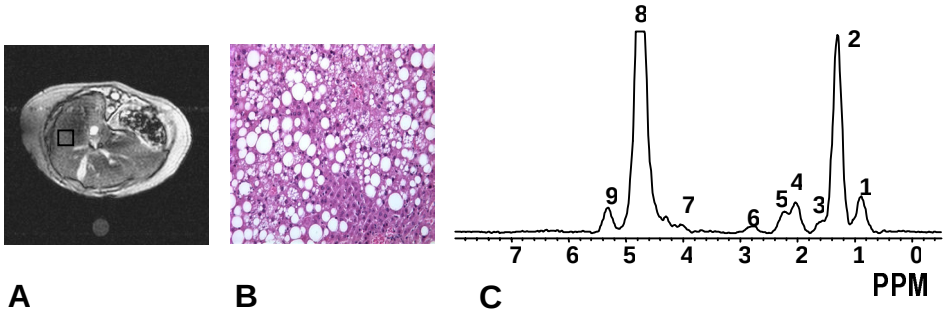

Figure 1. (A) Axial gradient echo images through the abdomen of ob/ob mouse. (B) Hematoxylin and eosin stained liver section (x200). (C) Localized single voxel STEAM proton MR spectrum. Spectrum is scaled to set lipid -CH_2 as the largest peak. Neutral lipid resonances from fat are labeled: **1**: triglyceride terminal methyls; **2**: methylene (CH_2)n ;**3**: C**H**$_2$CH$_2$CO; **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

The abdominal MR image of the ob/ob mouse (Fig 1A) shows substantial subcutaneous fat and hepatomegaly. A respective histologic section of liver is displayed in Fig 1B. It can be seen that the liver from the ob/ob mouse has extensive accumulation of fat that appear as clear intracellular lipid droplets. Localized *in vivo* MR spectroscopy (Fig 1C) 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). The lipid to water ratio of these two resonances is approximately equal (0.84 ± 0.09) which is markedly greater than normal liver (~0.03, data not shown). The spectral resolution obtained 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. I*n vivo* spectroscopic examinations of the liver have generated limited results due to poor spectral resolution. Broad resonances from water and from the methylene envelope can typically be observed in both murine and human *in vivo* spectra of liver. In this study, spectral resolution was optimized on the ob/ob mouse using a birdcage coil design with high filling factor from which shim values of < 50 Hz were routinely achievable. Respiratory gating was used to reduce motion artefacts and outer volume suppression was implemented to eliminate subcutaneous fat contributions. The effectiveness of the outer volume suppression was confirmed by obtaining spectra composed primarily of water from the gall bladder, which is surrounded by fatty liver in obese mice (data not shown). The overall result is excellent spectral resolution was obtained on steatotic liver. The high neutral lipid levels provide ample signal for high quality spectra within reasonable scan time allowing profiles of the relative content and degree of unsaturation of liver triglycerides to be obtained non-invasively. This may prove to be of diagnostic importance during progressive degenerative fatty liver disease. In addition, this technique provides a unique opportunity to evaluate the turnover of neutral lipids within the liver in response to endogenous and exogenous effectors of hepatic lipid metabolism.

Table 1: Fatty Acid Composition of Steatotic Liver from ob/ob Mouse

	Fatty Acid Moiety: FA	(n=7)
Lipid to Water Ratio	(CH_2)n /H_2O	0.84 ± 0.09
Lipid CH_2/CH_3 Ratio	(CH_2)n /CH_3	9.18 ± 0.25
Mean Polyunsaturation	3(CH=CHCH$_2$CH=CH)/ 2(CH_3)	0.29 ± 0.07
Mean Unsaturation	3(CH=CH) /2(CH_3)	1.56 ± 0.10

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.