Non-invasive Monitoring of Hepatic Steatosis via Acoustic Structure Quantification of US with MR Spectroscopy as a Reference Standard

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ABSTRACT

Purpose: To prospectively evaluate whether monitoring hepatic steatosis by untrasonography with an acoustic structure quantification (ASQ) technique is feasible when using magnetic resonance spectroscopy (MRS) as reference standard

Methods: Thirty-six patients with suspected fatty liver disease underwent both untrasonography with ASQ and MRS on the same day. After a mean follow-up period of 11.4±2.5 months, follow-up ultrasonography with ASQ and MRS were performed on 27 patients to evaluate whether hepatic steatosis improved. The focal disturbance (FD) ratio, as calculated using ASQ, and the hepatic fat fraction (HFF), estimated by MRS, were obtained at both initial and follow-up examinations. Pearson’s correlation coefficient was calculated to assess correlations between ordinal values.

Results: The FD ratio showed a strong, negative linear correlation with the HFF after logarithmic transformation of both variables from the initial examinations of 36 patients (ρ=-0.888; P<0.001) and the follow-up examinations of 27 patients (ρ=-0.920; P<0.001). There was also a significant, negative linear correlation between the change in the logarithm of the FD ratio and the change in the logarithm of the HFF by MRS over the follow-up period (ρ=-0.645; P<0.001). In 16 patients with increased FD ratio on follow-up, HFF on follow-up MRS significantly decreased, and HDL level significantly increased whereas LDL shows tendency of decrease.

Conclusions: The FD ratio was significantly correlated with the HFF at both the initial and follow-up examinations, and there was also a significant correlation between the changes in the FD ratio and the changes in the HFF over the follow-up period.
Keywords: Hepatic Steatosis; Acoustic Structure Quantification; MR Spectroscopy;

Monitoring Hepatic Steatosis
INTRODUCTION

Hepatic steatosis is defined as the excessive and abnormal accumulation of intracellular lipids, primarily in the form of triglycerides. Clinically, nonalcoholic fatty liver disease (NAFLD) is the most common type of hepatic steatosis, and it includes a broad spectrum of diseases ranging from simple steatosis to nonalcoholic steatohepatitis (NASH). In addition, NASH can progress to fibrosis/cirrhosis, and end-stage liver disease requiring liver transplantation can eventually develop when it is not appropriately managed [1-4]. Currently, NAFLD is the most common cause of chronic liver disease in Western countries, with a prevalence of approximately 30% in the general population, and the incidence of NAFLD is known to be increasing [5,6]. Therefore, NAFLD is a major emerging health problem, and the early detection and quantitative assessment of NAFLD are of crucial importance for preventing progression to advanced stages, such as cirrhosis.

Traditionally, liver biopsy has been considered the gold standard for the diagnosis and quantification of hepatic steatosis [7]. However, due to its invasive nature and procedure-related complications and mortality, repeated biopsies for monitoring NAFLD should rarely be performed. Therefore, hydrogen 1 (1H) magnetic resonance spectroscopy (MRS), which is an accurate and reproducible method for assessing hepatic steatosis, has been used as an alternative, non-invasive standard of reference [8,9]. In addition, 1H MRS can be used to monitor hepatic steatosis [10]. However, its high cost and the limited availability of MR compared to other non-invasive imaging modalities, such as ultrasonography (US), constitute the downsides of MRS.

Recently, acoustic structure quantification (ASQ), which is a newly developed quantitative analysis method for US reported by Toshiba Medical Systems (Otawara, Japan), has been
introduced to clinical practice. ASQ is based on comparisons between theoretical and real
echo amplitude distributions [11-13], and the theoretical echo amplitude distribution of the
liver region is considered by using the Rayleigh distribution function, based on the
assumption that the speckle pattern is generated by only ultrasound beam interference from
very small scattering objects that are located closer than the ultrasound beam wavelength [11-
13]. However, the real echo amplitude distribution of the liver parenchyma does not actually
fit a Rayleigh distribution because of the presence of small structures, such as the hepatic
vessel walls, which scatter the ultrasound beam and cause heterogeneity in the echo
amplitude [11-13]. By comparing the theoretical echo amplitude distribution to a real one,
ASQ can provide quantitative information regarding the parenchymal echotexture changes in
diffuse liver diseases, such as steatosis. According to the results of recent studies, the focal
disturbance (FD) ratio calculated using the ASQ technique was significantly associated with
the degree of hepatic steatosis assessed by histopathology, and there was a strong, negative
correlation between the FD ratio and the estimated fat fraction assessed by MRS [12,13].
Given these findings, the FD ratio calculated using the ASQ technique could be used to
monitor hepatic steatosis. However, the utility of the ASQ technique as a tool for monitoring
hepatic steatosis has not been evaluated. Therefore, the purpose of our study was to
prospectively evaluate whether monitoring hepatic steatosis by using US with ASQ was
feasible when using $^1$H MRS as the reference standard in a fatty liver disease patient cohort.
MATERIALS and METHODS

Study Population

Institutional review board of our Hospital approved this prospective study, and written informed consent was obtained from all of the enrolled patients. From November 2014 to April 2015, we enrolled 36 patients who visited our institution for suspected or verified hepatic steatosis and were subjected to routine US examinations. The electronic medical records and past medical histories of all patients were reviewed to confirm that they did not have any past medical histories or clinical symptoms or signs of liver disease or systemic or malignant diseases. The laboratory findings, including liver function tests obtained within 3 months before the imaging examinations, were also checked in all of the patients. All patients underwent both US examinations that used the ASQ technique and $^1$H-MRS examinations on the same day. After the initial imaging examination, lifestyle interventions were conducted in all of the enrolled patients, with dietary counseling aimed to reduce body weight. The patients were also instructed to perform at least 3 hours of moderate physical activity per week, and aerobic endurance exercise, including walking and swimming, was encouraged [14-17]. All of the enrolled patients were seen by a physician (M.S.P.) on a regular basis. After a mean and median follow-up of 11.4 ± 2.5 (standard deviation) months and 12.0 months (range, 6-15 months), respectively, follow-up US examinations using ASQ, $^1$H-MRS examinations and laboratory tests, including liver function tests, were performed to evaluate whether hepatic steatosis improved in 27 of the 36 patients; follow-up US and MRS examinations were done on the same day. Nine patients (9/36, 25.0%) refused to undergo follow-up imaging examinations. The characteristics of the patient cohort are summarized in Table 1.
US examinations

All of the US examinations were performed by one of the two authors (J.Y.L. and D.H.L. with 20 and 9 years of experience, respectively, in liver US imaging) using an US scanner (Aplio XG; Toshiba Medical Systems, Otawara, Japan) with a 5-MHz convex transducer. All patients fasted for at least 6 hours before the imaging examinations. US examinations were done with patients in the supine position with their right arm extended above their head to stretch the intercostal muscle for a better sonic window; the same patient position was used in both the initial and follow-up examinations. First, a B-mode US examination of the liver was performed, including transverse and sagittal images, to evaluate the presence of any possible focal liver lesions. Then, US images in ASQ mode were acquired five times each from the right intercostal view and the right subcostal view. All five regions of interest (ROIs) were placed in the same intercostal and right subcostal views. The display depth and transmit focus were set to 10 cm and 6 cm, respectively. The ROIs were as large as possible and were placed on the liver parenchyma; care was taken to avoid large hepatic vessels or artifacts. The value of the FD ratio was calculated by the system and was displayed on the monitor (Figure 1). The mean FD ratio of five measurements was calculated for each patient and recorded for further analysis. Detailed methods and a description of the ASQ technique and the calculation of the FD ratio are provided in the appendix.

$^{1}$H-MR Spectroscopy

All $^{1}$H-MRS examinations were performed on a clinical 3.0 T MR scanner (Trio A Tim System, Siemens Healthcare, Erlangen, Germany) with a 32-channel phased-array surface...
coil on the same day of the US examinations. First, sagittal, coronal and axial sections that covered the whole liver were obtained to position the spectroscopy acquisition voxel. Then, the 3 x 3 x 3 cm-sized signal voxel was placed in the right hepatic lobe dome area of Couinaud segment VII or VIII, with care taken to avoid large blood vessels or bile ducts and liver edges. For the fat fraction spectroscopy measurements, a modified stimulated-echo acquisition sequence (echo times msec/repetition time msec, 12, 24, 36, 48 and 72/3000) was employed within the single voxel. Each acquisition was performed during a single breath hold with a scan time of 15 seconds. T2 correction for both the water and lipids was performed to calculate the hepatic fat fraction (HFF), and each T2 value of water and fat was calculated separately. With the use of an exponential fit of the points acquired at five different echoes by automatic fitting of water (4.7 ppm) and the major fat peak of methylene (1.3 ppm), extrapolation of fat and water integrals for an echo time of 0 msec was performed for fat quantification [8,18]. The calculated HFF was automatically displayed as a percentage in Distal Imaging and Communications in Medicine format and was recorded for further analysis.

**Statistical analysis**

The estimated HFF by MRS and the FD ratio calculated using the ASQ technique are expressed as the mean ± standard deviation throughout the results. We used Pearson’s correlation coefficient to assess the correlation between the FD ratio and the HFF. After the initial examination, 27 of the 36 patients underwent follow-up US examinations using the ASQ technique and 1H-MRS examinations, and the changes in the FD ratio, as well as the
estimated HFF by MRS, between the initial and follow-up examinations were calculated. Using the changes in FD ratio calculated by ASQ over the follow-up period, patients were classified into two groups: patients with increased FD ratio during the follow-up and patients with decreased FD ratio during the follow-up. Differences in the estimated HFF on MRS as well as in other patients characteristics between the initial and follow-up examinations were assessed according to the patient groups using the Wilcoxon signed rank test. All statistical analyses were performed using SPSS software, version 21 (SPSS, Chicago, IL, USA), and MedCalc software, version 12.1.0.0 (MedCalc Software, Mariakerke, Belgium).
RESULTS

FD Ratio and Estimated HFF for Initial Examinations

The mean estimated HFF calculated from the initial MRS examinations of all 36 patients was 16.7 ± 9.7% (range: 1.8% to 39.3%), and the mean FD ratio calculated using the ASQ technique for the initial US examinations was 0.069 ± 0.097 (range: 0.02 to 0.56). The correlation coefficient (ρ) between the FD ratio calculated using the ASQ technique and the estimated HFF by MRS was -0.526 (95% confidence interval [CI]: -0.239, -0.729). However, a scatter plot of the estimated HFF and the FD ratio showed an exponential relationship. When these two variables were log-transformed, there was a strong, negative linear correlation between the logarithm of the FD ratio and the logarithm of the HFF (Figure 2), with a correlation coefficient (ρ) of -0.888 (95% CI: -0.790, -0.942, P<0.001).

FD Ratio and Estimated HFF for Follow-up Examinations

Follow-up US examinations using the ASQ technique and 1H-MRS examinations were performed on 27 patients, and the mean estimated HFF calculated from the follow-up MRS examinations was 14.0 ± 9.3% (range: 1.0% to 32.0%). The mean FD ratio calculated using the ASQ technique for the follow-up US examinations was 0.096 ± 0.113 (range; 0.002 to 0.53). The correlation coefficient (ρ) between the FD ratio calculated using the ASQ technique for the follow-up examinations and the estimated HFF according to the follow-up MRS examinations in these 27 patients was -0.637 (95% CI: -0.338, -0.819). A scatter plot of the estimated HFF and the FD ratio obtained from the follow-up examinations also showed an exponential relationship. There was a strong, negative linear correlation between the
logarithm of the FD ratio obtained from the follow-up US examinations and the ASQ technique and the logarithm of the HFF for the follow-up MRS examinations, and the correlation coefficient (ρ) was -0.920 (95% CI: -0.831, -0.963; P<0.001) (Figure 3).

**Changes in the FD ratio over the follow-up period and Correlation with the HFF**

Among the 27 patients who underwent follow-up examination, FD ratio calculated by ASQ was increased on follow-up US compared to initial US in 16 patients and decreased in 11 patients. The correlation coefficient (ρ) between the change in the FD ratio and the change in the estimated HFF was -0.553 (95% CI: -0.219, -0.771). A scatter plot of the change in the estimated HFF and the change in the FD ratio over the follow-up period also showed an exponential relationship. There was also a significant, negative linear correlation between the change in the logarithm of the FD ratio over the follow-up period and the change in the logarithm of the HFF over the follow-up period by MRS, and the correlation coefficient (ρ) was -0.645 (95% CI: -0.351, -0.823; P<0.001) (Figure 4).

**Changes in the patient characteristics according to patients group over the Follow-up Period**

Changes in the patient characteristics over the follow-up period according to patient group determined by changes in FD ratio calculated using ASQ are summarized in Table 2. Body weight was decreased on follow-up examination in both patient groups, but statistically significant difference was not achieved. The estimated HFF on follow-up MRS significantly
decreased in 16 patients with increased FD ratio on follow-up US with ASQ technique (P=0.003). In addition, high density lipoprotein (HDL) level was significantly increased on follow-up examination in 16 patients with increased FD ratio (P=0.035). There was a tendency of reduction in low density lipoprotein (LDL) level in follow-up examination in 16 patients with increased FD ratio (P=0.084). In 11 patients with decreased FD ratio on follow-up US with ASQ technique, estimated HFF increased on follow-up MRS without statistically significant difference (P=0.248). Other patient characteristics were not significantly different on follow-up examination in 11 patients with decreased FD ratio.
DISCUSSION

In our study, the FD ratio calculated using the ASQ technique showed a significant, negative correlation with the estimated HFF by MRS at both the initial and follow-up examinations, and this result was well correlated with the results of a previous study by Son et al [13]. In addition, there was a significant correlation between the change in the FD ratio over the follow-up period and the change in the HFF over the follow-up period by MRS. Increased FD ratio on follow-up US with ASQ technique compared to initial examination was significantly associated with the decreased HFF on follow-up MRS. Moreover, HDL level was significantly increased and LDL level showed tendency of decrease in 16 patients with increased FD ratio on follow-up examination which could mean the improvement of lipid panel.

Because NAFLD encompasses a broad spectrum ranging from simple steatosis to cirrhosis and can progress into more advanced stages during the disease course, the quantitative assessment and monitoring of this disease are important for patient management. US is a non-invasive imaging modality for assessing liver disease with a relatively lower cost compared to MR, and it can be performed repeatedly during the disease course with no risk to the patient. Given this finding, US could potentially be a good imaging modality for monitoring fatty liver disease. When the echogenicity of the liver parenchyma increases compared to that of the renal cortical parenchyma on B-mode US, it is usually considered to indicate the presence of hepatic steatosis. However, the assessment of hepatic parenchymal echogenicity by B-mode US is subjective in nature. In addition, quantifying the degree of hepatic steatosis is only barely possible by using B-mode US images alone. To overcome these drawbacks of B-mode US, several amendments, including the hepatorenal ratio, have been suggested for
the quantitative assessment of hepatic steatosis, with relatively good performance [19-21]. However, although Mancini et al reported that the hepatorenal ratio showed an excellent correlation with the degree of steatosis evaluated by MRS [22], whether this ratio has sufficient diagnostic performance for assessing hepatic steatosis or could be used an alternative tool to MRS remains questionable [13]. In addition, other studies have reported that additional processes, such as the standardization or use of artificial neural networks, would be needed to attain the sufficiently high diagnostic performance of the hepatorenal ratio for the quantitative assessment of hepatic steatosis [21,23].

In the ASQ technique, the theoretical echo amplitude distribution, which is believed to be a function of the Rayleigh distribution, is compared to a real echo amplitude distribution, which does not actually fit a Rayleigh distribution, mainly due to the presence of small structures, including hepatic vessels, that scatter the US [11-13]. As the degree of hepatic steatosis progresses, the small structures in the liver, including small vessels, are blurred with the increased hepatic parenchymal echogenicity; in other words, hepatic parenchymal echogenicity might become more homogeneous. In such cases, the real echo amplitude distribution of the liver parenchyma can become similar to the theoretical echo amplitude distribution (i.e., the Rayleigh distribution) [13]. Comparing the theoretical echo amplitude distribution to a real one, the FD ratio calculated by using the ASQ technique could provide quantitative information regarding the hepatic parenchymal echo texture changes that occur during the progression of hepatic steatosis. Our study results clearly showed a significant correlation between the FD ratio calculated by the ASQ technique and the estimated HFF by MRS at both the initial and follow-up examinations. In addition, the change in the FD ratio over the follow-up period was significantly correlated with the change in the estimated HFF.
by MRS, and increased FD ratio on follow-up examination was significantly associated with the decreased HFF on follow-up MRS. Considering this result, the FD ratio calculated by using ASQ technique could potentially be used to quantitatively monitor fatty liver disease.

Nowadays, NAFLD has been considered as hepatic manifestation of metabolic syndrome which is characterized by concomitant existence of type 2 diabetes, hypertension, dyslipidemia and atherosclerotic cardiovascular disease [24]. In addition, Sinn et al recently reported that NAFLD was significantly associated with the development of coronary calcified plaque independent of cardiovascular and metabolic risk factors [25]. Therefore, accurate evaluation and monitoring of NAFLD could be quite important for reducing the potential mortality and morbidity from cardiovascular disease. In this regards, quantitative assessment of hepatic steatosis using US with ASQ technique would be helpful in management of NAFLD patients as our study results showed FD ratio calculated by ASQ technique was significantly correlated with estimated HFF on MRS which is reference of standard non-invasive diagnostic method of NAFLD. In addition, increased FD ratio on follow-up examination was also associated with significantly increased HDL level and tendency of decrease in LDL level on follow-up examination which could mean the improvement of lipid panel. Considering our study results, US examination using ASQ technique could be a promising monitoring method not only for hepatic steatosis but also for the metabolic syndrome.

Our study had some limitations. First, in our study, $^1$H-MRS examination, instead of histologic examination, was used as a reference standard to quantitatively assess hepatic steatosis. Therefore, the relationship between the FD ratio calculated using the ASQ technique and histologic changes could not be evaluated in our study. However, many clinical
trials still use $^1$H-MRS as the reference standard to assess the degree of hepatic steatosis due to its accuracy and its quantitative and noninvasive nature. Second, the number of patients was relatively small, especially those undergoing follow-up examinations. Third, we did not perform liver biopsy nor elastography. Therefore, we could not assess the effect of fibrosis/inflammation on the FD ratio. In addition, according to the result of recent animal study done by Lee et al., the degrees of both steatosis and fibrosis were significant factors affecting the FD ratio calculated by ASQ although the correlation coefficient for steatosis was higher than that for fibrosis [26]. Indeed, it is unclear whether the FD ratio calculated using the ASQ technique can provide good diagnostic performance for quantitatively assessing hepatic steatosis in patients with both steatosis and inflammation/fibrosis. Further studies with prospective designs and large numbers of patients with various stages of fatty liver disease, including steatosis and cirrhosis, are warranted to address this issue. Fourth, as the MRS signal voxel was placed on the right liver dome where accurate US evaluation would be difficult, there could be some differences in the area evaluated using the ASQ technique from the area assessed by MRS; therefore, there would be some limitations regarding the accurate evaluation of the correlation between the FD ratio calculated by ASQ and the estimated HFF by MRS.

**Conclusions**

the FD ratio calculated using the ASQ technique was significantly correlated with the estimated HFF by MRS at both the initial and follow-up examinations, and there was a significant correlation between the changes in the FD ratio and the changes in the HFF over
the follow-up period, which suggest the ability of the FD ratio to quantitatively monitor fatty liver disease.
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hepatic/renal ratio and hepatic attenuation rate to quantify liver fat content: an improvement method. Obesity (Silver Spring) 2012;20:444-452.


## TABLES

Table 1. Baseline characteristics of the 36 patients with suspected fatty liver disease.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>20-71 (50.4 ± 13.3)</td>
</tr>
<tr>
<td>Range (mean ± SD)</td>
<td></td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>21 (58.3%)</td>
</tr>
<tr>
<td>Female</td>
<td>15 (41.7%)</td>
</tr>
<tr>
<td><strong>Diabetes, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>10 (27.8%)</td>
</tr>
<tr>
<td>No</td>
<td>26 (72.2%)</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17 (47.2%)</td>
</tr>
<tr>
<td>No</td>
<td>19 (52.8%)</td>
</tr>
<tr>
<td><strong>Hyperlipidemia, n (%)</strong></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>21 (58.3%)</td>
</tr>
<tr>
<td>No</td>
<td>15 (41.7%)</td>
</tr>
<tr>
<td><strong>Serum albumin (mg/dL)</strong></td>
<td>4.5 ± 0.2 (4.1-4.9)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Total bilirubin level (mg/dL)</strong></td>
<td>0.90 ± 0.31 (0.6-1.7)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Alanine aminotransferase (ALT, IU/dL)</strong></td>
<td>49.6 ± 43.2 (14-220)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Body weight (kilograms)</strong></td>
<td>80.4 ± 18.6 (55-124)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Body mass index</strong></td>
<td>28.4 ± 4.62 (22-38.5)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Total Cholesterol (mg/dL)</strong></td>
<td>194.2 ± 38.5 (125-267)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Triglyceride (mg/dL)</strong></td>
<td>164.8 ± 92.3 (45-433)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Low density lipoprotein (mg/dL)</strong></td>
<td>124.3 ± 44.3 (54-216)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>High density lipoprotein (mg/dL)</strong></td>
<td>49.9 ± 12.5 (32-90)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
<tr>
<td><strong>Estimated hepatic fat fraction by MRS (%)</strong></td>
<td>16.7 ± 9.7 (1.8-39.3)</td>
</tr>
<tr>
<td>mean ± SD (range)</td>
<td></td>
</tr>
</tbody>
</table>

Note: SD=standard deviation; MRS=magnetic resonance spectroscopy
Table 2. Changes in parameters in the 27 patients who underwent follow-up examinations according to the patient group determined by change of FD ratio calculated by ASQ technique on follow-up examination

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Increased FD ratio on Follow-up (n=16)</th>
<th>Decreased FD ratio on Follow-up (n=11)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>Follow-up</td>
</tr>
<tr>
<td>Body weight (kilograms)</td>
<td>82.8±16.9</td>
<td>79.8±13.5</td>
</tr>
<tr>
<td>Albumin (mg/dL)</td>
<td>4.6±0.2</td>
<td>4.5±0.2</td>
</tr>
<tr>
<td>Total Bilirubin (mg/dL)</td>
<td>1.08±0.37</td>
<td>0.99±0.25</td>
</tr>
<tr>
<td>ALT (IU/dL)</td>
<td>59.8±56.5</td>
<td>47.8±32.9</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>188.5±40.9</td>
<td>185.9±33.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>146.3±71.9</td>
<td>136.4±44.4</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>128.6±44.4</td>
<td>109.8±35.7</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>47.1±8.4</td>
<td>50.7±9.8</td>
</tr>
<tr>
<td>Fat fraction on MRS (%)</td>
<td>17.6±11.8</td>
<td>13.3±8.8</td>
</tr>
</tbody>
</table>

†: P-value was determined by Wilcoxon signed rank test

Note FD ratio=focal disturbance ratio; ASQ=acoustic structure quantification; ALT= Alanine aminotransferase; LDL=low density lipoprotein; HDL=high density lipoprotein; MRS=magnetic resonance spectroscopy
Legends for Figures

Fig. 1. Initial and follow-up US examinations using the ASQ technique and MRS examinations in a 29-year-old man with fatty liver. (a) Upon initial examination, the patient’s body weight was 115 kg, and he had a BMI of 36. On an ASQ mode US image, the ROI was placed on the liver parenchyma, and care was taken to avoid large hepatic vessels or artifacts. The FD ratio calculated by ASQ in this patient was 0.03. (b) The estimated HFF according to the initial MRS examination of this patient was 10.14%. (c) During the 10-month follow-up period, the patient lost 21 kg of his body weight, and his BMI at the follow-up examination was 30. The FD ratio calculated by ASQ for the follow-up examination was 0.25. (d) The estimated HFF for the follow-up MRS examination was 2.93%.

Fig. 2. Correlation between the FD ratio and the HFF for the initial examinations of all 36 patients. Regression plot showing a strong negative linear correlation between the FD ratio and the HFF after the logarithmic transformation of both variables; the correlation coefficient was -0.888 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.

Fig. 3. Correlation between the FD ratio and the HFF for the follow-up examinations of 27 patients. Regression plot showing a strong negative linear correlation between the FD ratio and the HFF after the logarithmic transformation of both
variables; the correlation coefficient was -0.920 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.

**Fig. 4. Correlation between changes in the FD ratio and the HFF over the follow-up period in the 27 patients who underwent follow-up US with ASQ and MRS.** Regression plot showing a significant negative linear correlation between changes in the logarithm of the FD ratio and the logarithm of the HFF; the correlation coefficient was -0.645 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.
Fig. 1. Initial and follow-up US examinations using the ASQ technique and MRS examinations in a 29-year-old man with fatty liver. (a) Upon initial examination, the patient’s body weight was 115 kg, and he had a BMI of 36. On an ASQ mode US image, the ROI was placed on the liver parenchyma, and care was taken to avoid large hepatic vessels or artifacts. The FD ratio calculated by ASQ in this patient was 0.03. (b) The estimated HFF according to the initial MRS examination of this patient was 10.14%. (c) During the 10-month follow-up period, the patient lost 21 kg of his body weight, and his BMI at the follow-up examination was 30. The FD ratio calculated by ASQ for the follow-up examination was 0.25. (d) The estimated HFF for the follow-up MRS examination was 2.93%.
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Figure 1d

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Figure 2

Fig. 2. Correlation between the FD ratio and the HFF for the initial examinations of all 36 patients. Regression plot showing a strong negative linear correlation between the FD ratio and the HFF after the logarithmic transformation of both variables; the correlation coefficient was -0.888 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.
Figure 3

Fig. 3. Correlation between the FD ratio and the HFF for the follow-up examinations of 27 patients. Regression plot showing a strong negative linear correlation between the FD ratio and the HFF after the logarithmic transformation of both variables; the correlation coefficient was -0.920 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.
Fig. 4. Correlation between changes in the FD ratio and the HFF over the follow-up period in the 27 patients who underwent follow-up US with ASQ and MRS. Regression plot showing a significant negative linear correlation between changes in the logarithm of the FD ratio and the logarithm of the HFF; the correlation coefficient was -0.645 (P<0.001). The solid line indicates the regression line, and the lines with small dots are the 95% confidence intervals.