Molecular Imaging of Non-Alcoholic Fatty Liver Disease using an Endogenous Hyperpolarized Redox Sensor

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INTRODUCTION: Non-alcoholic fatty liver disease (NAFLD) is recognized as the most prevalent liver abnormality in the United States, with nearly 10% of the population demonstrating some form of the disease. Incidence can reach as high as 70% in patients who are obese and/or have type II diabetes [1]. Many rodent models have been developed to study NAFLD, which are induced by both diet and genetic manipulation [2]. The methionine choline deficient (MCD) model has been used to develop NAFLD, with onset of disease readily visible after 2 weeks on the diet. Changes in reduction and oxidation (redox) have been implicated in the development of this disease as well as its response to therapy [3]. Methods to detect redox changes in these animal models non-invasively are limited. Recent development of hyperpolarized (HP) [1-^{13}C] dehydroascorbate (DHA), using the dissolution dynamic nuclear polarization (DNP) technique, provides a new redox sensor to address NAFLD and its treatment. HP DHA is readily transported into the cell and reduced to Vitamin C (VitC) *in vivo*. The aim of this study was to use HP [1-^{13}C] DHA to image redox changes in a standard model of NAFLD and correlate these findings with standard ^{1}H imaging and histopathology.

METHODS: Prior to and post diet, high-resolution T_2 and fat-water imaging was conducted at 14T using a Varian WB600 micro-imager and 40mm ^{1}H millipede coil (Varian Instruments, Palo Alto, CA). Lipid percentage maps were calculated from fat and water images acquired using a conventional spin-echo sequence using the Dixon method [4]. 350µL of hyperpolarized DHA was injected each mouse (n=4) at baseline and 2 weeks of MCD diet using a Hypersense (Oxford Instruments), as previously described [5]. ^{13}C MRSI data was acquired using an EPSI readout, variable flip angle scheme, matrix size 16 x 8 x 8 and final voxel resolution of 6mm isotropic on a 3T GE MRI (GE Healthcare, Waukesha, WI) equipped with a multinuclear package and dual-tuned ^{1}H-^{13}C imaging coil. MR data was processed offline using custom software written in IDL 8 (ITT Visual Information Solutions, CO, USA) and Matlab 2009b (MathWorks, MA, USA). Ratios of HP DHA and VitC were calculated from the peak integrals in 3D MRSI data. Mice were then sacrificed and liver tissue was sectioned for staining with hematoxylin and eosin (H&E, structure) and Oil Red (lipid presence). Livers of normal mice were used for histologic comparison.

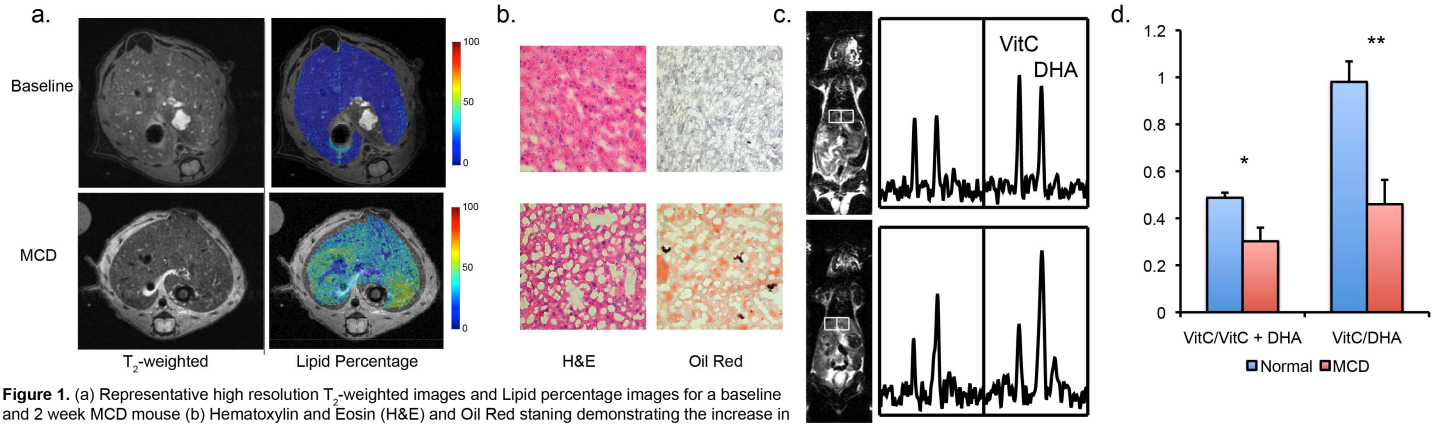

Figure 1. (a) Representative high resolution T_2-weighted images and Lipid percentage images for a baseline and 2 week MCD mouse (b) Hematoxylin and Eosin (H&E) and Oil Red staning demonstrating the increase in lipid droplets at 2 weeks. (c) T_2-weighted images and corresponding hyperpolarized ^{13}C MR spectra post-injection of HP DHA, in a representative mouse at baseline and at 2weeks of MCD diet (d) Significant increase in VitC/ VitC + DHA as well as VitC/DHA after 2 weeks on the MCD diet (P=0.01 and 0.005).

RESULTS AND DISCUSSION: The normal liver demonstrates high conversion of HP DHA to Vitamin C (VitC/VitC + DHA of 0.49±02), indicative of high antioxidant capacity. We believe that this antioxidant capacity correlates most strongly with the concentration of reduced glutathione (GSH), which is high in the normal liver (on the order of 5mM) but decreased significantly in experimental fatty liver models [6]. When placed on the MCD diet for two weeks, the average mouse weight decreases 13% and the liver fat percentage dramatically increases 6 fold from baseline (**Figure 1a**). These changes are validated in histopatholgic sections of the same liver tissue (**Figure 1b**) as compared to those of normal. Lipid staining denotes large regions of fat accumulation in the liver (**Figure 1b**, *Oil Red staining*), which is also visualized by fat/water imaging at high field (**Figure 1a**), while the liver size does not change significantly. There is also a remarkable decrease in HP VitC after 2 weeks on the diet (**Figure 1c**) resulting in a **48%** decrease in the VitC/ VitC + DHA ratio from baseline (0.49±02 to 0.30±0.5, *P=0.01*) and **92%** in the VitC/DHA ratio (0.98 ± 0.09 to 0.46±0.1, *P=0.005*). Deregulation of lipid export and changes in oxidative stress are hallmarks of fatty liver disease, with overload of free fatty acids resulting in electron leakage during mitochondrial β-oxidation. Generation of lipid peroxides results in subsequent damage to hepatic membranes, proteins and DNA. Total anti-oxidant capacity, both enzymatic and non-enzymatic is insufficient to mitigate liver injury [7]. After 2 weeks of the MCD diet, increased oxidative stress leads to depletion in hepatic reducing capacity, thus resulting in lowered production of HP VitC. The non-invasive observation of changes in redox provides a means to evaluate not only the extent of this liver injury but also potential response to antioxidant therapeutics.

CONCLUSIONS: This study demonstrates the application of HP MR to a non-oncogenic liver pathology, namely NAFLD that impacts 1 out of 10 Americans in their lifetime. Redox has been implicated in the progression of this disease and remains an active target for NAFLD treatment. HP DHA conversion to Vitamin C provides a potential non-invasive probe for changes in redox. In this study, we demonstrate changes in redox with onset of NAFLD in a MCD deficient mouse model. Future studies aim to extend the use of HP DHA for fatty liver disease characterization in other models as well as response to both therapeutics and diet.

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