Original Article
Quantification of MRI-PDFF by complex-based MRI: phantom and rabbit study at 3.0T

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Abstract: This paper aimed to evaluate the accuracy of magnetic resonance imaging-proton density fat fraction (MRI-PDFF) in fat-water-iron phantom and rabbit with hepatic steatosis using complex-based chemical shift-encoded MRI technology. Phantoms of varying fat, water and iron content were constructed. Rabbit model with hepatic steatosis was successfully established by high-fat and high cholesterol diet. All MR examinations were performed on a 3.0T MR system. PDFF values of phantoms and rabbits were calculated from IDEAL-IQ. Fat assessment of rabbit liver biopsy was performed as reference standard. MRI-PDFF of four phantom groups with different iron content has no significant difference with the known fat contents (F=0.011, p=1.0), and extremely close correlations were observed (r=0.998, 0.998, 0.999, 0.998, respectively, all P<0.001). The existence of magnetic microspheres in phantoms did not affect fat measurement accuracy. MRI-PDFF showed significant differences between different steatosis grades with medians of 3.72% (normal), 5.43% (mild), 9.11% (moderate) and 11.17% (severe) except normal with mild steatosis. Close correlation between MRI-PDFF and histological steatosis was observed (r=0.78, P<0.01). IDEAL-IQ provides robust and promising fat quantification and can be considered potential alternative to biopsy for chronic patients where available.

Keywords: MRI, fat quantification, proton density fat fraction (PDFF), phantom, liver

Introduction
Hepatic steatosis (HS) is defined as excessive fat accumulation in hepatocytes. As the earliest manifestation of most common health problems such as nonalcoholic fatty liver disease (NAFLD) [1], steatosis is also considered playing important role in pathogenesis of many hepatic and systemic disorders, and may accelerate disease progression and reduced therapy efficacy [2, 3]. Assessment of liver fat is important for early detection, monitoring and treatment of patients with HS. Biopsy remains gold standard for fat diagnosis, with limitations including invasiveness, sampling bias and subjective variability. Biopsy is not the optimal choice for patients who need long-term clinical follow-up and observations. Among noninvasive surrogates for hepatic steatosis quantification, magnetic resonance (MR) has been confirmed accurate and become a hot spot in clinical researches [4-8].

To date, magnetic resonance imaging-proton density fat fraction (MRI-PDFF) has emerged as a standardized, reproducible and promising biomarker for quantification of hepatic steatosis. It has been a highly active topic and is considered potential alternative to biopsy for chronic patients [9-18].

The multi-echo 3D gradient recalled echo (GRE) sequence named iterative decomposition of water and fat with echo asymmetry and least square estimation (IDEAL-IQ, GE Medical systems, USA), is a complex-based chemical shift encoded MRI quantitative method for diffusive liver diseases in a single breath-hold [9, 19]. Through acquisition of six different echoes, water, fat, in-phase (IP), out-of-phase (OP), R2*, and fat fraction images can be created. PDFF and R2* can be simultaneously obtained by fat fraction and R2* maps to reflect fat and iron content respectively. With simple data processing, IDEAL-IQ has a good prospect of clinical application.
Previous clinical researches have shown accurate quantification of hepatic steatosis using IDEAL-IQ [9, 20-23]. Many researches also use IDEAL-IQ to assess fat deposition of different organs of patients [23, 24]. To our knowledge, current state-of-the-art researches on animal model are limited, and researches on efficiency of IDEAL-IQ taking iron influence into account are few in number. This study was aimed to evaluate the accuracy of MRI-PDFF in fat-water-iron phantom and rabbit with hepatic steatosis using IDEAL-IQ. R2* in phantoms was also calculated to investigate efficiency of iron quantification by IDEAL-IQ.

Materials and methods

Phantom construction

In order to test MRI fat quantification in the presence of iron which shortened T2*, phantoms of varying fat, water and iron content were constructed. With similar proton nuclear magnetic resonance (NMR) spectrum to triglyceride, peanut oil was selected [25]. Superparamagnetic iron oxide (SPIO) was applied to investigate influence of iron on fat quantification accuracy of IDEAL-IQ.

According to phantom construction method from Bernard [26], 2 g of carrageenan was dissolved in 400 ml of purified water heated to 50°C by a temperature-controlled magnetic stirrer. 1.8456 g of sodium dodecyl sulfate was added to the solution in order to create homogenous oil-in-water emulsions. Four groups (A, B, C, D) of phantoms (20 ml) were constructed, each of which is composed of eight homogeneous fat-water test tubes with fat volume ratio at 0%, 5%, 20%, 35%, 50%, 65%, 80% and 95% respectively. SPIO content of each phantom group was as follows: A, none; B, 0.05 mg; C, 0.1 mg; D, 0.15 mg. These phantoms were emulsified by a homogenizer to ensure stability of the gels.

Animal model

This work was performed with approval from the Animal Research Committee. Forty New Zealand white rabbits (half male, half female, 2.0-2.5 kg) were randomly divided into four groups: ten rabbits in control group (group Gc) were fed with a standard diet, experimental groups (group GE1, GE2, GE3) were given a high-fat high-cholesterol diet (standard diet with additional 10% lard oil, 2% cholesterol and 5% maltose) for an interval of 4 weeks (group GE1, 4 weeks; GE2, 8 weeks; GE3, 12 weeks) [27, 28].

MRI acquisition

All MRI examinations were performed on a 3.0T MRI system (Discovery MR750 3.0T, GE Medical systems, USA) using an eight-channel phased-array knee coil. IDEAL-IQ sequence was acquired in phantoms and rabbits. Following parameters were chosen: field of view (FOV), 14×14 cm; matrix, 96×96; bandwidth, 100 kHz; flip angle, 5°; slice thickness, 4 mm; repeated measurements (NEX), 3; repetition time (TR), 11.5 ms. The first echo time (TE1)/ΔTE were 1.2/1.9 ms.

Rabbits in the control group (Gc) were divided into three groups to receive MR scanning at different time-points (Gc1, 3 rabbits, 4 weeks; Gc2, 3 rabbits, 8 weeks; Gc3, 4 rabbits, 12 weeks) in order to investigate whether different feeding time affects liver fat content with standard diet, rabbits of each experimental group (GE1, GE2 and GE3) underwent MR scanning after different feeding periods. The animals were anaesthetized with intramuscular injection of 0.3 ml xylazine/kg and 0.2 ml ractecanisodamine hydrochloride to inhibit stomach and intestine peristalsis, and were immobilized with fixed limbs during MRI examinations. The MRI protocol of rabbits also included routine transverse T2 fast-recovery fast spin-echo (FRFSE) sequence for axial images, and parameters were: FOV,
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Table 1. MRI-pDFF of different phantom groups at different known fat volume ratio

<table>
<thead>
<tr>
<th>Group</th>
<th>0%</th>
<th>5%</th>
<th>20%</th>
<th>35%</th>
<th>50%</th>
<th>65%</th>
<th>80%</th>
<th>95%</th>
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<tbody>
<tr>
<td>A</td>
<td>0.54±0.2</td>
<td>3.66±0.9</td>
<td>17.02±1.3</td>
<td>30.41±2.1</td>
<td>44.12±1.3</td>
<td>59.49±1.0</td>
<td>75.29±1.2</td>
<td>93.02±0.7</td>
</tr>
<tr>
<td>B</td>
<td>0.84±0.5</td>
<td>4.28±1.0</td>
<td>16.91±0.6</td>
<td>31.40±1.7</td>
<td>49.36±0.9</td>
<td>60.21±1.2</td>
<td>74.39±1.9</td>
<td>94.55±1.1</td>
</tr>
<tr>
<td>C</td>
<td>0.60±0.3</td>
<td>3.33±0.6</td>
<td>16.61±1.1</td>
<td>31.58±1.5</td>
<td>45.48±2.7</td>
<td>61.03±0.9</td>
<td>77.00±2.0</td>
<td>93.85±0.8</td>
</tr>
<tr>
<td>D</td>
<td>0.86±0.1</td>
<td>4.99±1.2</td>
<td>16.47±2.0</td>
<td>30.40±0.4</td>
<td>49.71±1.6</td>
<td>61.50±2.2</td>
<td>77.92±1.0</td>
<td>95.31±2.2</td>
</tr>
</tbody>
</table>

Values are given as mean ± SD from phantoms in each group.

Figure 2. MRI-pDFFs in different phantom groups plotted against known fat volume ratios. No statistically significant differences were observed between MRI-pDFF values of phantom groups (F=0.011, P=1.0) from one-way ANOVA analysis. Pearson correlation measurement showed close correlation between MRI-pDFF and known fat volume ratio in each group, r values were 0.998, 0.998, 0.999 and 0.998 respectively for group A, B, C and D. Each group was composed of eight homogeneous fat-water-iron test tubes (20 ml) with different fat volume ratios respectively. SPIO content: A, none; B, 0.05 mg; C, 0.1 mg; D, 0.15 mg.

Figure 3. R2* images of phantom groups (known fat volume ratio: first column from top to bottom, 95%, 80%, 65%, 50%; second column from top to bottom, 35%, 20%, 5%, 0%). Each group (A-D) was composed of eight homogeneous fat-water-iron test tubes (20 ml) with different fat volume ratios respectively. SPIO content: (A) None; (B) 0.05 mg; (C) 0.1 mg; (D) 0.15 mg.

Data analysis

The radiologist unaware of grouping and biopsy results did data analysis. For phantoms, elliptical region of interest (ROI) of 2 cm² was placed respectively on fat fraction and R2* image to measure MRI-pDFF and R2*. For rabbits, the radiologist placed five ROIs (average value as measured result) of 0.5 cm² on the fat fraction image to measure MRI-pDFF. The selected rabbit images covered the central liver at the level of portal vein. Blood vessels, bile ducts and artifacts should be avoided during ROI placement.

Measurements of the variables were repeated with the interval of a few days to assess calculation reliability. All the calculations were performed on a workstation (AW Volume Share 4; GE Healthcare).

Histological analysis

After MRI imaging, rabbits were deeply anaesthetized and sacrificed by intravenous injection of 5 ml potassium chloride. Liver specimens matching MRI slices to the greatest extent were processed by hematoxylin and eosin (HE) and Oil red O staining to assess the liver morphology and fat
droplets in the hepatocytes. According to the nonalcoholic steatohepatitis clinical research network (NASH CRN), hepatic steatosis was graded as following: grade 0, minimal steatosis or normal, <5.0% of liver cells with intracellular vacuoles of fat; grade 1, mild, 5.0%~32.0%; grade 2, moderate, 33.0%~65.0%; grade 3, severe, >65.0% [29]. Semi-automatic quantification (VP) was measured by the ratio of fatty content in liver to liver volume using an image analyzer system (Image Pro Plus V6.0). The pathologist blinded to the rabbits’ diet and MR values performed biopsy evaluation.

Statistical analysis

Kurtosis and skewness were applied to test the normality of the data distribution. Intraclass correlation coefficient (ICC) was used to assess reliability of IDEAL-IQ calculation. For statistical analysis of phantoms, one-way ANOVA was used to determine whether there were any significant differences between phantom groups in PDFF and R2* values. SNK-Q test performed pairwise comparisons between group values. Pearson correlation measured correlation between measured and known values.

For rabbits, Mann-Whitney U test was used to determine if there was gender difference between male and female rabbits. The comparisons of PDFF and VP among groups or steatosis grades were tested by Kruskal Wallis test followed by Dunn-Bonferroni test for post hoc multiple comparisons. Spearman’s correlation coefficient evaluated the degree of association between MRI-PDFF and histological results. All statistical analyses were performed using statistical software (IBM SPSS, version 21.0). P<0.05 has statistical significance.

Results

As a measure of reliability, ICC between repeated measurements in IDEAL-IQ PDFF calculations was 0.91 (95% confidence interval [CI]: 0.83-0.95). And ICC between repeated R2* calculation was 0.81 (95% confidence interval [CI]: 0.67-0.90). The measurements of PDFF...
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and R2* from IDEAL-IQ had good repeatability and reliability.

**Phantom study**

Fat fraction images of different phantom groups were of high quality (Figure 1). MRI-PDFF values from IDEAL-IQ of different phantom groups at different known fat volume ratio were showed in Table 1. Figure 2 shows MRI-PDFFs in different phantom groups plotted against known fat volume ratios. One-way ANOVA results indicated no statistically significant differences between MRI-PDFF values of phantom groups (F=0.011, P=1.0). And close correlation between MRI-PDFF and known fat volume ratio was observed in each group, r values were 0.998, 0.998, 0.999 and 0.998 respectively for group A, B, C and D.

R2* values of different phantom groups were also calculated. R2* images were shown in Figure 3. Mean R2* values of phantom group A, B, C, D were 28.77±14.62, 71.37±35.00, 108.08±24.38 and 144.22±41.86 (Hz) respectively. R2* values showed significant differences between phantom groups (F=20.71, P=0.000), while no correlation between R2* and iron level was observed (P=0.82). Figure 4 shows R2*s in different phantom groups plotted against known fat volume ratios.

**Animal model study**

A total of 37 rabbits (19 male, 18 female) were included in this study (3 died in experimental groups). Rabbits of control group (group Gc) grew well, whereas some rabbits of experimental groups (group Ge1, Ge2, Ge3) showed phenomenon of hair and appetite loss with high-fat high-cholesterol diet.

HS rabbit model was successfully established by high-fat high-cholesterol diet. MRI-PDFFs showed differences between the four groups with different diet and raising period except group Ge1 and Ge2. VPs showed significant differences between each two of the four groups except group Ge1 and Ge2 (Table 2). And there was no differences in variables between male and female rabbits, p values were 0.793, 0.307 respectively in sex comparison of MRI-PDFF and VP.

On the liver biopsy examination, rabbits were divided into different grades as following: 6 (16.2%, 6 from group Gc) with normal liver, 8 (21.6%, 3 from group Gc, 1 from Gc1, 2 from Ge2, 2 from Ge3) with mild steatosis, 13 (35.1%, 1 from group Gc, 2 from Gc1, 7 from Ge2, 3 from Ge3) with moderate steatosis, and 10 (27.1%, 6 from group Gc, 1 from Ge2, 3 from Ge3) with severe steatosis.

In contrast with liver in the normal group (Figure 5A), livers with HS were large-sized, dull red to light yellow, rough surfaces with blunt edges and greasy sections (Figure 5B-D). From histology analysis, normal livers did not show any abnormalities in cellular architecture. By contrast, typical histological lesions of steatosis were observed in HS livers. Diffuse severe fatty infiltration was noted in HS livers from HE staining. Fat droplet accumulation and fibrosis were obvious in HS samples stained with Oil red O (Figure 6).

Representative T2 weighted, fat fraction and colormap images in normal and HS livers were shown in Figure 7. MRI-PDFFs showed signifi-
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Figure 6. Histologic staining of rabbit liver tissue. The liver specimens were stained with HE (A-D), and Oil red O (E-H). Livers of normal group did not show any abnormalities in cellular architecture, while typical histological lesions of steatosis were observed in HS group. In contrast with normal liver, diffuse severe fatty infiltration was noted in HS group from HE staining. Fat droplet accumulation and fibrosis were obvious in HS samples stained with Oil red O, but not in the normal group.

Figure 7. Representative T2 weighted, fat fraction and color map images in normal and HS livers. Five elliptical region of interests (ROIs) of 0.5 cm² were placed on the fat fraction and R2* images to measure MRI-PDFF and R2* values.

cant differences among different steatosis grades except normal and mild steatosis. VPs showed significant differences among different steatosis grades (Table 3). There was a close correlation between MRI-PDFF and histological VP ($r=0.78$, $P=0.000$; Figure 8). Scatterplots between VP, MRI-PDFF and histopathologic steatosis grades were shown in Figure 9.
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Discussion

pDFF is defined as accurate separation of fat protons’ signal from other protons, proton density becomes the main factor affecting signal strength. T1 bias, T2* decay, spectral complexity of fat, noise bias and eddy currents are the confounding factors in signal acquisition. With correction for these confounders, fat fraction from MRI is equivalent to PDFF [30].

MRI-PDFF has been a robust imaging-based biomarker for hepatic steatosis quantification. Phantom, animal and clinical studies have confirmed accuracy of MRI-PDFF in liver fat quantification and indicated that PDFF is preferred fat quantification where available. Study from Artz demonstrated reproducibility of PDFF across field strengths and methods in obese subjects [31]. Runge compared MRI-PDFF and histopathology against reference standard of biochemically determined liver triglyceride content in mice, and showed that MRI-PDFF (r=0.75) had a higher correlation than histopathology (r=0.59) [32]. MRI-PDFF showed strong correlation with histology (r=0.85) in ex vivo human livers from Bannas [33]. Kukuk compared systematically different methods for hepatic steatosis quantification for patients and confirmed that MRI-PDFF provides the most reliable results [10]. MRI-PDFF has been used as reference or technical standard in many researches [34, 35].

As a complex-based corrected fat quantification technique, IDEAL-IQ uses both magnitude and phase information for accurate separation of fat and water. T1 bias is minimized using a very low flip angle. For correction of T2*, eddy currents and field inhomogeneity (Bn), iteration using magnitude information following complex-based reconstruction was applied in IDEAL-IQ algorithm [19, 24].

With convenient data processing, IDEAL-IQ becomes a hot spot of quantification research. Karcaaltincaba calculated PDFF and R2* using IDEAL-IQ in patients with hepatic iron deposition [36]. Idilman compared the efficiency of MRI-PDFF from IDEAL-IQ in hepatic steatosis quantification in NAFLD patients and observed a close correlation between MRI-PDFF and histology [37].

| Normal (0) | 6 | 3.72±2.36 | 6.58±3.08 |
| Mild steatosis (1) | 8 | 5.43±2.47 | 14.51±8.82 |
| Moderate steatosis (2) | 13 | 9.11±2.30 | 29.10±7.09 |
| Severe steatosis (3) | 10 | 11.17±2.21 | 46.69±1.74 |

Overall comparison (χ², p) 21.634, 0.000 30.043, 0.000

Multiple comparison (p)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>p</th>
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</thead>
<tbody>
<tr>
<td>0 vs 1</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>0 vs 2</td>
<td>&lt;0.01</td>
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<tr>
<td>0 vs 3</td>
<td>&lt;0.01</td>
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<td>1 vs 2</td>
<td>&lt;0.01</td>
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<tr>
<td>1 vs 3</td>
<td>&lt;0.01</td>
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<tr>
<td>2 vs 3</td>
<td>&lt;0.01</td>
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</tbody>
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Values are given as mean ± SD from phantoms in each group. *Kruskal Wallis test; Dunn-Bonferroni test.

Figure 8. Correlation between Liver MRI-pDFF and VP of histological steatosis. Spearman’s correlation analysis showed close correlation between MRI-pDFF and VP (r=0.78, p=0.000).
R2* calculation was also performed in fat-water-iron phantoms, and showed significant differences between different SPIO contents. Nevertheless, no correlation between R2* and iron level was observed. The R2* distribution in phantoms (Figure 4) showed differences between varying iron contents at specific fat content. Based on gradient echo acquisitions, R2* is calculated from exponential signal decay rate. Signal model, fat correction and B₀ field variations are critical for estimation of R2* values. R2* measurements with relevant confounding factors corrected have shown to be efficiency in iron evaluation. Liau evaluated performance of MRI-PDFF and R2* in liver and spine by intravenous infusion of superparamagnetic iron oxide (SPIO) contrast, and confirmed that IDEAL-IQ is robust to R2* evaluation [38]. But R2* does not measure iron content directly, and spatial variability of iron concentration lead to broad quantification confidence intervals. With respect to hepatic R2*-based iron evaluation, coexisting conditions such as fat, inflammation and fibrosis must be taken into account. From the results of phantoms, R2* value was affected by fat content, R2* values with same iron content fluctuate at different fat contents. Further studies to evaluate the efficiency of R2* from IDEAL-IQ are needed.

Figure 9. Scatterplots between VP, MRI-PDFF and histologic steatosis grade. The VP and PDFF values increased with the deeper changes of steatosis.
Accurate quantification of liver fat is critical for clinical diagnosis and treatment of important hepatic and systemic disorders, and facilitates research on related fields. The continuous development of MRI technologies, particularly the accomplishment of IDEAL-IQ measuring MRI-PDFF and R2* simultaneously, has provided prominent measures for diagnosis and quantification of steatosis and iron deposition, especially for patients who need long-term clinical follow-up and observations.

From this phantom and rabbit research, IDEAL-IQ provides robust and promising fat quantification and can be considered potential alternative to biopsy for chronic patients where available. Evaluations of the reliability and accuracy of the technology are still critical in order to determine whether they can substitute histological examination as the gold standard to assess hepatic steatosis deposition. R2* from IDEAL-IQ is sensitive to iron changes, however, R2* measurement and classification for iron deposition using IDEAL-IQ need further experimental investigation.

Disclosure of conflict of interest

None.

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