Feasibility of an Animal Model of Hepatic Steatosis for In Vivo Fat Imaging with IDEAL

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting more than 20% of all Americans, and can progress to non-alcoholic steatohepatitis (NASH)[1]. Steatosis is the earliest biomarker of NASH, and current fat quantification methods, such as biopsy, are inadequate due to high sampling variability, morbidity and cost. MRI may provide a potential strategy for rapid, non-invasive and accurate fat quantification to meet this growing unmet need. In this work, we propose an animal model for hepatic steatosis to provide an *in vivo* animal model for fat imaging and validation.

Methods: Three nine-week old male Sprague-Dawley rats with a body weight of 275-300g were fed a 1% orotic acid, 65% sucrose diet[2] (Harlan Teklad, Madison, WI) and one identical rat was fed standard rodent chow (Harlan Teklad, Madison, WI) for control. Rats remained on the 1% orotic acid diet for two weeks and were housed individually with controlled humidity, temperature, lighting and ventilation in accordance with a university-approved animal protocol. Prior to imaging, rats were sacrificed and perfused with phosphate buffered saline to eliminate motion and susceptibility artifacts from deoxygenated blood.

Fat quantification was performed using IDEAL (Iterative Decomposition with Echo Asymmetry and Least-squares Estimation), which provides robust separation of fat and water[3,6]. Combined with a 3D spoiled gradient echo (SPGR) imaging[6], the echo times of the three echoes are chosen to optimize the SNR performance of the water-fat decomposition[7,8]. Separated water and fat images are provided with an on-line reconstruction program that uses a region growing algorithm to prevent water-fat swaps[4].

All imaging was performed on a 3.0T Signa GE clinical scanner (V12.0, Waukesha, WI) with a home-built quadrature bird cage coil using the 3D-IDEAL-SPGR sequence and flip angle of 5° to minimize the effects of T_1 bias due to the differences in T_1 between fat and water. Imaging parameters included: TR = 8.6 ms, TE = 3.3, 4.9 and 4.1 ms, BW = ± 31.25 kHz, FOV =12 cm, slice = 1.5 mm, a 256 x 256 matrix, for a total scan time of 3:03. Calculation of fat fraction images was constructed offline with Matlab (Mathworks, Natick, MA) using the individual separated water and fat images obtained from the IDEAL reconstruction where:

$$Fat\ Fraction = 1 - \frac{W}{W+F}$$

This formulation for fat fraction was used to reduce noise bias when fat fractions are low (unpublished method to be reported elsewhere). After imaging, the rat livers were excised and sections were stained with Oil Red O, a stain for endogenous and exogenous lipid deposits. Additionally, fat was quantified in samples of the liver using a modified Folch lipid extraction[5]; half-lobe pieces were analyzed and the mass of total lipid per sample was obtained. Samples submitted for Histology were taken from the lobe subjected to lipid extraction.

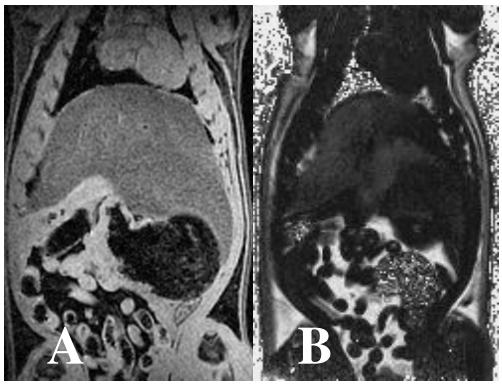

Figure 1: Steatotic model of rat fed 1% orotic acid, 65% sucrose diet for 2 weeks. A) Water image and B) fat fraction image of steatotic model. Lipid extraction determined liver to be 16.85% fat, whereas control was 3.87% fat.

Results: Figures 1A and 1B show the water and calculated fat fraction images of an orotic-acid diet rat, where the heterogeneous distribution of fat is visible as higher signal in the fat fraction image. After two weeks, total lipid content in the rat in Figure 1 was 16.85% and the corresponding region in the fat fraction images reported fat content to be 15.35%. Biochemical lipid extraction determined the control rat to be significantly lower than orotic acid-fed rats; total lipid content was 3.87%. Figure 2A shows a Histology section of the liver shown in Figures 1A and 1B, where red indicates the presence of fat. Figure 2B shows a histology slide of a control rat, with less fat content.

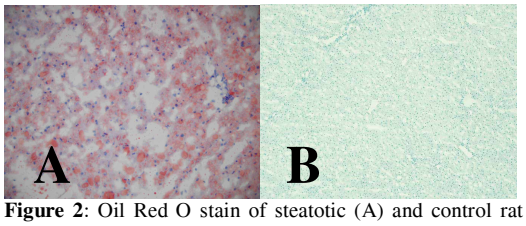

Figure 2: Oil Red O stain of steatotic (A) and control rat (B) where the presence of red indicates fat deposits. Nuclei are stained blue.

Discussion and Conclusion: Rats fed a 1% orotic acid, 65% sucrose diet serve as a potential model for hepatic steatotis and in vivo fat imaging using IDEAL; rats fed this diet displayed markedly higher fat content, as seen in fat fraction images, biochemical extraction and histology, as compared to controls. This diet has been previously established[2], but has not been utilized for quantification of steatosis with imaging. This diet and model can potentially produce controllable levels of hepatic steatosis with prolonged duration, and thus we propose that this model is feasible for *in vivo* imaging of hepatic steatosis.

References:

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