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Abstract. Quantitative fat-water MRI (FWMRI) methods provide valuable information about the distribution, volume, and composition of adipose tissue (AT). Ultra high field FWMRI of animal models may have the potential to provide insights into the progression of obesity and its comorbidities. Here, we present quantitative FWMRI with all known confounder corrections on a 15.2T preclinical scanner for noninvasive *in vivo* monitoring of an established diet-induced obesity mouse model. Male C57BL/6J mice were placed on a low-fat (LFD) or a high-fat diet (HFD). Three-dimensional (3-D) multiple gradient echo MRI at 15.2T was performed at baseline, 4, 8, 12, and 16 weeks after diet onset. A 3-D fat-water separation algorithm and additional processing were used to generate proton-density fat fraction (PDFF), local magnetic field offset, and R_2^* maps. We examined these parameters in perirenal AT ROIs from LFD and HFD mice. The data suggest that PDFF, local field offset, and R_2^* have different time course behaviors between LFD and HFD mice over 16 weeks. This work suggests FWMRI at 15.2T may be a useful tool for longitudinal studies of adiposity due to the advantages of ultra high field although further investigation is needed to understand the observed time course behavior. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JMI.3.2.026002]

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1 Introduction

A worldwide rise in the prevalence of obesity has led to higher risk of morbidities such as type 2 diabetes, cardiovascular disease, and other chronic illnesses.^{1,2} As onset of type 2 diabetes is correlated with obesity,³ the ability to quantitatively track changes in adipose tissue (AT) deposition can enhance our understanding of its etiology.^{4–9} *In vivo* human^{4–9} and animal^{10–12} volumetric imaging of AT depots is an active area of research. Animal models are valuable for longitudinal studies about adiposity changes caused by dietary and pharmacological interventions that would be difficult to perform in a human cohort.^{13–15}

Quantitative fat-water MRI (FWMRI) is unique among the available body composition imaging methods because it provides true three-dimensional (3-D) volumetric images. Because FWMRI uses nonionizing radiation, it is well suited for juvenile and adult population studies to avoid radiation exposure concerns. FWMRI has in recent years made significant advances in the quantification of spatial distribution, volume, and composition of AT in human and animal studies.^{11,12} Advanced quantitative FWMRI methods, based on deriving a proton-density fat fraction (PDFF) parametric map, can robustly quantify AT volume and distribution, fat content in tissues such as liver and muscle as well as identifying brown AT.^{16–19}

Animal studies are helpful as animal tissues can easily be harvested for rigorous testing and validation of imaging findings that may not be possible to perform in humans. Due to the

smaller size of animals in comparison to humans, preclinical MRI requires higher spatial resolution, which generally necessitates a move to higher magnetic fields.^{20,21} Aside from higher spatial resolution, other consequences of moving to higher magnetic fields that may be advantageous for FWMRI include increased chemical shift in Hz between component spectral peaks of different species such as water and fat, higher signal-to-noise ratio, and unique or improved image contrast such as those derived from magnetic susceptibility differences.²² As of the date of writing, FWMRI has not been widely implemented on the highest magnetic fields available for preclinical studies.

In this work, we present quantitative FWMRI with all confounder-factor corrections on a 15.2T preclinical scanner for noninvasive *in vivo* monitoring of mice over time. Using an established diet-induced obesity (DIO) mouse model,²³ we follow PDFF, field offset, and R_2^* parametric maps longitudinally in the same mice and explore the time course behavior of these parameters. In the preclinical setting, ultra high field FWMRI may have the potential to provide new insights into the progression of obesity and its contributors to comorbidities.

2 Methods

2.1 Mice and Diet

Animal care and experimental procedures were performed in accordance with and approval by the Vanderbilt University Institutional Animal Care and Use Committee. Following an

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established DIO mouse model protocol,²³ male C57BL/6J wild-type were bred in-house from mice originally purchased from the Jackson Laboratory (Bar Harbor, Maine) and maintained on a normal chow diet until 8 weeks of age. At 8 weeks of age, three mice were placed on a low-fat diet (LFD) (10% kcal from fat, cat. no. D12450B; Research Diets, Inc., New Brunswick, New Jersey) and three mice were placed on a high-fat diet (HFD) (60% kcal from fat, cat. no. D12492; Research Diets, Inc., New Brunswick, New Jersey) for a total of 16 weeks. The LFD and HFD both contain 10 g mineral mix (cat. no. S10026; Research Diets) per 4000 kcal digestible energy, with each 10 g mineral mix providing 37 mg iron. Mice had free access to food and water throughout the study, thus caloric intake was not measured or controlled.

2.2 MRI Experiments

MRI was performed on a 15.2T Bruker Biospec horizontal bore scanner (Karlsruhe, Germany) using a 35-mm diameter volume RF coil. During imaging, mice were anesthetized with isoflurane (Sigma-Aldrich, St. Louis), were kept warm using a circulating water bath, and both temperature and respiration were monitored. The mice were placed in a prone position and the kidneys were roughly centered within the RF coil. The center of the RF coil was positioned at isocenter. Imaging was performed at baseline, 4, 8, 12, and 16 weeks after LFD or HFD onset. A 3-D multiple gradient echo image with slab excitation was acquired on each mouse using the following parameters: $128 \times 128 \times 32$ acquisition matrix, $FOV = 32 \times 32 \times 48 \text{ mm}^3$, number of acquisitions = 2, excitation flip angle = 5 deg, echoes = 12, flyback gradients activated (for unipolar echoes), $TE/\Delta TE = 1.2/0.78 \text{ ms}$, sampling bandwidth (BW) = 500 kHz, and repetition time (TR) = 50 ms. TE stands for the echo time for the first echo and ΔTE represents the interecho spacing. With the given $TE/\Delta TE$ parameters, the echo times ranged from 1.2 to 9.78 ms. Respiratory gating was used and the isoflurane concentration was titrated to maintain a breath rate of 30 to 40 breaths/min. The resulting total scan time was 27 min. A long TR and small excitation flip angle were specifically chosen to reduce T_1 bias.

2.3 Image Processing

Quantitative FWMRI, as implemented in this work, relies on confounder-factor correction by simultaneous estimation of PDFF, the apparent transverse relaxation rate R_2^* , and magnetic field offset (ΔB_0) and uses the following signal model:²⁴

$$S(TE) = \left(\rho_W + \rho_F \sum_{m=1}^M \alpha_m e^{j2\pi f_{F,m} TE} \right) e^{-R_2^* TE} e^{i2\pi \Delta B_0 TE}, \quad (1)$$

where $S(TE)$ is the total signal at echo time TE, ρ_W and ρ_F are the water and fat signal amplitudes, respectively, and $\sum_{m=1}^M \alpha_m e^{j2\pi f_{F,m} TE}$ is the fat signal model consisting of multiple spectral peaks with relative amplitudes α_m (normalized such that $\sum_{m=1}^M \alpha_m = 1$), and frequencies $f_{F,m}$. Note that this model assumes a single R_2^* value for both water and fat. While water and fat will generally have different R_2^* constants and even the different lipid peaks will have different relaxation rates, modeling with a single R_2^* value improves the robustness of the fit.^{24,25} Therefore, the fitted R_2^* is not the true value for either water or fat. However, AT has a high PDFF (~90%) and a single-component R_2^* model may be an acceptable

approximation as the lipid spectrum is dominated by the single methylene peak as suggested by simulations.²⁶

3-D fat-water separation with the above signal model was performed using a recently proposed algorithm based on whole-image optimization.^{16,27} Only echoes 2 to 12 were used for fat-water separation to minimize possible eddy current effects that primarily confound the phase of the first acquired echo. Whole-image optimization was used to resolve signal ambiguity at the voxel level, but is generally computationally expensive. Computational demand was reduced by exploiting the periodicity of the magnetic field offset as described elsewhere.^{16,27} Here, a nine peak model (chemical shift and amplitude) of the lipid signal based on *in vivo* human liver measurements was employed.²⁸ The algorithm computed a complex water image, a complex fat image, a single component R_2^* map, and a magnetic field offset map. Fat-water separation is an important step in generating the magnetic field offset map as it corrects the field offset map for the presence of fat.²⁹ An PDFF map was computed from the water and fat magnitude images using the equation $PDFF = [|\text{fat}|/(|\text{water}| + |\text{fat}|)]$.

While PDFF is defined computationally as the signal fat-fraction, all confounding factors associated with FWMRI (T_1 bias, eddy currents, R_2^* , and magnetic field offset) have been mitigated in this work. Signal fat-fraction is, therefore, equivalent to PDFF, which is defined as the ratio of density of mobile protons from fat (triglycerides) and the total density of protons from mobile triglycerides and mobile water.³⁰ PDFF reflects the concentration of fat within a tissue.

The magnetic field offset map included effects from both background field inhomogeneities and local tissue field properties. Only the local tissue field may contain information on changes in AT iron levels, so the background field must be removed. While there are several different approaches to this step,^{31–33} polynomial fitting,^{34–37} which assumes that the background field was smooth and slowly varied across space, was chosen for its simplicity. The background field was estimated by fitting the magnetic field offset map with a fourth-order polynomial fit. The estimated background field was then subtracted from the magnetic field offset map to produce a local magnetic field offset map.

In this analysis, we focused on perirenal AT as a representative sample of visceral AT. It is known that visceral AT is an important marker of metabolic health.^{7,8} Manual ROIs were first located on the magnitude images by identifying AT dorsal and lateral to the kidneys. Voxels with a value greater than 80% on the PDFF maps were identified, and their corresponding PDFF, local magnetic field offset, and R_2^* values were recorded. A PDFF threshold of 80% was chosen after a preliminary inspection indicated that the majority of the PDFF values were in the range of 80% to 100%. The AT local field offset was normalized by subtraction with average local field offset values from ROIs in the kidney and erector spinae muscles. ROIs in the kidney and muscles were identified on PDFF maps (PDFF < 12%) and a local field offset value averaged across both kidney and muscles was recorded. This value was then subtracted from the average perirenal AT ROI values.

The time course behavior of the ROI PDFF, R_2^* , and local field offset parameters were normalized by subtracting the individual ROI baseline values. Therefore, week 0 parameter values are zero for all ROIs. This normalization was performed to emphasize the changes in parameter values over time, which was the focus of the work here.

Fig. 1 Representative magnitude images and PDFF maps of LFD and HFD mice at 4, 8, 12, and 16 weeks after diet. PDFF values range from 0% to 100% (see color bar). Kidneys are labeled in magnitude images with “k” and perirenal AT is marked with arrows on PDFF map. Dashed lines approximate outlines for perirenal ROIs. Average and standard deviation PDFF, averaged over left and right perirenal ROIs, are shown.

Fig. 2 Representative masked PDFF, R_2^* , and magnetic field offset (ΔB_0) maps of an HFD mouse at 16 weeks after diet. The background field was removed from the magnetic field offset map with a fourth-order polynomial fit to produce a local magnetic field offset map.

2.4 Statistical Analysis

All statistical analyses were performed using R Commander.³⁸ The goal was to investigate whether or not data from HFD mice showed different temporal behaviors than LFD mice. Average PDFF, local magnetic field offset, and R_2^* from each perirenal ROI were computed. Left and right perirenal ROI measurements were pooled together. The following statistical tests were performed on PDFF, local magnetic field offset, and R_2^* measurement. A repeated-measures two-way ANOVA [one within subjects factor (diet) and one between subjects factor (week)] with $\alpha = 0.05$ was performed to investigate any mean differences between HFD and LFD groups over time. A repeated-measures one-way ANOVA with $\alpha = 0.05$ was performed to investigate if either HFD or LFD group mean changed over time.

3 Results

Figure 1 shows representative magnitude images and PDFF maps of LFD and HFD mice at 4, 8, 12, and 16 weeks after start of diet. All images and maps are masked to remove background noise. Arrows mark the general perirenal AT regions and dashed lines approximate outlines for perirenal ROIs used in this analysis. It is clear that the visceral AT volume fraction increases dramatically in the HFD mice compared with LFD mice as does overall mouse size. Body weight of HFD mice increased $\sim 100\%$ over 16 weeks. Body weight of LFD mice increased $\sim 15\%$ over 16 weeks.

Figure 2 shows sample PDFF, R_2^* , and magnetic field offset maps of an HFD mouse at 16 weeks after diet. All parametric maps are masked to remove background noise.

Figure 3 shows local magnetic field offset histograms from kidney and erector spinae muscle as well as perirenal AT regions from an HFD mouse at 16 weeks after diet. The local magnetic field offset distribution from kidney and erector spinae muscle peaks around zero. On the other hand, the local field offset distribution for AT was offset by 0.09 ppm.

Figure 4 shows normalized perirenal AT ROI averages and standard deviations for PDFF, local magnetic field offset normalized to kidney and erector spinae, and R_2^* values from HFD and LFD mice across 16 weeks. Local field offset is reported in ppm.

Figure 5 shows correlation plots of R_2^* versus PDFF and local magnetic field offset versus PDFF for perirenal AT. The parameter values are not normalized to week 0 in this figure. Correlation plots illustrate the degree of mutual information between model parameters. Significant correlation was observed

Fig. 3 Local magnetic field offset (ΔB_0) histograms from kidney + erector spinae muscle and perirenal AT regions from an HFD mouse at 16 weeks after diet.

Fig. 4 Perirenal AT ROI average and standard deviation of PDFFF, local magnetic field offset (ΔB_0) normalized to kidney and erector spinae muscle, and R_2^* values for HFD and LFD mice across 16 weeks. All parameters are plotted in relative difference to week 0 values with standard deviation bars.

Fig. 5 Correlation plots of R_2^* versus PDFFF and local magnetic field offset (ΔB_0) versus PDFFF for perirenal AT. Each data point represents an average parameter value from one mouse (LFD = red, HFD = blue). Parameter values from weeks 0 (circle), 4 (square), 8 (diamond), 12 (triangle), and 16 (open square) are plotted. Note parameter values are not normalized to week 0. Significant correlation was observed between R_2^* and PDFFF ($R^2 = 0.56$, $p < 0.001$) but not between local magnetic field offset and PDFFF ($R^2 = 0.02$, $p = 0.45$).

between R_2^* and PDFFF ($R^2 = 0.56$, $p < 0.001$) but not between local magnetic field offset and PDFFF ($R^2 = 0.02$, $p = 0.45$). These results suggest that the signal model correctly accounts for the field offsets arising from the multiple fat peaks, i.e., local field offset contains no information on PDFFF, but, as expected, does not correctly account for R_2^* in AT. In other words, the single-component R_2^* contains information about the water and fat fractional composition.

Table 1 summarizes the p -values from the repeated-measures one-way and two-way ANOVA analysis of the normalized PDFFF, R_2^* , and local field offset data. Significant p -values ($p < 0.05$) are indicated in italics. The repeated-measures two-way ANOVA analysis indicated a significant interaction between diet groups (HFD versus LFD) and weeks on diet for normalized R_2^* and local field offset. This suggests that HFD and

LFD mice have different R_2^* and local field offset time course behaviors. No significant interaction between diet and weeks on diet was found for normalized PDFFF. However, diet-averaged normalized PDFFF values did significantly change over time. The repeated-measures one-way ANOVA analysis indicated that PDFFF, R_2^* , and local field offset in HFD mice significantly changed over time, while only PDFFF and R_2^* in LFD mice significantly changed over time.

4 Discussion

This work reports noninvasive *in vivo* monitoring of a DIO mouse model using quantitative FWMRI at 15.2T with all confounder-factor corrections to ensure accurate PDFFF, field offset, and R_2^* parametric maps. FWMRI of a DIO mouse model at

Table 1 Reported p -value for statistical analysis. Italicized p -value indicate significant values ($p < 0.05$).

p -value			
Repeated measures two-way ANOVA	PDFF	R_2^*	Local field offset
Between subjects			
Diet	0.549	0.095	<i>0.011</i>
Within subjects			
Week	$<10^{-4}$	$<10^{-5}$	<i>0.004</i>
Week $\times$ Diet	0.761	<i>0.022</i>	<i>0.008</i>
Repeated measures one-way ANOVA	PDFF	R_2^*	Local field offset
LFD: source of variation			
Mice	<i>0.034</i>	<i>0.017</i>	0.128
Week	<i>0.033</i>	<i>0.025</i>	0.862
HFD: source of variation			
Mice	<i>0.004</i>	<i>0.002</i>	0.073
Week	<i>0.002</i>	$<10^{-4}$	0.002

15.2T may offer advantages of improved image quality as well as of potentially new image contrast, which may aid in longitudinal studies of adiposity changes due to disease.

FWMRI studies on mouse models have been reported.^{39,40} These studies were performed at 3T and validated FWMRI biomarkers of hepatic steatosis. By focusing on a large organ such as the liver, the drawbacks of mouse imaging at 3T, namely lower spatial resolution, were acceptable. In order to study smaller structures, a move to preclinical MRI scanners may be advantageous. In this work, improved spatial resolution and image quality of the 15.2T scanner allowed for visualization of the small perirenal AT depots in LFD mice, particularly at early time points.

The higher magnetic fields of preclinical MRI have several advantages, which are compounded at 15.2T.²² Increased signal-to-noise ratio at higher fields can lead to improved spatial resolution and image quality. Higher magnetic fields also lead to increased sensitivity to different contrast such as those derived from magnetic susceptibility differences. Further, spectral resolution is improved from the increased chemical shift in Hz between peaks of different species. In the context of FWMRI, higher spectral resolution and signal-to-noise ratio are particularly useful for triglyceride characterization due to increased sensitivity to smaller lipid peaks as well as less peak overlap leading to improved lipid peak discrimination.⁴¹ However, a limitation of the 15.2T scanner used in this work is that the limited homogeneous region of the gradient and RF coils precluded the possibility of whole body mouse imaging.

In this study, we focused on perirenal AT as a representative sample of visceral AT, which is an important marker of metabolic health.^{7,8} Visceral AT is hormonally active and possesses unique biochemical characteristics that influence several normal

and pathological processes.⁴² Individuals with abnormally high deposition of visceral AT are at increased risk for development of type 2 diabetes, dyslipidemia, and coronary heart disease.⁴³ It has also been reported that obese individuals also have visceral AT inflammation, i.e., macrophage infiltration.⁴⁴

The results showed time course behavior differences in PDFF, local field offset, and R_2^* between HFD and LFD mice (Fig. 4). The results suggest a trend of gradually increasing PDFF for both HFD and LFD mice that plateaus after week 8. The local field offset showed no change over time for LFD mice, while HFD mice showed a trend of decreasing local field offset over time. R_2^* of LFD mice slowly decreased over time, while R_2^* of HFD mice sharply fell between weeks 0 and 8 before leveling off. This study was not designed to investigate the mechanisms of FWMRI contrast and further investigation is needed. While it is possible to speculate on potential mechanisms as we do below, without knowing the underlying causes of the time course behavior differences, the significance of our findings is unclear.

The DIO mouse model used in this work is known to exhibit cellular changes in AT that occur with obesity and inflammation including adipocyte enlargement, iron overload, macrophage infiltration, and apoptosis.^{23,45–49} Increases in adipocyte cell size are generally due to lipid droplet and not cytoplasmic enlargement,⁵⁰ which may in turn affect PDFF.^{47,51} Larger adipocytes may also affect R_2^* .⁵² Changes in iron levels may affect both local field offset^{53,54} and R_2^* .⁵⁵ PDFF may be affected by both macrophage infiltration and adipocyte apoptosis due to changes in the ratio of lipid to water volumes. PDFF, local magnetic field, and R_2^* could all be affected by multiple mechanisms that are not readily distinguishable by the data presented here and further investigation is required.

A confounding factor of PDFF is that low PDFF may be biased by image noise. Liu et al.⁵⁶ proposed a straight-forward solution they termed the “magnitude discrimination” method. To avoid this noise bias, this method calculates PDFF differently when water is the dominant component or when fat is the dominant component.

In addition, Figure 5 shows R_2^* is significantly correlated with PDFF indicating that there is significant mutual information between the two parameters. This makes it challenging to interpret what R_2^* and PDFF reflect about changes in AT. While a single R_2^* model was chosen to improve robustness of fit,^{24,25} an additional two-compartment R_2^* model may be needed in order to properly interpret the meaning of R_2^* and PDFF.

Another confounding factor for R_2^* estimates is macroscopic magnetic field inhomogeneity from poor shimming or sharp changes in susceptibility like air/tissue interfaces that increase R_2^* .⁵⁷ Field inhomogeneity is more severe at higher magnetic fields. Evidence of this R_2^* increase can be seen in the R_2^* map (Fig. 2) at the boundaries of the mouse body. While not accounted for in this work, it is possible to use the field offset map to correct R_2^* estimates.⁵⁸

Finally, there are limitations on what tissue information can be inferred from the local field offset. At a given voxel, the local field offset is affected by the magnetic susceptibility in that voxel as well as in all the surrounding voxels.^{29,59} Consequently, the local field offset is influenced by the magnetic susceptibility and shape of the tissue of interest as well as those of all the surrounding tissues. In order to unambiguously infer properties of a specific tissue, measurement of magnetic susceptibility itself would be desired. Computation of a magnetic

susceptibility map with additional and nontrivial processing of the local field map is possible,^{33,34,60} but it is beyond the scope of this current work.

5 Conclusion

In this work, we describe quantitative FWMRI with all confounder-factor corrections on a 15.2T preclinical scanner for noninvasive *in vivo* monitoring of DIO mice over time. We measured PDFF, local field offset, and R_2^* and observed differences in time course behaviors between HFD and LFD mice. Further work is needed to understand these observations. FWMRI at 15.2T may be a useful tool for longitudinal studies of adiposity due to the advantages of ultra high field.

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Biographies for the authors are not available.