Quantification of Hepatic Steatosis with MRI: Validation in the ob/ob Mouse at 3T

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, affecting up to 30% of all Americans[1], and can progress to non-alcoholic steatohepatitis (NASH)[2], an increasingly important cause of liver failure and hepatocellular carcinoma. Steatosis (fatty infiltration) is the hallmark of NASH and its earliest biomarker. Current fat quantification methods, such as biopsy, are inadequate due to high sampling variability, associated morbidity and high cost, so an alternative to biopsy is needed. MRI has great potential as a rapid, non-invasive quantitative biomarker to address this important unmet need. The purpose of this work was validation of new imaging biomarkers of steatosis using IDEAL (Iterative Decomposition with Echo Asymmetry and Least-squares estimation) in an animal model of hepatic steatosis, the *ob/ob* mouse.

Methods: A total of eighteen mice were included in this study. Twelve five-week old male leptin-deficient (*ob/ob*) mice were separated into two groups; the first group was fed standard rodent chow for four weeks, the second was fed a 60% fat diet for four weeks (Research Diets, Inc). Six lean littermate controls (*ob*/+) were fed standard rodent chow for four weeks, age-matched to the second group. Mice were anesthetized with pentobarbital prior to imaging.

Imaging was performed on a 3.0T Signa clinical scanner (Signa HDx, GE Healthcare, Waukesha, WI) using a 3D spoiled gradient echo (SPGR) IDEAL[4] sequence and a home-built quadrature bird cage coil. IDEAL provides robust separation of fat and water[3,4] and provides separate water and image that can be used to calculate a fat-fraction image for quantification of steatosis. The three echo times for IDEAL-SPGR were chosen to optimize the SNR performance of the water/fat decomposition[9,10]. A modified IDEAL water/fat reconstruction[4] using a pre-calibrated fat signal evolution model that takes all spectral peaks of fat into account (details submitted separately) was used for subsequent online calculation of fat fraction. This algorithm uses a magnitude discrimination method to calculate fat-fraction, free from noise bias[5].

A 5° flip angle was used to minimize bias in fat measurements caused by differences in T_1 between fat and water[5]. Other imaging parameters included: TE = 3.3, 4.1 and 4.9 ms, TR = 10.9 ms, FOV = 12 cm, phase FOV = 0.6, with nine slices covering the liver, 256 x 256 matrix, and BW = ± 27.78 kHz for a total scan time of 3:38 min.

Following imaging, mouse livers were excised and a portion of the left lateral lobe was sectioned and stained with H&E and graded subjectively by a Surgical Pathologist who was blinded to imaging results. Grading results were estimates made by the Pathologist as to the percent of cells affected by steatosis. Digitized H&E slides were also analyzed using Image Pro Plus 6.2 (MediaCybernetics, Bethesda, MD) to obtain a quantitative histological measurement of steatosis; a threshold intensity is established and the percent of the image area above the threshold is measured, resulting in a fat percentage by area[6]. Additionally, percent fat was quantified in five of the lobes of the liver using a modified Folch lipid extraction; entire lobes were analyzed and the mass of total lipid per lobe was obtained[7].

Results: Figures 1A and 1B display calculated coronal fat-fraction images in an *ob/ob* mouse and in a control mouse. Figures 2A and 2B show histological examples from a steatotic and control mouse, where white areas denote fat vacuoles. Subjective grading of the control was 0% of cells affected while the steatotic liver had 70% of cells affected. IDEAL estimated -6.27 ± 4.5% and 21.9 ± 7.1%, and lipid extraction showed 4.3% and 20.4%, respectively, for control and steatotic mouse. Histological steatosis grading indicated 0% of affected cells for all controls, and 45-90% for steatotic mice. Figure 3 displays a correlation between qualitative histology and IDEAL; the slope is 0.49 ± 0.03, intercept = -3.7 ± 2.1%, and R^2 = 0.92. Figure 4 displays a correlation between Folch lipid extraction and IDEAL; slope is 1.9 ± 0.1, intercept = -13.1 ± 2.8%, and R^2 = 0.92. It should be noted that small negative fat percentages in controls may have occurred due to absence of T_2* correction[8].

Discussion and Conclusion: Excellent correlation between IDEAL fat-fraction, lipid extraction and histological assessment (both quantitative and qualitative) was observed. These results demonstrate the ability of IDEAL to quantify absolute fat percentages in vivo, and demonstrate the potential of MRI as a biomarker of hepatic steatosis clinically. Future work will include correction for the effects of T_2*[8].

References:

[1] Brunt, et al. Hepatol Res 2005 33(2): 68-71. [2] Harrison, *et al.* Clin Liver Dis 2004; 8(4): 861-879. [3] Reeder, *et al.* MRM 2004; 51(1):35-45. [4] Reeder, *et al.* JMRI 2007; 25(3): 644-652 [5] Liu, *et al.* MRM 2007; 58(2):354-64. [6] Marsman, *et al.* Human Pathology; 35(4): 430-435. [7] Hara, *et al.* Analyt Biochem 1978; 90:420-426. [8] Yu, et al. JMRI 2007; 26(4): 1153-61.[9] Pineda, et al. MRM 2005; 54(3):625-35. [10] Reeder, et al. MRM 2005; 54(3):636-44.

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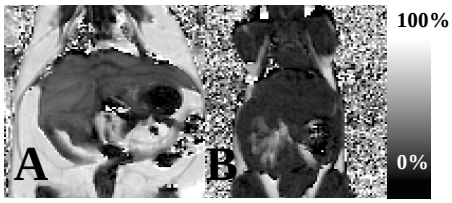

Figure 1: Coronal fat fraction images from control (right) and four week diet ob/ob (middle) show progressively increasing hepatic steatosis. Excellent quality images with high resolution were able to depict distribution of fat within the livers.

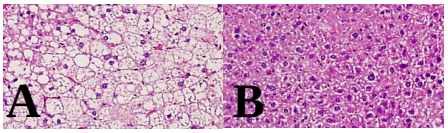

Figure 2: H&E slides (100x) of *ob/ob* mouse fed a high fat diet for 4 weeks (A) and control *ob*/+ (B) illustrate in the presence of fat vacuoles (white) in figure 3A only.

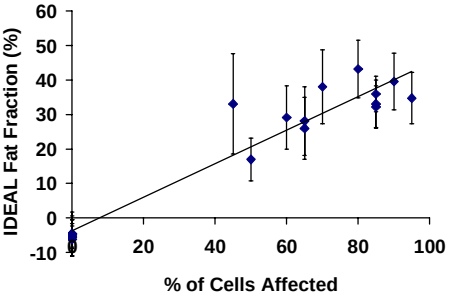

Figure 3: Fat-fraction measured from imaging correlates with qualitative histological estimates of the percentage of cells affected with steatosis. Correlation coefficient (R^2) = 0.92, and slope and intercept are 0.49 ± 0.03 and -3.7 ± 2.1%.

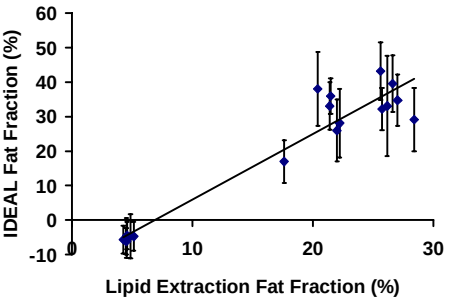

Figure 4: Fat-fraction measured from imaging correlates with Folch lipid extraction. Correlation coefficient (R^2) = 0.92, and slope and intercept are 1.9 ± 0.1 and -13.1 ± 2.8%.