Validation of Imaging Biomarkers of Steatosis in ob/ob Mice with Multiple SPIO Injections

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Introduction: Non-alcoholic fatty liver disease is the most common chronic liver disease in the US[1], and its earliest manifestation is intracellular accumulation of fat (steatosis). Recent work has shown that MRI can detect and accurately quantify fat in the liver[2,3], so long as T_2* correction and other known confounding factors (T_1 bias[4], spectral complexity of fat[5,6], eddy currents[7], noise bias[4]) are addressed. Importantly, iron overload often occurs concomitantly in patients with steatosis, confounding the ability of MRI to accurately quantify fat[6,8]. The purpose of this work is to validate the need for T_2* correction for fat quantification, in an animal model of steatosis with increasing iron overload, using triglyceride extraction as a reference standard.

Methods: Validation of in vivo hepatic fat quantification was performed using the ob/ob mouse model, a known model of hepatic steatosis. Ob/ob mice are leptin-deficient, exhibiting hyperphagia with subsequent development of obesity, diabetes and steatosis that worsens with age. After obtaining IACUC approval, 20 mice were divided into 3 groups to create three levels of steatosis ("high", "medium", and "low" fat). The high fat group were comprised of 8 week old ob/ob mice (n = 8), the medium fat group were 4 week old ob/ob mice (n = 6), and the low fat group were 8 week old wild-type (control) mice (n = 6). Prior to imaging, mice were sedated using pentobarbital, and a homemade catheter was placed into the tail vein.

Imaging was performed at 3T (MR750, GE Healthcare) using an eight channel wrist coil. Images were acquired with an investigational version of the multi-echo 3D SPGR IDEAL sequence[9] using the following parameters: first TE/TR=2.6/41.4ms, echo spacing=1.4ms, echo train length=15 using 3 interleaved TRs, 256x256 matrix, 0.8mm coronal slices, FOV=12x7.2cm, flip=5° to minimize T_1 bias[4], BW=±100kHz, and scan time=10:13. Imaging was performed before administration of SPIO ("Time 0"), and after two subsequent single doses of contrast ("Time 1", "Time 2"). SPIOs (Feridex, Bayer Healthcare, Wayne, NJ) were administered at 0.56mg/kg in 5% dextrose over 15 minutes, followed by 30 minutes of rest for SPIO uptake.

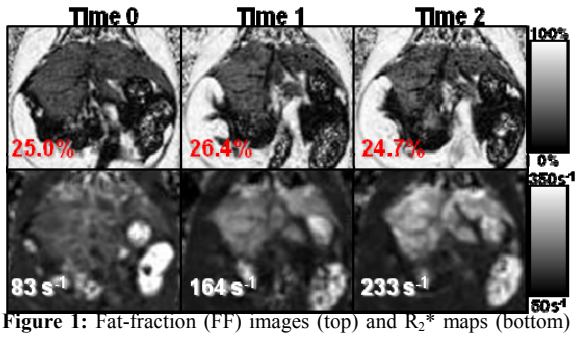

Figure 1: Fat-fraction (FF) images (top) and R_2* maps (bottom) of high fat mouse before SPIO (Time 0), after 1 (Time 1), and 2 injections (Time 2). FF remains relatively constant while R_2* values increase with increasing SPIOs. Measured FF and R_2* values are displayed above. Triglycerides were 156mg/g tissue and pathology reported 70% of cells affected by steatosis.

A modified IDEAL reconstruction with correction for T_2* decay[8], eddy currents[7] and noise bias[4], and accurate spectral modeling[5,6] was used to reconstruct fat-fraction ("FF") and R_2* maps. To demonstrate the necessity of T_2* correction, reconstructions were also performed without T_2* correction. Finally, reconstructions were also performed using the first six acquired echoes to investigate the feasibility of reducing scan time by shortening the echo train (and therefore, TR).

Following imaging, animals were sacrificed and livers were harvested. Triglyceride extraction was performed on the caudate lobe (AniLytics, Inc.) and histological grading according to Brunt et al[10] was performed on the left lateral lobe.

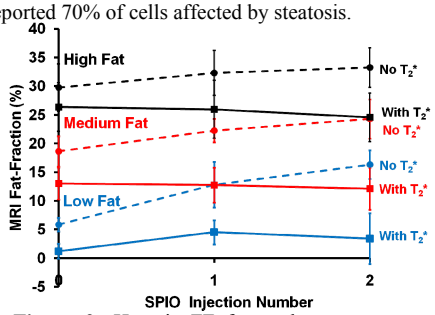

Figure 2: Hepatic FF for each mouse group with T_2* correction (solid lines) and without T_2* correction (dashed lines). FF remains relatively constant with increasing iron present when using T_2* correction, and are elevated when T_2* correction is omitted. Error bars are standard deviation

Results: Figure 1 shows example coronal fat-fraction (top) and R_2* maps (bottom) of an 8-week old ob/ob mouse prior to contrast injection, and after single and double doses of SPIO administration. R_2* values increased consistently with SPIO injection for all mice, as shown in the R_2* maps in Figure 1. No difference in the pattern of uptake was observed for mice with different gradations of steatosis. Liver triglycerides in this mouse were 156mg/g, and pathology reported 70% of cells affected by steatosis for this mouse.

Images reconstructed using 6 and 15 echoes (both with T_2* correction) showed no statistically significant differences in measured fat-fractions or R_2* values. Therefore, only data from the 6-echo reconstructions are shown.

Figure 2 plots average fat-fractions with T_2* correction (solid lines) and without T_2* correction (dashed lines) for low fat (blue), medium fat (red), and high fat (black) mice vs SPIO injection number. Without T_2* correction, apparent fat-fraction increased with increasing SPIOs, particularly for low fat mice, emphasizing the necessity of T_2* correction. As can also be seen in Figure 2, it is important to perform T_2* correction even in the absence of SPIOs, as fat-fractions without T_2* correction are elevated before SPIO injection. When T_2* correction is used, fat-fractions remain constant.

Figure 3 plots measured fat-fractions at Time 0 using 6 echoes and T_2* correction against the measured triglyceride concentrations. Excellent correlation is seen (r^2=0.94), indicating that MRI fat-fraction is highly predictive of triglyceride concentrations. As expected, histological grading of steatosis displayed excellent agreement with fat-fraction measurements. For brevity, comparisons with histological interpretation have been omitted.

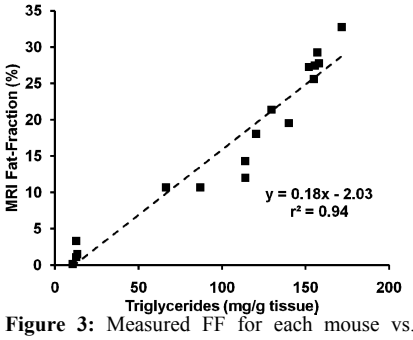

Figure 3: Measured FF for each mouse vs. amount of quantified triglycerides. Excellent correlation between MRI FF and triglycerides is seen. Dashed line is line of best fit.

Discussion: Hepatic fat-fraction measured using a T_1-independent, T_2*-corrected chemical shift-based fat-water separation with spectral modeling of fat and correction for eddy currents was validated in vivo with triglyceride extraction in the presence of increasing SPIO. These results show fat-fraction is highly predictive of liver triglyceride concentration. Results clearly demonstrate the need for T_2* correction when quantifying liver fat, even in the absence of iron overload.

References: [1] Browning Hepatology 2004 [2] Meisamy Radiology in press [3] Yokoo Radiology 2009 [4] Liu MRM 2007 [5] Yu MRM 2008 [6] Bydder MRM 2008 [7] Yu ISMRM 2009 #462 [8] Yu JMRI 2007 [9] Reeder JMRI 2007 [10] Brunt Am J Gastroenterol 1999

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