In Vivo Lipid Quantitation in Mouse Liver Using the Gradient Reversal Water-Fat Imaging Method

H. Tang[1], C. Miller[1], R. Kennan[1], E. X. Wu[2], D. S. Williams[1], and H. Liu[1]

[1]Imaging, Merck Research Laboratories, Rahway, NJ, United States, [2]Electrical & Electronic Engineering, The University of Hong Kong, Hong Kong SAR, China, People's Republic of

Introduction

Gradient reversal technique for chemical shift selective imaging was proposed by Park et al in 1987 [1] and was applied to water-fat imaging by utilizing the water-fat chemical shift in the slice direction. In the same year, Volk et al proposed a gradient reversal based chemical-shift-specific slice-selection technique [2] which allows imaging of separated water and fat during a single acquisition by interleaving water and fat excitations at the same locations. Because MR imaging methods for effective and accurate water-fat quantitation are scarce, a multi-echo gradient reversal water-fat imaging [3] allowing simultaneous acquisition of water and fat images is implemented and validated at 11.7T for *in vivo* lipid quantitation in mouse liver. Compared to conventional water-fat imaging, the proposed imaging method provided qualitative as well as quantitative spatially registered fat and water images with high imaging efficiency. Image quantitation was verified with water/lipid emulsion phantoms. Despite yielding limited spatial information, proton MR spectroscopy has been widely used for characterization of fatty tissue. The *in vivo* MRS study of liver lipid in mouse has been reported in [4]. To evaluate the MRI liver lipid measurement, a single voxel MRS was implemented and applied to the same mice studied, and liver lipid quantitation from both methods were compared with the *ex vivo* liver triglyceride (TG) levels analyzed by conventional wet-chemistry method.

Methods

Gradient reversal water-fat imaging During slice selection, water and fat slices could be completely offset when $\Delta\omega_{rf} < \Delta\omega_{off}$, where $\Delta\omega_{rf}$ is the bandwidth of selective RF excitation or refocusing pulses, and $\Delta\omega_{off}$ is the water-fat offset frequency. The chemical shift imaging using a spin echo sequence with reversal of slice gradient during the refocusing pulses allows pure water and fat images from the same slice location to be obtained in a single acquisition without increasing scan time [2, 3].

Animals Animal procedures were approved by the Institutional Animal Care and Use Committee. Mice from several mouse models of diabetes/obesity (db/db, db/+, db/+ on high fructose diet, NONcNZO on high fat diet) were used in the study (n=3/group), which cover a wide range of liver lipid content. Mice were euthanized and the portion of the liver was excised and immediately frozen in liquid nitrogen for chemical analysis of triglycerides following MR imaging and spectroscopy.

MR imaging experiments Imaging experiments were performed on a Bruker Biospin 500WB (11.7T) spectrometer (Bruker NMR, Inc., Billerica, MA) with an 89 mm vertical bore and a shielded gradient system up to 150 G/cm. For quantitative water-fat imaging, a temporally interleaved water and fat selective multi-echo spin echo gradient reversal sequence was implemented. Gaussian-shaped RF pulses were selected for both excitation and refocusing because they are short and produce slice profiles that contain no lasting ripples. The duration of RF pulses were chosen such that the bandwidths of the slice excitations were matched and the slice profile was approximately 1750Hz (water-fat shift 3.5ppm at 11.7T) full-width at 20% of maximum. This is necessary to reduce the fat contamination during water selection and vice versa. A multi-echo train was used to generate proton density images for more accurate quantitation, and the lipid percentage was estimated according to [5].

To demonstrate the quantitative aspects of the simultaneous water-fat imaging, a phantom study was conducted. The phantom was based on a water/lipid emulsion (mayonnaise) which contained about 75% fat, and was diluted with water from 20% to 100% mayonnaise to vary lipid concentration. Separated water and fat selective images at identical slice location were acquired in a single acquisition with TR/TE = 10s/4.5 ms, number of echoes = 8, and slice thickness = 1.2 mm.

During *in vivo* liver imaging, mice were anesthetized with 1.5~2.5% isoflurane/O_2 gas mixture (respiratory rate ~30-50 breaths/min), and positioned in a 30mm ID RF coil. Water and fat selective images at a single slice location were acquired using respiratory-gating (per slice triggering), with TR~4s, TE = 4.5 ms, matrix = 128 × 128, number of echoes = 8, NEX = 1, and slice thickness = 1 mm. Spoilers at both sides of the reversal gradient were optimized to minimize the flow artifacts in liver.

MR spectroscopy experiments For *in vivo* MRS of mouse liver, localized proton spectra from the same MR imaging slice were acquired using the PRESS (Voxel size = $(2.5\text{mm})^3$, TR~4s, TE=20ms, and NEX=16). Manual shimming was performed resulting in linewidths of 50-70 Hz (~1.2 ppm). Single voxel was selected because initial experiments showed lipid levels vary little with voxel position within the liver. T2 values were estimated for the water (11ms) and fat (25ms) peaks by varying TE.

Results

Figure 1 shows the fat (a) and water (b) images of the water/lipid emulsion phantom. As expected, the signal intensity varied with the lipid concentration. In the fat image (a), no water contamination was found in the location of center tube containing only water. Fig. 1c is the summation of the fat and water free of the chemical shift artifacts. In comparison, Fig. 1d shows the image acquired using the conventional spin echo sequence, with the in-plane and through-plane chemical shift artifacts (blurring) clearly seen at the edges. The MRI lipid% correlated linearly with the water/lipid emulsion concentrations (Fig 1e). Figure 2 shows the water (a) and fat (b) proton density images of a db/db mouse, Fig. 3c is the lipid% map, and the color coded lipid% in liver is shown in Fig. 3d. The MRI lipid% in liver was in a range of 2-25% in the preliminary study that contains 12 mice, and the MRI measured lipid% highly correlated with the MRS estimations (r=0.98, see Fig. 2e).

Conclusions and discussions

The gradient reversal water-fat imaging was implemented at 11.7T to assess quantitative lipid distribution in mouse liver *in vivo*. Image quantitation was verified with phantom study. *In vivo* MRI liver lipid% measurements were compared with MRS estimations and are being validated by biochemical triglyceride analysis. More mice will be included to validate the MRI lipid measurement. The high selectivity, efficiency, and quantitative nature indicates that the proposed MRI method is applicable to longitudinal studies of lipid modifying therapies, and allows sufficient throughput for evaluation of the effects of diet and/or pharmaceutical agents.

Reference

1. Park HW, Kim DJ, Cho ZH. Magn Reson Med 1987; 4:526-536. 2. Volk A, Tiffon B, et al. J Magn Reson 1987; 71:168-174. 3. Tang H, Wu EX, et al. Proc. ISMRM 14, 2006. 4. Garbow JR, Lin X, et al. J Lipid Research 2004; 45:1365-71. 5. Poon CS, Szumowski J, et al. Magn Reson Imaging 1989;7:369–382.

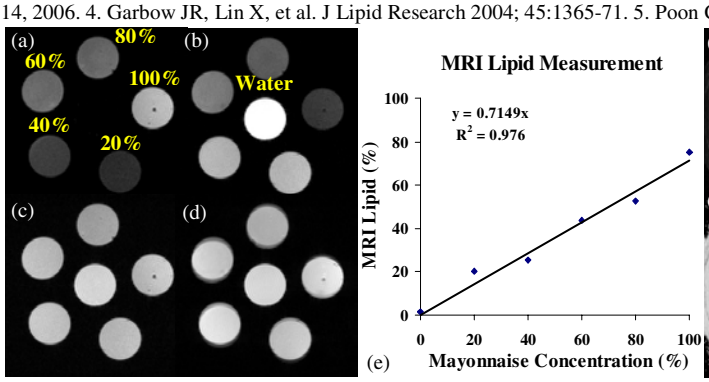

Fig. 1. Water and fat phantom images acquired using the gradient reversal water-fat imaging sequence. (a) fat image; (b) water image; (c) summation of fat and water; (d) conventional spin echo image; and (e) comparison of MRI lipid % and mayonnaise concentrations in the water/lipid emulsion phantoms.

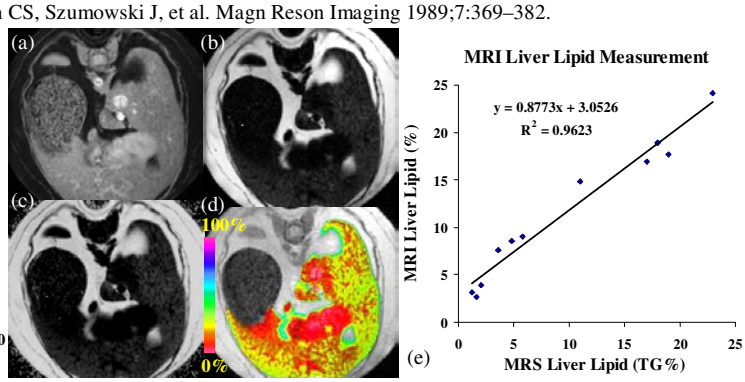

Fig. 2. *In vivo* water and fat images and lipid quantitation in mouse liver using the gradient reversal water-fat imaging sequence. (a) water proton density image; (b) fat proton density image; (c) lipid percentage map; and (d) color coded MRI lipid% in liver of a db/db mouse; and (e) comparison of MRI and MRS lipid% measurements.