Interleaved Water and Fat Imaging and Applications to Lipid Quantitation Using the Gradient Reversal Technique

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Purpose: To implement and evaluate the gradient reversal-based chemical shift imaging technique to obtain qualitative and quantitative spatially-registered fat and water images with high imaging efficiency at very high field.

Materials and Methods: A multiecho gradient reversal-based sequence allowing interleaved water–fat imaging during a single acquisition and quantitation of fat/water content is presented. The sequence was optimized and implemented at 11.7T. The quantitation was verified with water–fat phantoms and applied to lipid measurement in an in vivo mouse model.

Results: Results from phantoms, in vivo lipid measurement in mouse liver and hind limb muscle, and ex vivo rat knee imaging experiments demonstrated the robustness and high selectivity of this technique for interleaved and quantitative water and fat imaging at very high field.

Conclusion: The proposed MRI technique permits interleaved water and fat imaging, with which spectrally well-separated water and fat images at the identical slice locations could be obtained in a single acquisition without increasing scan time. The technique could be used for in vivo quantitative mapping of lipid content and applied to investigations using small animal experiment models.

Key Words: MRI; water and fat imaging; chemical-shift; gradient reversal; liver; muscle


MR IMAGING of water and fat is important in a wide range of biomedical studies including obesity and diabetes research, cartilage delineation in joint imaging, body composition measurement, and evaluation of effect of drugs on lipids. A rapidly evolving and extremely useful area is evaluation of lipid content in genetically altered mice. At very high field, the commonly used spectral selection or saturation techniques are often hampered by strong susceptibility effects at tissue–air interfaces, as well as other sources of field inhomogeneity (1,2). The spatial-spectral selective excitation techniques (3,4) are limited in practice by their requirement for high gradient amplitude and switching rate during the short radio frequency (RF) pulse train. The alternative phase sensitive techniques such as the Dixon method (5–7) may not be desirable for applications at very high field because the chemical shift during slice-selective excitation causes a misregistration of water and fat slices. Furthermore, phase sensitive methods require multiple data acquisitions for separation of water and fat signal. The recent three-point Dixon (6)-based iterative decomposition of water and fat with echo asymmetry and least-squares estimation (IDEAL) technique (8,9) maximized the noise performance of the water–fat decomposition and thus improved the scan efficiency by combining the acquisition of asymmetric echoes with respect to the spin echo (SE) and an iterative least-squares separation algorithm. However the implementation of IDEAL at very high field and the related data acquisition issues require further investigation.

In 1987, Park et al (10) proposed a gradient reversal technique for single-slice two-dimensional (2D) or single-slab 3D chemical shift–selective imaging. It was applied to obtain water and fat images by utilizing the fat–water chemical shift separation in the slice direction. This technique is applicable to spin echo 2D or 3D sequence that involves both excitation and refocusing slice-selective RF pulses. Because a single spectral resonance is selected in an imaging scan, two acquisitions are required to obtain both water and fat images. In the same year, Volk et al (11) proposed the chemical shift–specific slice-selection technique and demonstrated the use of the gradient reversal technique for interleaved water and fat imaging during a single acquisition by interleaving water and fat excitations at the same locations. Because such an approach relies on the strong chemical shift effect along the slice direction for com-
plete separation of water and fat, the method is best suited for high field imaging in which larger chemical shifts enable the use of slice-selective RF pulses of reasonable durations.

Since 1987, no further studies using the gradient reversal technique for fat/water imaging have been reported. On the other hand, besides proton MR spectroscopy-based methods that provide limited spatial information (12), MR imaging methods that allowed accurate fat–water quantitation have been scarce. Imaging lipid content remains an area of high priority for the pharmaceutical industry in diabetes and obesity research as well as in safety assessment of drugs (7). The technical issues of existing techniques include susceptibility gradients, effective spectral separation of fat and water, imaging the same slice location for fat and water, and imaging efficiency in terms of number of acquisitions and scan time required. Given the increasing availability of high-field scanners, and the need for small animal imaging, the gradient reversal–based chemical shift imaging techniques (10,11) were reevaluated in this study with a view toward obtaining quantitative, spatially-registered fat and water images with high imaging efficiency. An interleaved water- and fat-selective imaging sequence based on the gradient reversal technique designed for qualitative and quantitative water and fat imaging purposes was implemented at 11.7 T to assess the robustness of this approach for in vivo imaging at very high field. The proposed imaging sequence is based on spin echo and is capable of multiecho and multislice acquisitions, allowing well-separated water and fat images at the identical slice locations to be acquired in a single acquisition without increasing acquisition time. The multiecho train is used to generate true proton density (PD) images for better quantitation of fat and water. Image quantitation was verified with a water/fat phantom, and its application to in vivo high-field small animal imaging was demonstrated in liver and in hind limb muscle–fat quantitation, in obese vs. lean mice. Finally, ex vivo high-field musculoskeletal imaging was demonstrated with a formalin-fixed rat knee sample.

**MATERIALS AND METHODS**

**Gradient Reversal Technique for Water and Fat Imaging Within a Single Acquisition**

In conventional proton MR imaging, the chemical-shift effect of water and fat occurs not only in the frequency-encoding direction, but also in the slice-selection direction. Provided the slice selection profile is approximately rectangular, water and fat slices could be completely separated in the slice direction when $\Delta \omega_f \leq \Delta \omega_{off}$, where $\Delta \omega_f$ is the bandwidth of slice-selective RF excitation or refocusing pulse, and $\Delta \omega_{off}$ is the frequency offset between water and fat resonances. Under such circumstances, the gradient reversal method (10,11), a spin echo sequence with reversal of slice gradient polarity during the refocusing pulse, allows pure water and fat images from the same slice location to be obtained as long as a slice at the center of the gradients is selected. If the RF pulses are applied on water resonance, only the water slice will be excited and refocused to produce a spin echo because it experiences both 90° and 180° pulses. Repeating this with RF frequency on fat resonance will allow fat from the same slice location to be excited and refocused. Since fat (or water) spins in the slice are not excited when RF offset is set on water (or fat), the scheme can easily be implemented in a multislice acquisition mode to obtain water and fat images in the same scan without increasing acquisition time. Extending the scheme to multislice acquisition requires the reversal of frequency offset for the refocusing pulse and also places restrictions on slice gap.

To help illustrate this, a schematic of the location of excited slices for a two-slice imaging protocol using the gradient reversal sequence is shown in Fig. 1 when RF applied is on resonance with water (Fig. 1a), and with fat (Fig. 1b) of the same slice location. Diagonal lines show the variation of resonances for water (thick line, W) and fat (thin line, F) for positive (solid line) and negative (dashed line) polarity gradients. Note that an echo will be formed from a slice location only if both 90° and 180° pulses excite the same slice location. W1 and W2 in (Fig. 1a), and F1 and F2 in (Fig. 1b) are excited water and fat slices with a solid-line frame, representing slices that experience both 90° and 180° pulses; while F1’ and F2’ in (Fig. 1a) and W1’ and W2’ in (Fig. 1b) represent slices that experience only the 90° (solid-line frame) or the 180° (dotted-line frame) pulses. With the scheme, water and fat slice excitations can be interleaved within a TR period to image both water (Fig. 1a) and fat (Fig. 1b) species at the identical slice locations during a single acquisition without increasing overall scan time. However, as seen on Fig. 1, the multislice acquisition requires the slice interval (center-to-
center) frequency to be greater than \(\Delta w_{rf} + \Delta w_{pp}\) to avoid interference between adjacent slices. Finally, if during signal detection, the frequency is set to the Larmor frequency of the species excited, the fat images will no longer be shifted along the readout direction, and water and fat images will be spatially registered with one another.

This technique is generally applicable to the 2D and 3D sequences that involve at least two slice-selective RF pulses, such as spin echo–related sequences. It is especially suited for high-field applications. At low field, the requirement of \(\Delta w_{rf} \leq \Delta w_{pp}\) leads to unacceptably long RF pulse duration and restricts the practical applications.

**Quantitative Water and Fat Imaging Sequence Development**

For all phantom and in vivo experiments, optimized Gaussian-shaped RF pulses were used for both excitation and refocusing because they are short and produce slice profiles that contain no lasting ripples. The durations of the 90° and 180° RF pulses were chosen such that the slice excitation bandwidth of the pulses were matched, and the spin echo slice profile bandwidth was approximately 1750 Hz full-width at 20% of maximum, corresponding to the water–fat chemical shift (3.5 ppm) at 11.7T. This is necessary to reduce the fat contamination during water selection and vice versa. Effective bandwidth for the given RF pulse shape and durations was experimentally verified by examining the actual slice profile obtained by Fourier transform of the echo formed by slice gradient reversal.

For quantitative water/fat imaging, a temporally interleaved water- and fat-selective sequence capable of multiecho and multislice acquisition was implemented using the gradient reversal technique illustrated in Fig. 1. In a single acquisition, the sequence provided a series of multiecho water- and fat-selective images. Multiecho data were then fitted to monoeXponential decay to generate fat and water PD images for accessing lipid quantitation and distribution. Fat/water content was calculated according to Ref. 13.

**Imaging Experiments**

All imaging experiments were performed on a Bruker Biospin 500WB spectrometer (Bruker Biospin Corporation, Billerica, MA, USA) with an 89-mm 11.7T vertical bore magnet (Oxford Instruments Ltd., UK) and a shielded gradient system capable of up to 100 G/cm gradients.

In the first phantom study to demonstrate the effectiveness of fat and water separation, a tube of 29 mm inner diameter (ID) was filled with olive oil, and contained two smaller tubes of water doped with 1:100 dilution gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA) (Magnevist). The phantom was imaged using a 30-mm ID RF coil. Interleaved fat and water images were obtained during the same scan using the proposed chemical shift–selective and gradient reversal–based sequence, with slice thickness = 2 mm, and TR/TE = 1000 msec/4.5 msec. To demonstrate the quantitative aspects of the interleaved water/fat imaging, another phantom study was conducted, in which water and fat concentrations were varied at a subvoxel scale. The phantom was based on a water/lipid emulsion (mayonnaise) that contained approximately 75% fat (14), and was diluted with water from 20% to 100% mayonnaise to vary lipid concentration. Separated water- and fat-selective images of single slices were acquired with TR/TE = 10 seconds/4.5 msec, number of echoes = 8, and slice thickness = 1.2 mm.

To demonstrate quantitative water and fat imaging in vivo, the interleaved water- and fat-selective multiecho and multislice imaging sequence was applied to a mouse. For in vivo lipid quantitation, water and fat proton densities were obtained by fitting the multiecho images pixel by pixel to a monoeXponential, from which relative lipid percentage maps were generated. Lipid measurements in mouse liver and hind limb muscle were performed in wild-type lean mice and obese mice that were on a high fat diet for five months. All in vivo experiments were approved by the Institutional Animal Care and Use Committee. Mice were anesthetized with 1.5% isoflurane/O2 gas mixture delivered through a nose cone during imaging.

For in vivo liver imaging, a 30-mm ID RF coil was used. A total of six water and fat images at three slice locations were acquired with respiratory gating (per slice triggering) in a single acquisition, resulting in a TR = 6 seconds. Strengths of the spoilers at both sides of the reversal gradient were reduced, and flow saturation in the descending aorta was applied to minimize the flow artifacts. Other parameters were TE = 4.5 msec, number of echoes = 8, field of view (FOV) = 30 mm, acquisition matrix = 168 \(\times\) 168, slice thickness = 1.5 mm, slices spacing = 3.2 mm, and number of averages = 2. The total acquisition time was approximately 40 minutes.

Unlike the mouse liver, in which a wide range of lipid levels could be detected, the in vivo MRI lipid detection and quantification in skeletal muscle is challenging because of the low lipid content and heterogeneous spatial distribution. To increase sensitivity, muscle lipid studies were carried out with a 10-mm ID RF coil. High-resolution water and fat images at a single slice location were acquired from the left hind limbs, with TR/TE = 2000 msec/4.5 msec, number of echoes = 8, FOV = 12.8 mm, acquisition matrix size = 128 \(\times\) 128, slice thickness = 1 mm, and number of averages = 32. The acquisition time was 137 minutes.

As a further demonstration of this technique to high-field musculoskeletal imaging, water and fat imaging of an ex vivo formalin-fixed rat knee was also performed. Water and fat images were acquired at three slice locations, with TR/TE = 2000 msec/5.3 msec, FOV = 16.5 mm, acquisition matrix size = 256 \(\times\) 256, slice thickness = 0.6 mm, slices spacing = 1.3 mm, and number of averages = 8. The acquisition time was 68 minutes.

**RESULTS**

Figure 2 shows fat (Fig. 2a) and water (Fig. 2b) images of the first phantom acquired at the same slice location in a single acquisition using the gradient reversal tech-
The chemical shift effect along the frequency encoding direction was eliminated by setting the resonance frequency on either water or fat during acquisition. Figure 2c is the chemical shift artifact–free image obtained by the summation of Fig. 2a and b. Figure 2d is the conventional spin echo image, containing both in-plane and through-plane chemical shift artifacts. In comparison to the conventional spin echo image, a slight signal inhomogeneity is noted at the edge of the coil in Fig. 2c, indicating that the gradient reversal technique is sensitive to B₀ field inhomogeneity, thus requiring good shimming over the imaging region. The image in Fig. 2e was acquired with the same shimming, using the conventional spin echo sequence with presaturation of fat. In comparison to the conventional spin echo image, a slight signal inhomogeneity is noted at the edge of the coil in Fig. 2c, indicating that the gradient reversal technique is sensitive to B₀ field inhomogeneity, thus requiring good shimming over the imaging region. The image in Fig. 2e was acquired with the same shimming, using the conventional spin echo sequence with presaturation of fat. It is seen that poor fat suppression is achieved because the spectral saturation technique is sensitive to both B₀ and B₁ field inhomogeneities. Moreover, spectral saturation or selection techniques do not lend themselves to interleaved water and fat imaging in a single acquisition. Finally, it should be noted that a small signal in the oil region in Fig. 2b and 2e was caused by a small fraction of olefinic fatty acids having resonance frequency very close to that of water, thus it could not be suppressed (15) with any frequency-sensitive imaging technique including the gradient reversal–based water and fat imaging technique.

Figure 3 shows the fat (Fig. 3a) and water (Fig. 3b) images (PD weighted, echo train spacing = 4.5 msec) of the water/lipid emulsion phantom acquired using the proposed interleaved and quantitative water and fat imaging sequence. As expected, the signal intensity varied with the lipid concentration. It should be noted that the center tube of the phantom contains only water, and there is no water contamination found in the center tube location in the fat image. Figure 3c is the chemical shift artifact–free image obtained by summation of the fat and water images. In comparison, Figure 3d shows the phantom image acquired using the conventional spin echo sequence, in which the in-plane and through-plane chemical shift artifacts (blurring) were clearly seen at the edges. Figure 3e is the lipid percentage map generated from the PD images of water and fat calculated by extrapolating the multiecho images. Figure 3f shows that the MRI lipid content measurement correlated linearly with the water/lipid emulsion concentrations.

Figure 4 demonstrates the in vivo water (Fig. 4a) and fat (Fig. 4b) transaxial images (PD weighted, echo train spacing = 4.5 msec) in the liver region of an obese mouse acquired in an interleaved acquisition. Figure 4c shows the chemical shift artifact–free water and fat image combined. In Fig. 4d, a lipid percentage map was generated from the PD images of water and fat. With the multiecho imaging capability, the transverse relaxation rate (R2) maps of the water and fat could also be obtained separately and simultaneously in a single acquisition. Figure 4e and f are the calculated R2 maps of water and fat at the identical slice location, and the R2 appears much larger for the water component (54 ± 4.51/second) than the lipid component (7.5 ± 2.51/second) in liver. The increase relaxation rate of liver water is likely due to the ferritin that is mainly existed in the water component. To better visualize the spatial distribution of lipid concentration in mouse liver, the lipid percentage was color-coded in liver and the surrounding tissue. Figure 4g shows a color-coded liver lipid percentage map of the obese mouse. For comparison, a color-coded liver lipid percentage map of the lean mouse is shown in Fig. 4h. About 30% lipid was de-
tected in the obese liver, compared to about 8% lipid in the lean liver. These images demonstrate that the gradient reversal–based water and fat imaging method can provide high-resolution lipid distribution in mouse liver.

Figure 5 shows the high-resolution cross-sectional water and fat images and the lipid percentage map in hind limb muscle of the obese and lean mice. Figure 5a and c are water images of the obese and lean mice, respectively; Fig. 5b and d are fat images. Several regions of interest marked in Fig. 5a are the tibialis anterior (TA); soleus (S); and medial head of the gastrocnemius (MHG). The color-coded lipid percentage maps in the muscle of the left hind limbs are shown in Fig. 5e.

**Figure 4.** In vivo water and fat images (PD weighted, echo train spacing = 4.5 msec) and lipid quantification in mouse liver. a: Water image of an obese mouse; (b) fat image of the obese mouse; (e) fat plus water image; (d) lipid percentage map; (e) R2 map of water; (f) R2 map of fat; (g) color-coded liver lipid percentage map of the obese mouse; and (h) color-coded liver lipid percentage map of the lean mouse.

**Figure 5.** In vivo water and fat images (PD weighted, echo train spacing = 4.5 msec) and lipid quantification in mouse hind limb muscle. a: Water image of an obese mouse, with regions of interest marked: TA, tibialis anterior; S, soleus; MHG, medial head of the gastrocnemius; (b) fat image of the obese mouse; (c) water image of a lean mouse; (d) fat image of the lean mouse; (e) color-coded lipid percentage map in left hind limb muscle of the obese mouse; (f) color-coded lipid percentage map in left hind limb muscle of the lean mouse; and (g) normalized histogram of lipid percentage in MHG. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
An Interweaved Water and Fat Imaging Technique

This technique, does not demand a high gradient amplitude or spatial-spectral–selective RF pulses. Its implementation achieved at 11.7T in several applications requiring separation of water and fat, and chemical shift artifact–free water and fat imaging could be highly effective separation of water and fat, and chemical shift–selective imaging was evaluated for applications at very high field. An interleaved water and fat imaging sequence with in-plane chemical-shift correction was successfully implemented at 11.7T, by which complete separation of water and fat was achieved in a single acquisition without increasing acquisition time. The ability for quantitative lipid measurements was verified in phantoms and demonstrated in mouse liver and hind limb muscle. Compared to the phase-related method such as the Dixon method, the gradient reversal–based technique can image both water and fat at the identical slice locations and does not require multiple acquisitions. At very high field, this water and fat imaging approach is simple to implement, robust, and quantitative. It is applicable to the study of lipid-modifying therapies in animal models as well as lipid quantification in humans.

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REFERENCES