IMAGING OF HEPATIC STEATOSIS AND HYPERPOLARIZED CARBON METABOLISM AT 14T - APPLICATIONS TO A MURINE MODEL OF NON-ALCOHOLIC FATTY LIVER DISEASE

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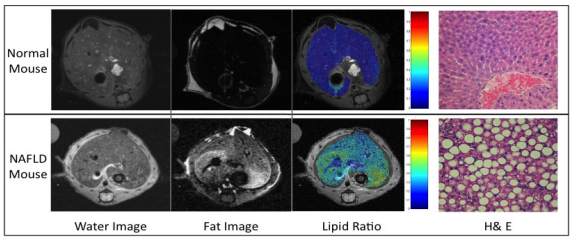
INTRODUCTION: Non-Alcoholic Fatty Liver Disease (NAFLD) is the most common cause of chronic liver disease in North America, with a prevalence approaching 35% in some population groups. The disease is closely associated with obesity and the metabolic syndrome.[1] Although the etiology is multifactorial, current understanding of the metabolic abnormalities of NAFLD, as well as of the factors that lead some patients to progress to hepatic inflammation and end-stage liver cirrhosis while others do not, is limited. Magnetic resonance imaging at high field allows the acquisition of anatomic images with exquisite spatial resolution and detail. Additionally, specialized pulse sequences can be used to determine the degree of adiposity of tissues *in vivo*, providing a method to visualize the severity of steatosis within the liver. Hyperpolarized ^{13}C MR continues to be a valuable tool for the investigation of metabolic and biochemical processes in a variety of organs and pathologic conditions. The goal of these studies was to utilize hyperpolarized ^{13}C MR to identify and quantify metabolic derangements in mice fed a diet deficient in methionine and choline (MCD diet), an animal model of Non-Alcoholic Fatty Liver Disease.

Figure 1. Quantitative Lipid/Water Imaging



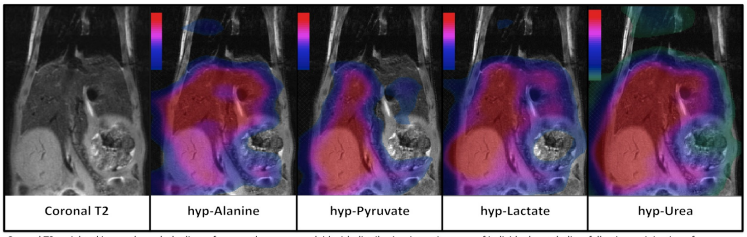
Axial water-sensitive and fat-sensitive images through the liver of a normal mouse (top) and of a mouse after two weeks on the MCD diet (bottom). Ratio maps demonstrate a marked increase in the amount of lipid signal in the NAFLD mouse, which is confirmed by the presence of massive steatosis on pathologic section.

METHODS: Hepatic Lipid/Water Imaging: Mice were anesthetized using isoflurane anesthesia and placed in a custom-built animal holder. Imaging was performed in a 600MHz (14.1 T) wide bore spectrometer using a 40mm ^{1}H probe. Interleaved fat- and water signal images were produced using a previously published gradient-reversal spin-echo technique.[2] Regions of interest were drawn within the liver using MRSC-Image software, and lipid ratio maps were subsequently generated in MATLAB.
Hyperpolarized Studies: Hyperpolarized ^{13}C studies were performed using a preparation of 14.2 M [1-^{13}C]pyruvate and 6.4 M ^{13}C-urea combined with OX63 radical and polarized in an Oxford Hypersense system. Spectroscopic imaging was performed on a 600 MHz (14.1 T) system using a 40 mm ^{13}C probe using frequency specific pulses (for alanine, lactate, pyruvate and urea) implemented with an echo-planar imaging sequence.[3] Resulting 3D images of the distribution of each metabolite were overlaid on the spatially matched anatomic images, and regions of interest were drawn to isolate the hepatic parenchyma for analysis of metabolite ratios, taking care to exclude the great vessels.

RESULTS AND DISCUSSION: Co-localized water- and fat-sensitive axial images acquired simultaneously through the livers of both normal and NAFLD mice demonstrate a marked increase in the degree of fat signal within the hepatic parenchyma after 14 days of feeding the MCD diet when compared to the livers of normal mice **(Figure 1)**. Additionally, characterization of the pulse sequence using phantom lipid/water emulsions of varying concentrations demonstrated a linear relationship between signal intensity and fat percentage in lipid-sensitive images (data not shown), allowing quantification of the deposition of microscopic fat in the liver.

3D frequency-specific acquisition **(Figure 2)** following injection of hyperpolarized [1-^{13}C]pyruvate generates metabolic maps with high spatial (1.25 mm^3 voxel size) and temporal resolution (acquistion time 140ms per metabolite), with the previously described large increase in signal to noise over standard spectroscopy.[4] This allows the use of respiratory gating during acquisition, minimizing motion artifact. Initial experiments comparing normal mice (n=3) with mice on the fatty liver diet (n=3) demonstrate no significant change in metabolism of hyperpolarized [1-^{13}C]-pyruvate substrate within the liver **(Table 1)**. It has been reported previously [5] that although mice on the MCD diet are hypermetabolic, this appears to be related to increased fatty acid flux through β-oxidation pathways. Thus, pyruvate metabolism via the action of pyruvate dehydrogenase and ALT may remain unchanged in this model of fatty liver disease.

Figure 2. Anatomic Distribution of Hyperpolarized Metabolites



Coronal T2-weighted image through the liver of a normal mouse, overlaid with distribution-intensity maps of individual metabolites following an injection of hyperpolarized [1-^{13}C]-pyruvate and ^{13}C-urea. Hyperpolarized pyruvate is converted into alanine, primarily within the liver. Alanine within the visualized right kidney is presumed related to serum alanine aminotransferase activity. Lactate is the product of the conversion of pyruvate by lactate dehydrogenase, ubiquitous within tissues. Urea is introduced primarily as a perfusion marker and spectroscopic reference.

CONCLUSION: Early studies from this project have demonstrated the feasibility of noninvasive monitoring of steatosis progression through the use of quantitative lipid:water imaging sequences. Hyperpolarized ^{13}C metabolic imaging of NAFLD using hyperpolarized [1-^{13}C]-pyruvate has been performed with high temporal and spatial resolution and with high signal-to-noise. These initial studies demonstrate no significant change in pyruvate metabolism as assessed by hyperpolarized lactate, pyruvate and alanine levels following intravenous injection in the fasting state, a finding that appears consistent with previously published work in this model. Additional experiments to assess hepatic oxidative stress in MCD-NAFLD mice are ongoing.

Table 1: Hyperpolarized metabolite Ratios

Metabolite Ratio	MCD Mean	Normal Mean
Pyruvate:TC	0.26	0.26
Lactate:TC	0.46	0.51
Alanine:TC	0.28	0.24

Hyperpolarized metabolite ratios compared to total injected carbon (TC, pyr + lac + ala) in MCD-fed mice and normal nice (n = 3, each group)

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