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Liver Steatosis: Concordance of MR Imaging and MR Spectroscopic Data with Histologic Grade[1]

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Purpose: To determine if the concordance of magnetic resonance (MR) imaging and MR spectroscopic data with histologic measures of steatosis is affected by histologic magnification level, tissue heterogeneity, or assessment of tissue area versus that of hepatocytes.

Materials and Methods: This study was institutional review board approved and HIPAA compliant. Written informed consent was obtained. In- and out-of-phase MR imaging and MR spectroscopic measures of steatosis were compared in 33 patients with nonalcoholic fatty liver disease and in 15 healthy volunteers. Concordance of MR imaging and MR spectroscopic data with histologic findings was assessed for *(a)* histologic examination at standard (×40 and ×100) versus high magnification (×200 and ×400), *(b)* heterogeneity and homogeneity of livers, and *(c)* percentage of tissue and hepatocytes that contained lipids. Evaluations included linear regression and Fisher exact tests.

Results: In- and out-of-phase MR imaging and MR spectroscopic data were well correlated (R^2 = 0.93) and generally concordant with histologic measures. Patients in whom MR fat fractions were higher than expected compared with steatosis grades at standard magnification histologic examination were upgraded significantly more often when high magnification was used than were the remaining patients (100% [10 of 10] vs 47% [7 of 15], $P < .01$). MR imaging and MR spectroscopic data of homogeneous livers were significantly more likely than those of heterogeneous livers to be concordant with steatosis grades when high magnification was used (81% [13 of 16] vs 47% [8 of 17], $P < .05$). For all patients, percentage of fat in tissue was lower than that in hepatocytes, which affected individual patients, but not the overall correlation.

Conclusion: MR imaging and MR spectroscopic data were generally concordant with histologic measures of steatosis. Discordance between them may reflect differences in magnification at histologic examination and in liver heterogeneity.

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Nonalcoholic fatty liver disease is common, affecting 20%–30% of the U.S. population (1). The reference standard for determining the severity of steatosis (ie, fat deposits in hepatocytes) is histologic analysis of a liver biopsy specimen. In- and out-of-phase magnetic resonance (MR) imaging and MR spectroscopy have been shown to have potential for noninvasive assessment of steatosis. Although some studies have demonstrated good correlation of MR measures with histologic measures of steatosis (2–5), results have varied, with R^2 values as low as 0.5 (2).

Advances in Knowledge

- Magnification used at histologic examination affected concordance of MR measures with histologic measures of steatosis in that standard magnification (×40 and ×100) resulted in steatosis grades that were discordant with MR spectroscopic data, which showed higher fat fractions than expected in 40% (10 of 25) of patients with steatosis grades 0–2: All of these patients (100%, 10 of 10) were upgraded to a higher steatosis grade when high magnification (×200 and ×400) was used at histologic evaluation, whereas only 47% (7 of 15) of the remaining cases were upgraded ($P < .01$).
- MR imaging and MR spectroscopic data for homogeneous livers were significantly more likely to be concordant with steatosis grades when high magnification was used at histologic examination than were data from heterogeneous livers (81% [13 of 16] vs 47% [8 of 17], $P < .05$).
- Patients with nonalcoholic fatty liver disease and grades 1 or 2 steatosis were likely to be upgraded to the next higher steatosis grade if high magnification was used at histologic evaluation (81% [17 of 21]).

An inherent problem with validating MR imaging and MR spectroscopic data with histologic measures is that there are several differences among the techniques as they are currently performed. MR imaging and MR spectroscopy measure all the fat in the sample studied, whereas histologic estimation of fat, in current clinical practice, is an examination of a small biopsy specimen at ×40 and ×100 magnification. In- and out-of-phase MR imaging provide a global assessment of the liver. Single-voxel MR spectroscopy is an evaluation of a portion of the liver (typically 8 cm^3), but the samples used for histologic comparison may be 10^5 times smaller, which could result in a large sampling error. MR imaging and MR spectroscopy provide quantitative assessments of the percentage of tissue that contains fat. Histologic examination, however, is a visual assessment of the percentage of hepatocytes that contain fat vacuoles. Finally, MR imaging and MR spectroscopy provide continuous measures, whereas histologic measures are categorized into grades 0–3.

The limitations in correlation of MR imaging and MR spectroscopic data with histologic measures and the differences among the techniques motivated our study. Our goal was to determine if the concordance of MR imaging and MR spectroscopic data with histologic measures of steatosis is affected by histologic magnification level, tissue heterogeneity, and assessment of the amount of fat in tissue area versus that in hepatocytes.

Implications for Patient Care

- Patients with nonalcoholic fatty liver disease may receive higher steatosis grades when high (×200 and ×400) rather than standard (×40 and ×100) magnification is used at histologic examination: Of the 21 patients with steatosis grades 1 or 2, 17 (81%) were upgraded at high-magnification histologic examination.
- Patients may receive higher steatosis grades when MR imaging is used: Of the 21 patients with steatosis grades 1 or 2, 10 (48%) had higher MR fat fractions than suggested by the standard-magnification histologic grade.

Materials and Methods

Patients

Our retrospective study was approved by our institutional review board and was compliant with the Health Insurance Portability and Accountability Act. Written informed consent was obtained from all patients. Forty-eight participants, including 33 patients who were suspected of having nonalcoholic fatty liver disease and 15 healthy volunteers, were examined during a 6-year period as part of a research study. Healthy volunteers were included to help in determining MR imaging thresholds between steatosis grades 0 and 1. Research patients were included if they underwent a biopsy within 70 days of MR imaging and if MR imaging data and pathologic slides were available for retrospective review. The participant age (mean ± standard deviation) was 41 years ± 14. There were 27 men (aged 36 years ± 12) and 21 women (aged 47 years ± 15) studied. Women were significantly older than the men in the

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Potential conflicts of interest are listed at the end of this article.

patient group and in the total group ($P < .01$). Twenty-six of these patients were part of a different study (6). There was no overlap in reported results between these studies.

MR Imaging

MR imaging was performed by using a 1.5-T clinical MR imaging unit (GE Healthcare, Waukesha, Wis) with a torso phased-array coil for signal reception. MR imaging included coronal breath-hold T1-weighted imaging. For MR spectroscopy, fat-suppressed T2-weighted fast spin-echo axial imaging was performed (echo time, 100 msec; echo train length, eight; section thickness, 8 mm; section gap, 1 mm). The in- and out-of-phase (two-point Dixon technique) MR images were acquired by using a dual-echo (echo times, 2.2 and 4.4 msec) fast spoiled gradient-recalled echo sequence (repetition times, 90 or 120 msec; flip angle, 75°; field of view, 32–44 cm; section thickness, 8 mm; section gap, 1 mm). For MR spectroscopy, an 8 cm^3 voxel was placed in the liver, avoiding blood vessels and the edges of the liver in all dimensions. The MR spectroscopic data were acquired by using a chemical shift–selective water-suppressed 128-acquisition time series of point-resolved spectroscopic single voxels (repetition time msec/echo time msec, 2500/30 [n = 46] or 2500/27 [n = 2]). Unsuppressed water spectra with eight acquisitions were also acquired at each location. An additional four spectra were acquired at the start of each acquisition but were not recorded, to ensure that an equilibrium state had been reached.

Histologic Analysis

All 33 patients who were suspected of having nonalcoholic fatty liver disease underwent liver biopsy as part of clinical care. The median time between MR imaging and biopsy was 11 days, with 11 patients having undergone the biopsy within a day of imaging. Liver biopsy was not obtained from the 15 healthy volunteers.

A single pathologist (M.M.L., with 3 years of experience) retrospectively reviewed all the histologic slides under the guidance of a senior liver pathologist (L.F., with 31 years of experience). Slides were reviewed by using an Olympus B53 microscope (Olympus, Hamburg, Germany) during a 1-year period. Steatosis was assessed histologically by using four techniques. First, by following standard clinical practice, steatosis was graded by estimating the approximate percentage of hepatocytes that contain fat, as visible by using standard magnification (defined as ×4 and ×10 lenses and ×10 eyepieces; [×40 and ×100 magnification]). Steatosis grades were then assigned as described in the literature (7,8) on the basis of the following percentages: grade 0, fewer than 5% of hepatocytes containing fat; grade 1, 5% to 33%; grade 2, 33% to 66%; and grade 3, more than 66%. Second, steatosis was regraded by using both standard and high magnification (the latter defined as ×20 and ×40 lenses and ×10 eyepieces [×200 and ×400 magnification]). Large droplets of fat were visible at both magnification levels, and, additionally, small droplets of fat were visible at high magnification. Third, the percentage of hepatocytes that contained fat was visually estimated to the nearest 5% and not assigned to grades. Fourth, the percentage of the whole tissue sample (hepatocytes and other tissue such as fibrosis) that contained fat as viewed with both low and high magnification was visually estimated to the nearest 5%. These alternative histologic methods are not in standard clinical use but were performed for comparison with MR imaging for our study.

MR Data Analysis

The MR spectra were postprocessed to reduce the effects of respiratory motion (6). First, each spectrum was individually Fourier transformed and phase and frequency shifted on the basis of the water peak. Next, spectra with water or lipid peaks more than 25% different from the median spectral peaks were identified and not included in the data. The remaining artifact-free spectra were averaged (6). To calculate the total lipids, the integrated peak areas attributed to CH_2 lipids (at 1.2, 2.0, and 2.2 ppm) and CH_3 lipids (0.8 ppm) were summed. The CH lipids peak at 5.3 ppm was not included, because it was complicated by overlap with water. The peak at 4.6 ppm, representing water, was integrated. The unsuppressed water spectra were similarly analyzed. Area ratios (total lipids/[total lipids + unsuppressed water]) were calculated for each patient. These ratios were corrected for differential T2 decay by using global values for T2 of 66 msec for lipids and 45 msec for water, on the basis of measurements in our population and in the literature (2,3,9). At the repetition time of 2500 msec, signals from both lipids and water were presumed to be at full longitudinal relaxation. Automated spectral results were reviewed by an MR spectroscopist (S.M.N., with 17 years of MR spectroscopy experience), who was blinded to the histologic results.

The in- and out-of-phase MR images were analyzed to obtain estimates of hepatic steatosis. A 2-cm circular region of interest was placed on one 8-mm section of the in- and out-of-phase images in the right lobe midaxillary plane at the level of the portal vein, to approximate a typical biopsy location. An estimated fat fraction was calculated from the in- and out-of–phase images by using the following equation: $(SI_{in} - SI_{out})/(2 \cdot SI_{in})$, where SI_{in} and SI_{out} represent the signal intensities on the in-phase and out-of-phase images, respectively. Assuming that the signal intensity followed the standard, closed-form spoiled gradient-recalled acquisition equation, these measurements were affected by this relationship and the T1 and T2* of water and fat. Global values for T1 and T2* were approximated as 570 msec and 42 msec for liver water and 420 msec and 19 msec for liver fat, on the basis of literature values and measurements in sample patients (2,9). Then, the fat fractions were corrected for T1 and T2* decay given the echo times and repetition times. In addition, the out-of-phase measurement did not take into account that the different lipid peaks are not all exactly out of phase. We adjusted the fat measures for this by assuming

that the percentages of total fat for the peaks were 0.8 ppm, 8%; 1.2 ppm, 84%; 2.0 ppm, 7%; and 2.2 ppm, 1%; on the basis of average relative contributions of peak areas to total lipids in 10 patients with fatty liver disease. This is similar to other correction techniques for multiple fat peaks (10,11). These corrections were performed by an MR physicist (S.M.N., with 17 years of MR imaging experience), who was blinded to the histologic results.

Steatosis Heterogeneity Assessment

The livers were classified as heterogeneous or homogeneous in steatosis grade on the basis of color maps made from the in- and out-of-phase MR images. Liver image pixels were assigned colors that represented values ascribed to the different grades of steatosis (grades 0–3) and to threshold values for grades 0–1, 1–2, and 2–3. This color-mapping assignment was based on a comparison of in- and out-of-phase MR imaging data with standard histologic steatosis grading of biopsy samples as developed, assessed, and reported in Nystrom et al (12). This color-mapping system was designed to provide simple rapid assessment of severity and heterogeneity of liver steatosis. An MR imaging researcher with 3 years of experience in liver MR imaging visually assessed these color maps for heterogeneity. Livers were deemed homogeneous when greater than 90% of the liver was assigned to one steatosis grade. More varied livers were classified as heterogeneous (12).

Comparisons of MR Imaging, MR Spectroscopy, and Histology

The corrected fat fractions measured with MR spectroscopy and in- and out-of-phase MR imaging were compared to confirm similarities. MR measures were then compared with histologic measures. If the MR imaging and MR spectroscopic measures were highly correlated, only the MR spectroscopic measures were used. The MR fat fractions were expected to increase as the levels of fat identified at histologic examination increased. The effect of each of the following was assessed for all patients: *(a)* high magnification versus standard magnification histologic examination, *(b)* heterogeneous versus homogeneous livers, and *(c)* percentage of tissue area containing fat versus percentage of hepatocytes with lipids.

Thresholds to separate grades 0 from 1, 1 from 2, and 2 from 3 were used to define concordance of MR measures with histologic measures. The threshold values were determined on the basis of maximizing the accuracy of concordance between the MR measures and the high-magnification histologic measures. When a range of MR measures yielded the same accuracy (no data points in that range), the threshold was chosen as the midpoint between the two neighboring points. Datapoints that were higher or lower than these thresholds were labeled as high or low discordance, respectively.

The concordance of MR measures with high- versus standard-magnification histologic measures was studied. MR imaging and MR spectroscopy were expected to delineate both large and small droplets of fat in the hepatocytes, as would high-magnification histology. However, small droplets of fat were not visible when standard magnification was used. For patients with small droplets of fat, the MR measures and high magnification histologic measures were expected to reflect higher levels of fat than standard-magnification histologic measures.

Concordance of MR imaging data with high-magnification histologic measures was also compared between heterogeneous and homogeneous livers. Because biopsy samples were taken of a very small portion of the liver, measures of heterogeneous livers were anticipated to show lower concordance between MR imaging and histology.

Last, concordance of MR measures with high-magnification histologic measures was examined by comparing the percentage of tissue area with lipids with the percentage of hepatocytes with lipids (standard practice measure of steatosis). MR measures were anticipated to reflect the percentage of tissue with lipids but not the percentage of hepatocytes with lipids. Differences from these two techniques arise when the amount of lipids in the hepatocytes differs greatly from that in tissues or when the tissue consists of more than hepatocytes (ie, fibrosis). Particularly in patients with fibrosis, MR measures were expected to be more concordant with the percentage of tissue with lipids rather than the percentage of hepatocytes with lipids.

Statistical Analysis

The ages of the men and women were compared for the patient, healthy volunteer, and total populations by using a Student *t* test with Bonferroni correction for multiple comparisons. The corrected fat fractions measured with MR spectroscopy and in- and out-of-phase MR imaging were compared by using linear regression analysis to confirm similarity. MR imaging measures were then compared with histologic measures. When the MR imaging and MR spectroscopic measures were highly correlated, only the MR spectroscopic measures were used. The Fisher exact test was used to assess the rate of upgrade in groups that were concordant with standard histology versus discordant. It was also used to assess the role of heterogeneity versus homogeneity of steatosis concordance versus discordance with high-magnification histologic examination. A P value of .05 was deemed to indicate a statistically significant difference. The JMP statistical software package (SAS Institute, Cary, NC) was used for statistical analyses.

Results

Concordance of MR Imaging and MR Spectroscopic Data with Histologic Measures

The two MR measures of fat fraction, in- and out-of-phase MR imaging and MR spectroscopy, showed similar results (Fig 1). The correlation between the two was high (R^2 = 0.93), although the slope was less than 1.0 (0.89). Because of this strong correlation, comparisons with pathologic measures were performed by using the MR spectroscopic data only.

Figure 1

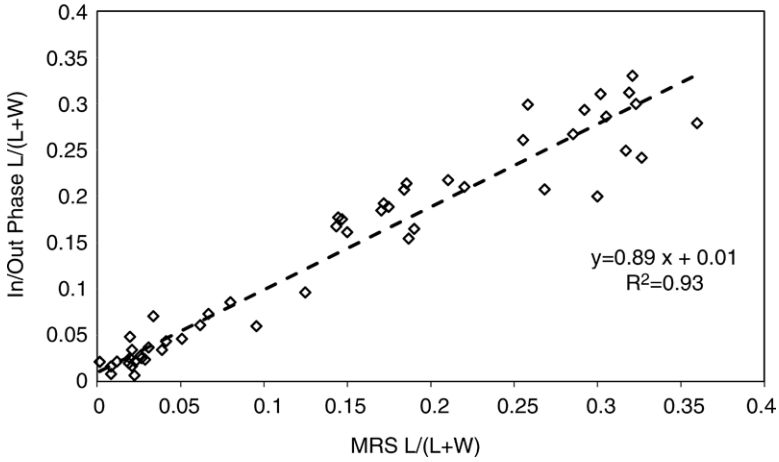

Figure 1: Scatterplot shows good correlation between MR imaging fat fraction and MR spectroscopic fat fraction ($R^2 = 0.93$). In- and out-of-phase MR imaging fat fraction estimates include global corrections for multispectral fat and T1 and T2*. MR spectroscopic fat fraction estimates include global T2 corrections. *L* = lipids; *W* = water.

In the comparison of the MR spectroscopic fat fractions with the standard histologic measures, some MR measures were high in each steatosis grade, and grades 2 and 3 overlapped to a large extent (Fig 2, Table 1). A linear correlation yielded an R^2 of 0.77. When high magnification was used, grades 2 and 3 became better separated, but grade 1 overlapped with grades 0 and 2, in large part because few biopsy specimens remained grade 1 when high magnification was used. Even with these changes, the linear correlation coefficient, R^2, remained at 0.77.

Threshold points of 0.064, 0.134, and 0.205 to separate grades 0 from 1, 1 from 2, and 2 from 3, respectively, were used to define concordance of MR measures with histologic findings. The ranges for each grade are indicated in different shades of gray in Figure 2. When viewed at high magnification, 100% (10 of 10) of specimens from livers that showed high discordance (MR spectroscopic data that were higher than expected compared with steatosis grade at standard-magnification histologic examination) in grades 0–2 were upgraded to the next higher steatosis grade (Fig 2, Table 2). This was a significantly higher rate of upgrading than in the remaining samples in grades 0–2, for which 47% (seven of 15) were upgraded ($P < .01$). In total, 74% (17 of 23) of patients with grades 0–2 steatosis were upgraded, including 63% (five of eight) of those in whom MR measures seemed to be concordant with histologic findings (Fig 2). Cases that were evaluated as grade 3 at standard-magnification histologic examination were excluded from these analyses because they could not be upgraded.

Steatosis Heterogeneity

In our investigation of liver heterogeneity, we determined that 52% (17 of 33) of the patients with steatosis showed heterogeneous and 48% (16 of 33) showed homogeneous steatosis. Of the patients with low discordance of MR measures and histologic findings who were upgraded at high-magnification histology, 86% (six of seven) were heterogeneous, which was a higher percentage than that for the remaining patients (42% [11 of 26]) ($P < .051$). In general, discordance between MR measures and histologic findings was significantly more likely than concordance (75% [nine of 12] vs 38% [eight of 21], $P < .05$) in heterogeneous livers. Homogeneous livers were significantly more likely than heterogeneous livers to show concordance of MR measures with high-magnification histologic findings (81% [13 of 16] vs 47% [eight of 17], $P < .05$).

Fat as Percentage of Tissue Area versus as Percentage of Hepatocytes

In addition, histologic measures of fat as a percentage of tissue area were compared with measures of fat as a percentage of hepatocytes (Fig 3). Fat as a percentage of area was lower than fat as a percentage of cells, but they were roughly linearly correlated. In one case, however, 50% of the hepatocytes had fat, but this represented only 10% of the area. The use of percentage of area for a histologic classification made a difference in this case, in which the MR spectroscopic fat fraction was much lower than expected for grade 2 steatosis but matched that expected for grade 1 steatosis. However, this did not improve the overall correlation in our study.

Examples of MR Imaging, MR Spectroscopy, and Histology

An example fat fraction map based on in- and out-of-phase MR imaging is shown in Figure 4a, and the corresponding MR spectrum is shown in Figure 4b. These MR measures were high compared with the steatosis grade of 2, which was determined at standard magnification histologic examination. The grade assigned at high-magnification examination was grade 3. Respective histologic slides are shown in Figure 4c and Figure 4d. Note that more lipids are visible in the high-magnification slide, leading to an increased percentage of hepatocytes with lipids.

A second example is shown in Figure 5, in which the high magnification steatosis grade is 3, although the fat fractions determined at in- and out-of-phase MR imaging and MR spectroscopy seemed to indicate lower-grade steatosis. This liver was classified as heterogeneous; therefore, the area from which the biopsy sample was taken may have shown a different grade of steatosis than the overall liver or the area examined at MR spectroscopy.

Discussion

Our study showed that, although MR measures and histologic findings of steatosis were generally concordant, discordance between the techniques may partly reflect their different methods of estimating fat percentage. Magnification level played an important role; for cases in which the MR spectroscopy fat fractions were higher than expected, compared with the standard-magnification histologic findings, steatosis grades were significantly more likely to be upgraded at high-magnification histologic examination than were the remaining cases ($P < .02$). Liver heterogeneity also played a role and significantly affected concordance ($P < .05$). Although using the percentage of tissue area with fat as opposed to the percentage of hepatocytes with fat was shown to improve the concordance of MR measures and histologic findings for one case, it did not significantly improve the overall correlation in our study. The patients in our study tended to have low stages of fibrosis. Higher fibrosis stages may lead to more disparate estimates of the percentage of hepatocytes versus the percentage of tissue with fat. The percentage of the tissue with fat may be more important in validating MR measures in a population with greater amounts of fibrosis.

High-magnification histologic evaluation and MR evaluation of steatosis reflected higher levels of fat than did standard-magnification histologic grading. Our study demonstrated that patients with nonalcoholic fatty liver disease had higher steatosis grades when histologic evaluation was made *(a)* at a high-magnification level (81% [17 of 21] of patients with steatosis grades 1 or 2 were upgraded when high magnification was used) or *(b)* when MR imaging was used (48% [10 of 21] of patients with steatosis grades 1 or 2 had higher MR imaging fat fractions than suggested by the standard-magnification histologic grade). Whether this has a clinical effect for these patients is not known.

The correlation of in- and out-of-phase MR imaging with MR spectroscopy in our study was high and was similar to that of others in the literature (4,10,13). Higher correlations have also been reported when additional acquisition and postprocessing approaches that reduce artifacts were used (14,15). These studies further support the idea that the use of MR imaging and MR spectroscopy can yield comparable metrics of liver fat fraction. In the comparison of MR imaging and

Table 1

MR Spectroscopic Fat Fractions and Standard Magnification Histologic Measures of 33 Patients with Biopsy Results

MR Spectroscopic Thresholds	Grade 0	Grade 1	Grade 2	Grade 3	Total
<0.064	4*	3	0	0	7
<0.134	0	3*	0	0	3
<0.205	0	4	5*	1	10
>0.205	0	1	5	7*	13
Total	4	11	10	8	33

Note.—Data are numbers of patients. Data for 15 healthy volunteers are not included; MR spectroscopic fat fraction for these volunteers was <0.064.

* Indicates concordance of MR spectroscopic and histologic data (19 of 33 [58%]).

Figure 2

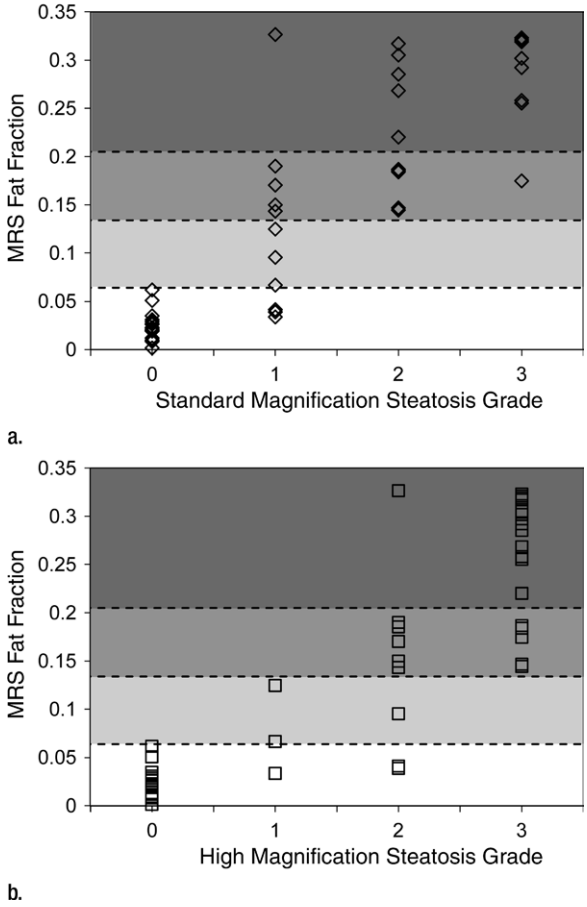

Figure 2: Liver MR spectroscopic fat fractions compared with histologic steatosis grades determined by using **(a)** standard magnification and **(b)** high magnification. Dotted lines = threshold points of steatosis grades.

Table 2

MR Spectroscopic Fat Fractions and High-Magnification Histologic Measures of 33 Patients with Biopsy Results

MR Spectroscopic Thresholds	Grade 0	Grade 1	Grade 2	Grade 3	Total
<0.064	4*	1	2	0	7
<0.134	0	2*	1	0	3
<0.205	0	0	5*	5	10
>0.205	0	0	1	12*	13
Total	4	3	9	17	33

Note.—Data are numbers of patients. Data for 15 healthy volunteers are not included; MR spectroscopic fat fraction for these volunteers was <0.064.

* Indicates concordance of MR spectroscopic and histologic data (23 of 33 [70%]).

Figure 3

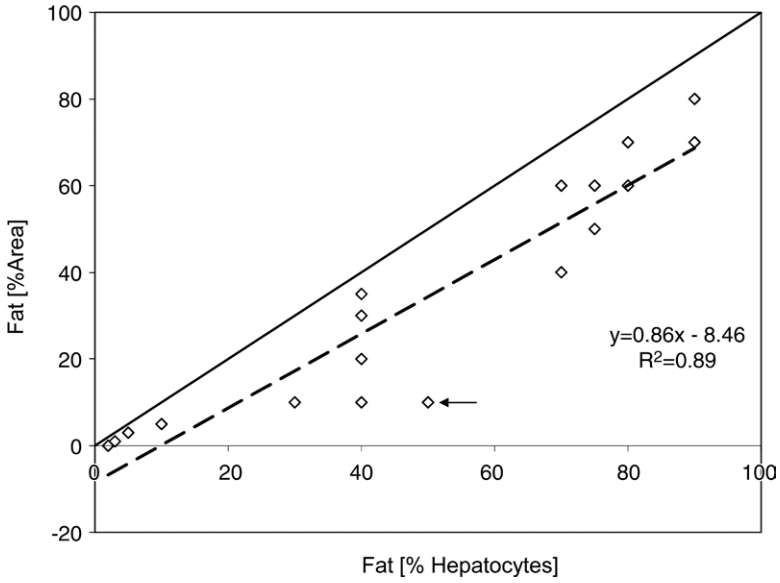

Figure 3: Graph shows that histologic estimates of fat as percentage of tissue area were lower than fat as percentage of hepatocytes. Solid line = unity. Results show good correlation of measures (dashed line = linear fit), except for individual cases. Arrow = outlier in which 50% of hepatocytes and 10% of tissue area were fat.

MR spectroscopic measures to histologic findings, the correlation coefficient we obtained, R^2 = 0.77, was similar to or better than those that others have reported in the literature (R^2 values of 0.5–0.68) (2,3). The authors of these prior studies do not report the magnification levels used for the histologic reference standard; however, the standard protocol is that low- to medium-power evaluation should be used (7). Individual pathologists may, in practice, use higher magnification, but this may not necessarily be recorded. Other researchers have demonstrated a higher correlation than our series, with r_s = 0.93 and r = 0.85, when comparing MR spectroscopy to percentage hepatocytes with fat at histologic examination (4,5).

Fifty-two percent (25 of 48) of the participants in our study were considered to have heterogeneous steatosis. This percentage is similar to those in other reports in the literature, with rates of 20% for grade 1 and 75% for grades 2 and 3 steatosis, in a large, ultrasonography-based study (16) and rates of 45% in an MR imaging–based study (17).

Validation of MR measures of steatosis may be enhanced by modified histologic measures of steatosis rather than standard, clinical methods for increased concordance of the data. Furthermore, our study suggests that most patients with grades 1 or 2 steatosis would be upgraded if they were evaluated histologically at higher magnification. The clinical effect of this is not known. MR measures seem to reflect the presence of both large and small droplets of fat, and they provide data for a larger portion of the liver or the entire liver, which may exhibit heterogeneous steatosis. Thus, our results suggest that MR measures may be more accurate and robust as estimates of steatosis than standard histologic grades. This may be particularly valuable for interventional studies in which an assessment of less than a grade level change in steatosis would be valuable, because MR spectroscopic measures can be provided on a continuous scale. The prevalent heterogeneity in this population also suggests that histology may be more valuable if guided by a prior MR imaging or MR spectroscopic examination. Future studies are needed to validate this.

Our study had several limitations. First, there were a limited number of patients for each steatosis grade, particularly when the high-magnification grading was performed, leaving only 3 patients with grade 1 steatosis. However, we were still able to detect significant findings with this population. Second, tissue sampling errors may have limited our concordance between MR measures and histologic findings. Histologic examination was performed on a single standard biopsy sample. This represented perhaps 1/50000 of the liver and may not have been an adequate representation of the histologic findings in the region of interest examined at MR spectroscopy. Heterogeneity can exist on a small scale, not represented

Figure 4

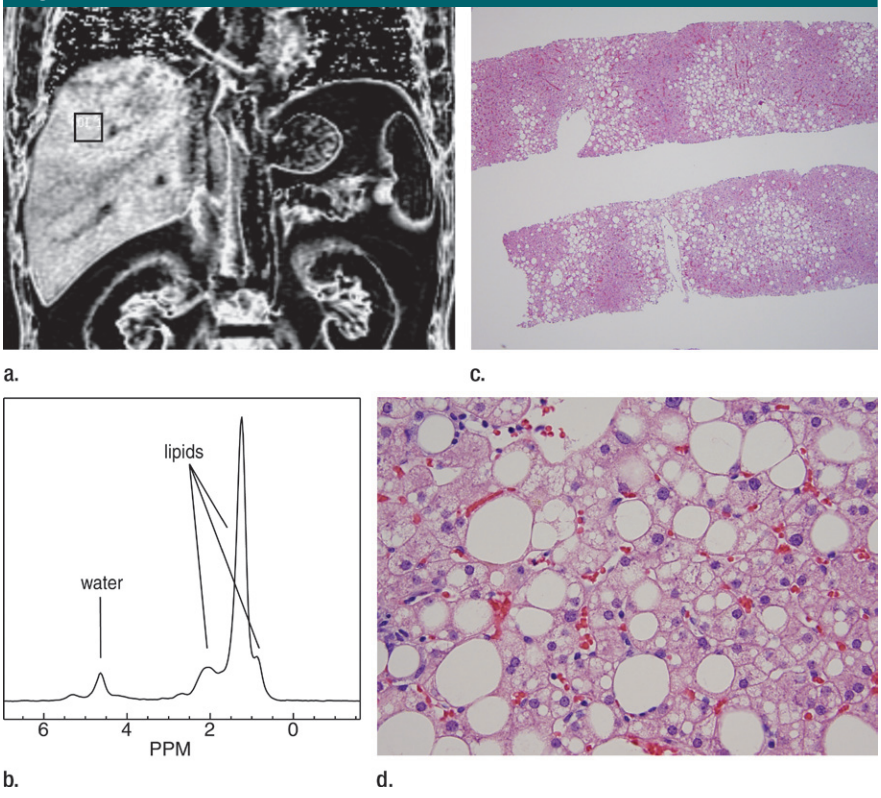

Figure 4: Example of high-magnification histologic examination measures that showed higher steatosis grade, which was concordant with MR imaging data. **(a)** Fat fraction map based on coronal in- and out-of-phase MR imaging (90/2.2 and 90/4.5) yields value of 0.29 in region of interest. **(b)** MR spectrum (2500/27) yields fat fraction of 0.31. **(c)** Histologic slide of liver biopsy specimen viewed at standard magnification highlights only large droplets of fat and yields grade 2 steatosis. (Hematoxylin-eosin stain; original magnification, ×40) **(d)** Same histologic slide at high magnification also shows small droplets of fat and yields grade 3 steatosis (Hematoxylin-eosin stain; original magnification, ×400).

Figure 5

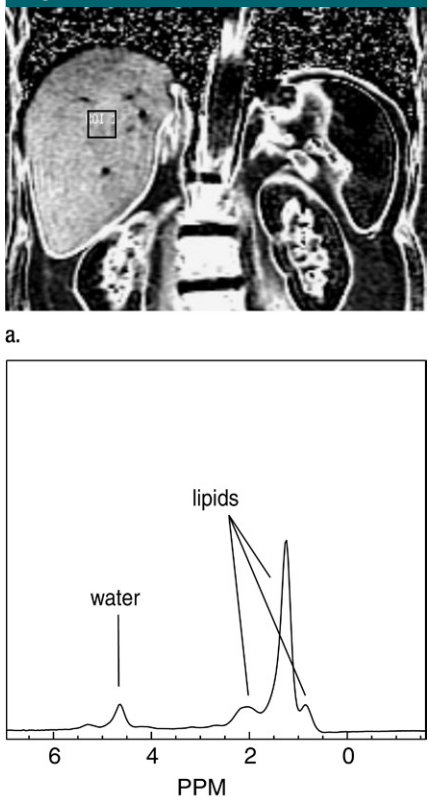

Figure 5: Example of heterogeneous steatosis distribution in which MR measures (fat fraction from in- and out-of-phase MR imaging, 0.15; fat fraction from MR spectroscopy, 0.19) are lower than expected given high-magnification histologic grade (grade 3). **(a)** Fat fraction map based on coronal in- and out-of-phase MR images (120/2.2 and 120/4.4) indicate location of MR spectroscopic voxel. Note low intensity in superior and inferior medial area and high intensity in lateral area. **(b)** MR spectrum (2500/30) corresponds to region of interest in **a**.

by the entire liver determination of heterogeneity. Even in a single biopsy sample, variability in steatosis grade exists and has been shown to be higher with higher levels of steatosis (18). Third, steatosis was visually estimated at histologic examination, and the estimates were grouped into broad grades; errors may have occurred, particularly at the thresholds of steatosis grades. Fourth, we did not take the CH resonance close to water into account in our MR spectroscopic measures of liver fat. This resulted in our MR spectroscopic measures of fat fraction being slightly lower than true values. This effect was anticipated to be negligible. Last, there were factors not taken into account that may have affected the MR imaging estimates of steatosis, such as T1, T2, and T2* relaxation times for the water and lipid peaks that were different from the global values used for corrections and differences in water concentration, because water was used as a normalization factor in these estimates.

In conclusion, our study demonstrated that MR measures and histologic findings of steatosis were generally concordant. Discordance between MR measures and histologic findings of steatosis may partly reflect magnification-related differences in histologic estimation of steatosis and in liver heterogeneity. The percentage of hepatocytes with fat versus the percentage of tissue with fat affected the concordance in only one patient in this population.

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