MRI Can Quantify Hepatic Steatosis for Treatment Monitoring During Pharmacological Intervention in the ob/ob Mouse

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease, and may progress to non-alcoholic steatohepatits (NASH)[1], and eventually cirrhosis and liver failure. NAFLD is commonly associated with type II diabetes because insulin resistance can lead to fatty infiltration (steatosis) of hepatocytes[2,3]. Insulin sensitizing drugs are widely used to treat type II diabetes, and can also be used to treat hepatic steatosis[4]. Biopsy is the current gold standard for evaluating the progression of NAFLD, although this technique is risky[5] and suffers from high variability between successive biopsies[6]. The purpose of this work is to demonstrate the use of MRI as a means of non-invasive longitudinal treatment monitoring during treatment with an insulin-sensitizing drug (Metformin) in a mouse model of hepatic steatosis.

Methods: Seven- to eight-week old male leptin-deficient (*ob/ob*) mice were separated into two groups for daily intraperitoneal administration of metformin (200mg/kg IP) or saline[7]. Four mice were administered metformin for four weeks (Group 1), and three mice were administered saline for four weeks (Group 2). Mice were imaged at Day 0 before administration of saline or metformin, and at Days 14 and 28. Mice were anesthetized with 40mg/kg pentobarbital prior to imaging. Following imaging on Day 28, mice were sacrificed and livers immediately excised.

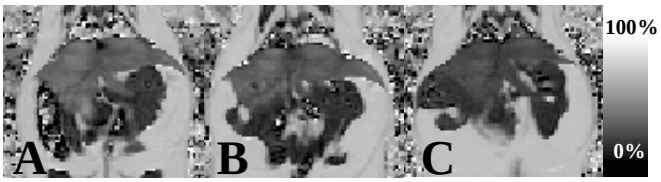

Figure 1: Coronal fat-fraction images of a mouse treated with metformin for four weeks, shown at (A) Day 0, (B) Day 14, and (C) Day 28, with fat-fractions of 35.9%, 33.6%, and 20.1% on Days 0, 14, and 28, respectively.

Fat quantification was performed using a chemical shift-based water-fat separation method known as IDEAL (Iterative Decomposition with Echo Asymmetry and Least-squares Estimation)[8,9]. This technique provides separate water and fat images that can be used to calculate a fat-fraction image for quantification of steatosis. Imaging was performed on a 3.0T clinical scanner (Signa HDx TwinSpeed, GE Healthcare, Waukesha, WI) using an investigational version of the 3D spoiled gradient echo (SPGR) IDEAL[8] sequence and a home-built quadrature bird cage rodent coil. Data were reconstructed using a modified IDEAL water/fat reconstruction that corrects for T_2* decay[10] and uses accurate spectral modeling of fat[11]. This algorithm also uses a magnitude discrimination method to avoid noise bias[12]. Six echoes were acquired with a spacing of 1.5 ms by using three interleaved TRs, each with an echo train length of 2 (TE_{min} = 1.6 ms). Other imaging parameters included: flip = 5° to minimize T_1 bias[5], TR = 29.7 ms, FOV = 16 x 8 cm, with 18 slices covering the entire liver, 256 x 128 matrix, and BW = ± 125 kHz for a total scan time of 4:04 min. True spatial resolution was 0.63x0.63x0.90mm.

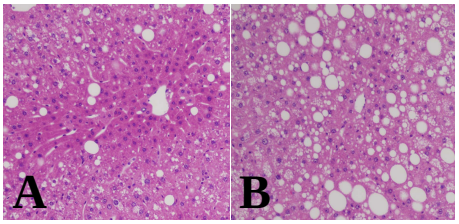

Figure 2: H&E slides (100x) of *ob/ob* mouse treated with metformin after four weeks (A) and ob/ob mouse receiving saline only for four weeks (B). Grading results were 65% of cells affected by steatosis for (A) and 70-75% for (B).

In order to assess reproducibility of fat-fraction measurements, mice were removed from the coil, repositioned, and imaging was repeated. The average percent deviation of fat-fraction measurements between each mouse on all three days was calculated, where percent deviation is defined as the absolute difference between exams, divided by their average. Longitudinal measurements were also taken using the first exam of each day. The left lateral lobe of the excised livers were sectioned, stained with H&E and graded subjectively by a Surgical Pathologist blinded to imaging results. Grading results were estimates made by the Pathologist as the percent cells affected by steatosis.

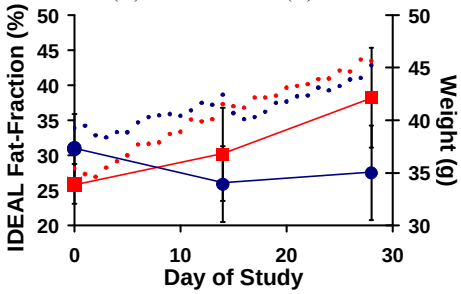

Figure 3: Group average IDEAL fat-fractions for Groups 1 (blue circles) and 2 (red squares) on Days 0, 14, and 28 display the progression and decline of steatosis for mice treated with saline and metformin, respectively. Average daily weights for Groups 1 (blue) and 2 (red) are dotted lines.

Results: Figures 1A, B and C display coronal fat-fraction images on Day 0, 14, and 28, respectively, in an *ob/ob* mouse treated with metformin for four weeks. MRI demonstrated 35.9 ± 4.8%, 32.6 ± 6.7%, and 20.1 ± 4.7% fat for this mouse on Day 0, 14, and 28, respectively. Figure 2A displays the H&E slide of this mouse, whereas Figure 2B displays the H&E slide of a mouse treated with saline only for four weeks. Subjective grading of the liver in Figure 2A was reported to be 65% of cells affected by steatosis, and 70-75% for Figure 2B.

Group 1 averages at Days 0, 14, and 28 were 31.0 ± 5.0%, 25.9 ± 5.4%, and 27.5 ± 6.7%, respectively. Group 2 averages at Days 0, 14, and 28 were 25.9 ± 2.9%, 30.1 ± 6.7%, and 38.2 ± 7.1%, respectively. Group average fat-fractions and daily weights are shown in Figure 3. Metformin inhibited the progression of hepatic steatosis; group average fat-fractions dropped 9.6% compared to Day 0 for Group 1, and increased 50.5% for Group 2.

Finally, repeated exams demonstrated good reproducibility of 5.3% (percentage error, not fat-fraction error), indicating that changes greater than 5.3% likely represent meaningful changes in hepatic fat-fraction.

Discussion and Conclusion: MRI can be used to quantify hepatic steatosis for non-invasive treatment monitoring with insulin sensitizing agents such as metformin. Final imaging fat-fractions agreed with histological grading at the conclusion of the experiment. In addition, we also demonstrated good reproducibility (5.3% percentage error), which was a critical feature in determining whether fat-fractions displayed a meaningful longitudinal change. These results demonstrate the ability of MRI to monitor treatment of hepatic steatosis during the course of pharmacological intervention. Future work will investigate the ability of MRI to monitor treatment intervention in patients.

References: [1]Sass, *et al.* Dig Dis Sci 2005;50(1):171-180. [2]Harrison, *et al.* Clin Liver Dis 2004;8(4):861-879. [3]Marchesini, *et al.* Am J Med 1999;2007(5):450-455. [4]Nar, *et al.* Acta Diabetol, epub ahead of print. [5]Bravo, *et al.* NEJM 2001;344(7):495-500. [6]Ratziu, et al. Gastroenterology 2005;128(7):1898-1906. [7]Bergheim, *et al.* Gastroenterology 2006;316(3)1053-1061. [8]Reeder, *et al.* JMRI 2007; 25(3):644-652. [9]Reeder, *et al.* MRM 2004; 51(1):35-45. [10]Yu, et al. JMRI 2007;24(6):1153-61.[11]Yu, *et al.* MRM 2008; 60(5):1122-1134. [12] Liu, *et al.* MRM 2007; 58(2):354-64.

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