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Cross-sectional and longitudinal evaluation of liver volume and total liver fat burden in adults with nonalcoholic steatohepatitis

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Abstract

Purpose—To explore the cross-sectional and longitudinal relationships between fractional liver fat content, liver volume, and total liver fat burden.

Methods—In 43 adults with non-alcoholic steatohepatitis participating in a clinical trial, liver volume was estimated by segmentation of magnitude-based low-flip-angle multiecho GRE images. The liver mean proton density fat fraction (PDFF) was calculated. The total liver fat index (TLFI) was estimated as the product of liver mean PDFF and liver volume. Linear regression analyses were performed.

Results—Cross-sectional analyses revealed statistically significant relationships between TLFI and liver mean PDFF ($R^2 = 0.740$ baseline/ 0.791 follow-up, $P < 0.001$ baseline/ $P < 0.001$ follow-up), and between TLFI and liver volume ($R^2 = 0.352/0.452$, $P < 0.001/ < 0.001$). Longitudinal analyses revealed statistically significant relationships between liver volume change and liver mean PDFF change ($R^2 = 0.556$, $P < 0.001$), between TLFI change and liver mean PDFF change ($R^2 = 0.920$, $P < 0.001$), and between TLFI change and liver volume change ($R^2 = 0.735$, $P < 0.001$).

Conclusion—Liver segmentation in combination with MRI-based PDFF estimation may be used to monitor liver volume, liver mean PDFF, and TLFI in a clinical trial.

Keywords

Liver; Fatty Liver; Magnetic Resonance Imaging; Organ Size; Biological Markers

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is now recognized as the hepatic component of the metabolic syndrome, a cluster of conditions including obesity, dyslipidemia, type 2 diabetes, and hypertension [1,2]. The hallmark feature of NAFLD is steatosis, the presence of intra-hepatocellular fat vacuoles. NAFLD is associated with an increased risk of cirrhosis, hepatocellular carcinoma, cardiovascular disease, and diabetes [3–7]. Clinical trials in the treatment of NAFLD and nonalcoholic steatohepatitis (NASH) have examined the effect of behavior modification [8,9] and pharmacologic therapy [10–14] on liver fat content.

Currently, virtually all methods to evaluate liver fat content, whether tissue- or radiology-based, assess fat content on a fractional basis – the amount of fat per unit area or volume. The most common tissue-based method is biopsy with histology analysis. This method assesses fractional liver fat content as the percentage of hepatocytes containing fat droplets [15,16] or the percentage of the surface area in a histological section ascribed to fat droplets [17,18]. A less common tissue-based method is biochemical lipid extraction, which estimates fractional liver fat content as the concentration (i.e., fraction by weight) of lipids within a small tissue specimen [19]. The most direct radiology-based methods to assess fractional liver fat content are magnetic resonance (MR) spectroscopy (MRS) [20,21] and MR imaging (MRI) [22–26]. Performed properly, these methods estimate the liver proton density fat fraction (PDFF), defined as the proportion of mobile protons in liver attributable to fat [27–29,23–25,30,31]. Other radiology methods assess fractional liver fat content indirectly. For example, computed tomography (CT) and ultrasound (US) can measure the attenuation of x-rays (CT) [32,33] or the attenuation and backscatter of an ultrasound beam (US) [34,35] by hepatic tissue. These CT- and US-based parameters may be used to estimate fractional fat content based on empirical calibration with a reference measurement of fractional fat content such as histology or MRI/MRS [32,36,37].

Measuring liver fat content on a fractional basis may be problematic, however, as liver fat may be distributed heterogeneously, and sampling variability may occur when fractional fat content is quantified only in select portions of the liver. Moreover, measuring fractional liver fat content may provide an incomplete picture of total liver fat burden, which relates not only to the fractional fat content but also to liver volume. Liver volumes vary across the population, and individuals with similar fractional fat content but dissimilar liver volumes will have different total liver fat burdens, which conceivably could convey different cardiovascular or metabolic risk. Moreover, longitudinal human studies suggest that changes in fractional fat content are accompanied by changes in liver volume [38–40]. The changes in liver volumes vary across individuals [38–40], and individuals with similar changes in fractional fat content but dissimilar changes in liver volume will have different changes in total liver fat burden, which conceivably could be clinically meaningful.

The cross-sectional relationships between fractional liver fat content, liver volume, and total liver fat burden and the longitudinal relationships between changes in these measurements are not yet understood, in part because until recently the technology to properly measure these parameters non-invasively was not available. Advanced MRI techniques that estimate the PDFF may be suitable for this purpose as they can acquire images of the entire liver and

thereby permit simultaneous estimation of fractional fat content, liver volume and, by integrating the fractional fat content over the liver volume, total liver fat burden.

The purpose of this proof-of-concept study was to explore in adults with NASH the cross-sectional relationships between fractional liver fat content, liver volume, and total liver fat burden as well as the longitudinal relationships between changes in these measurements. To assess total liver fat burden, we developed and evaluated a novel biomarker, total liver fat index (TLFI), defined as the product of the segmented liver volume by the liver mean PDFF within the segmented volume.

MATERIALS AND METHODS

Study Design

This was a secondary analysis of a recently conducted randomized, double-blind, placebo-controlled clinical trial that investigated the efficacy of colesevelam, a bile acid sequestrant, in the treatment of adults with NASH. The clinical trial included, at baseline and at end of treatment, MRI examinations incorporating a liver PDFF estimation sequence. One of the clinical trial endpoints was change in MRI-estimated liver PDFF in user-defined regions of interest (ROIs) within each Couinaud segment; these results are published elsewhere [references omitted for submission to maintain blinding to authors]. In this secondary analysis, we retrospectively analyzed the clinical trial MR images to calculate for each subject and time point three parameters not previously measured or reported: liver volume, mean PDFF across the entire volume, and TLFI. Both the clinical trial and this secondary analysis were approved by the [institution name withheld to maintain blinding to authors] IRB and is HIPAA compliant. Written informed consent was obtained from all patients.

Subject Selection

Between September 2009 to January 2011, fifty adult patients with biopsy-proven NASH were randomized to treatment for 24 weeks with either colesevelam or placebo. Two of the 25 patients randomized to colesevelam and three of the 25 randomized to placebo discontinued treatment. Additionally, in two patients randomized to colesevelam, the MRI PDFF estimation sequence did not provide whole-liver coverage and hence was unsuitable for calculation of the three parameters listed above. Thus, this study included 21 patients in the colesevelam group and 22 in the placebo group with baseline and end-of-treatment MRI examinations suitable for the required analyses (Table 1).

MRI PDFF Estimation Sequence

To estimate liver PDFF, patients were examined supine with a standard torso eight-channel phased-array coil centered over the liver at 3.0 T (Signa Excite HD; GE Healthcare, Waukesha, WI). Unenhanced contiguous axial images covering the entire liver, including at least one slice above the superior edge of the liver and at least one slice below the inferior edge, were obtained in a single breath hold at end-inspiration by using a two-dimensional multiecho spoiled gradient-recalled-echo sequence with all array coil elements. Acquisition time ranged from 12 to 34 seconds. To minimize T1 effects, a low flip angle (10°) was used [41,27,42] at a repetition time of 120 - 270 ms, adjusted by the technologist to individual

breath-hold capacity. To permit estimation of and correction of T2* effects [41,43,27], six echoes were obtained at serial opposed-phase and in-phase echo times (1.15, 2.3, 3.45, 4.6, 5.75, and 6.9 ms at 3.0 T). As this was a multiecho sequence, images at each echo time were co-registered. Other imaging parameters were 8 mm section thickness, 0% interslice gap, 1480 Hz/pixel receiver bandwidth, one signal average, base matrix of 192×160 – 192 , and rectangular field of view adjusted to individual body habitus and breath-hold capacity.

Segmentation and image analysis

A trained image analyst (one year of experience) and an abdominal radiologist (six years of experience) independently segmented the liver contour manually on the MR images using a custom-built plug-in developed in MATLAB (MathWorks, Natick, MA), excluding large vessels abutting the liver periphery such as the inferior vena cava and main portal vein, but not vessels surrounded by liver parenchyma. Segmentation was performed on the first out-of-phase echo of the multiecho gradient-recalled-echo sequence, as this set of images consistently delineated the liver boundary. A contraction of one pixel was applied around the periphery to exclude chemical-shift artifact at the edge of the liver. Liver volume was calculated by summing the liver surface area of each segmented slice, and multiplying the sum by the nominal slice thickness. The segmentation was then propagated automatically onto the co-registered source images acquired at the other echo times. PDFF parametric maps of the entire liver then were generated pixel by pixel from the segmented multi-echo source images using a nonlinear least-square fitting algorithm. This algorithm measures and corrects for T2* signal decay while taking into account the multi-frequency interference effects of protons in fat using a triglyceride model from spectroscopy measurements of human liver fat *in vivo* [23,44,20,45]. The processing steps described above are illustrated in Figure 1. It took each reader about 20 minutes per case to perform the processing. The exact times were not recorded.

TLFI Calculation

The TLFI (units: % • mL) was calculated as the product of liver volume and liver mean PDFF:

$$TLFI = (\text{Liver volume}) \frac{\sum_{i=1}^n (PDFF_i)}{n}$$

where *liver volume* is the total segmented liver volume, *n* is the total number of voxels in the segmented volume, and $PDFF_i$ is the PDFF in the *i*th voxel. This is mathematically equivalent to the integration of the fat content in each voxel, defined as the product of voxel volume and voxel PDFF, over the entire liver volume.

Intra- and inter-observer agreement

To estimate intra- and inter-observer agreement of liver volume, liver mean PDFF, and TLFI measurements, the image analyst and radiologist independently segmented 10 examinations from 5 cases, randomly selected. Repeated segmentation was performed one week later using identical methods. Observers were blinded to their first measurement results, and the results of the other observer.

Liver biopsy and histopathological analysis

Liver biopsy was performed as part of the clinical trial to which the present study is ancillary. Steatosis, lobular inflammation, hepatocellular iron, fibrosis, steatohepatitis, and NAFLD activity score were scored by a single expert hepatopathologist using the NASH Clinical Research Network histologic scoring system [16].

Statistical Analysis

Statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, Ill).

Baseline characteristics—Study subjects' demographic, laboratory, imaging and histologic information were summarized, as previously described [reference omitted for submission to maintain blinding to authors]. Categorical variables were expressed as numbers and percentages. Continuous variables were expressed as mean ($\pm$ standard error).

Liver volume, liver mean PDFF, and TLFI—Linear regression was used to evaluate the cross-sectional relationships at baseline and at follow-up between the three variables (liver volume, liver mean PDFF, and TLFI), as well as the longitudinal relationships between the changes from baseline to follow-up in these three variables.

Reader agreement—The agreement between and within readers for liver volume, liver mean PDFF, and TLFI was reported according to the Bland-Altman method as bias $\pm$ 1.96 standard deviations (SD) of the differences, followed by the 95% limits of agreement interval.

Group comparison—Comparisons within treatment groups were made using paired t-tests. Comparisons between treatment groups were made using independent sample t-tests assuming equal variance for continuous/ordinal variables and Fisher's exact test for categorical variables. A two-tailed *P* value ≤ 0.05 was considered statistically significant.

RESULTS

Clinical Characteristics

Twenty-four (55.8%) of forty-three patients were men. The mean $\pm$ SD age was 48 ± 11.7 years. The mean body mass index was 31 ± 4.8 kg/m². Eighteen (42%) of 43 patients were Caucasian, 12 (28%) Hispanic, eight (19%) Asian, and three (7%) multi-racial. Both groups had similar baseline characteristics as shown in Table 1.

Imaging Characteristics

At baseline, subjects had a mean liver volume of 1918.9 mL (range: 1139.7–3146.7 mL), a mean PDFF of 16.6% (range: 5.2–31.8%), and mean TLFI of 323.2 %•mL (range: 93.5–685.8 %•mL).

Cross-sectional analyses

As summarized in Figures 3 and 4, cross-sectional analyses at both baseline and at follow-up revealed a weak positive relationship between liver volume and liver mean PDFF ($R^2 =$

0.022 to 0.107, $P = .045$ to $.369$), but statistically significant, positive, moderate or strong relationships between TLFI and liver mean PDFF ($R^2 = 0.740$ to 0.791 , $P < .001$) and between TLFI and liver volume ($R^2 = 0.352$ to 0.452 , $P < .001$). The regression equations, including intercepts and slopes, are listed in the corresponding figures.

Longitudinal changes

As summarized in Figure 5, longitudinal analysis of changes between baseline and follow-up measurements revealed statistically significant, positive, moderate or strong relationships between liver volume change and liver mean PDFF change (liver volume = 26.3 (liver mean PDFF) + 57.8 ; $R^2 = 0.556$; $P < 0.001$), between TLFI change and liver mean PDFF change (TLFI = 23.3 (liver mean PDFF) + 15.1 ; $R^2 = 0.920$; $P < 0.001$), and between TLFI change and liver volume change (TLFI = 0.6 (liver volume) - 24.2 ; $R^2 = 0.735$; $P < 0.001$).

Group Comparison

The effect of colesevelam and placebo on liver mean MRI-PDFF, liver volume, and TLFI are summarized in Table 2.

The liver mean PDFF increased by 2.0% points from 15.1% to 17.1% in patients who received colesevelam ($P = 0.067$) and decreased by 2.9% points from 17.8% to 14.9% in patients who received placebo ($P = 0.044$). Colesevelam, as compared with placebo, significantly increased liver mean PDFF with a difference of 4.8% points ($P = 0.007$).

The liver volume increased from 1882.1 mL to 2012.6 mL in patients who received colesevelam ($P = 0.007$). There was a trend toward volume reduction in the placebo group from 2027.4 mL to 1964.3 mL ($P = 0.149$). Colesevelam, as compared with placebo, significantly increased liver volume with a difference of 193.5 mL ($P = 0.003$).

The TLFI increased by 67.1 %•mL from 292.9 %•mL to 360.0 %•mL in patients who received colesevelam ($P = 0.016$). There was a trend toward TLFI reduction in the placebo group by 59.2 %•mL from 352.9 %•mL to 293.7 %•mL ($P = 0.068$). Colesevelam, as compared with placebo, significantly increased TLFI with a difference of 126.3 %•mL ($P = 0.003$).

Reader Agreement

Bland-Altman analysis showed good agreement between observers for each reading and the average of two readings for liver volume, liver mean PDFF and TLFI (Table 3). Overall, the 95% limits of agreement were narrower for cross-sectional than longitudinal changes in liver volume, average fat fraction and total liver triglyceride.

DISCUSSION

We performed a proof-of-concept, secondary analysis of a recently completed randomized placebo-controlled clinical trial in adult patients with biopsy-proven NASH, in which an MRI-PDFF estimation sequence was performed at baseline and at follow-up. Our analysis was aimed at exploring the cross-sectional and longitudinal correlations between fractional

liver fat content, liver volume, and total liver fat burden. Therefore, for our study we selected the subset of clinical trial patients in whom the MRI-PDFF sequence covered the entire liver, both at baseline and follow-up. This permitted simultaneous non-invasive measurements of fractional liver fat content and liver volume, from which the total liver fat burden could be derived.

MRI-PDFF was chosen as the biomarker of fractional liver fat content because of its high accuracy, reproducibility across field strengths and scanner platforms, and robustness to parameter changes over clinically relevant changes [46,47]. Hence, the findings observed here can be generalized to other field strengths and scanners. By comparison, the signal fat fraction (which can be calculated from conventional out-of-phase and in-phase imaging) is confounded by various factors and any results obtained using signal fat fraction might be scanner, field strength, and platform specific. Although MRS is widely regarded as the most accurate noninvasive method to estimate liver PDFF, it cannot evaluate the entire liver volume and therefore cannot evaluate total liver fat burden [22]. Moreover, numerous studies have shown such close agreement between MRI- and MRS-based PDFF estimation techniques [27,23,19,48,25,24] that the two approaches, MRI and MRS, can probably be considered equally valid. Non MR-based modalities are also not suitable for assessing these biomarkers. CT is less accurate than MRI for fat quantification because several factors other than fat affect CT attenuation values [49]. Additionally, because of associated ionizing radiation, CT is preferably avoided for the longitudinal monitoring of fat. Using currently available technology, ultrasound does not allow volumetric measurement of the entire liver nor quantitative measurement of the fractional liver fat content.

Rather than estimating liver PDFF from multiple small ROIs as done in the parent clinical trial (reference blinded) or in other prior studies [48,23,24,29,28], our ancillary study measured the mean PDFF across the entire segmented liver volume. A similar approach was previously described by d'Assignies et al [50] who performed semi-automated liver segmentation to calculate the mean liver fraction across the entire liver volume, although those authors calculated the signal fat fraction using conventional out-of-phase and in-phase imaging rather than the PDFF. A potential advantage of using whole-liver segmentation is that it reduces the subjectivity of ROI placement in the liver, and therefore may lessen sampling variability. This concept is a logical extension to the observation by Hines and et al [48] that the precision of fat fraction estimation can be improved by averaging the values derived from multiple ROIs across the liver. Another advantage is that whole-liver segmentation simultaneously permits estimation of liver volume, as also previously described by d'Assignies et al [50]. A potential disadvantage is that inclusion of non-fat-containing vessels within the segmented volume may introduce a systematic bias such as underestimation of the true PDFF of the liver parenchyma. However, the impact is likely to be small, as macro-vessels represent a small proportion of total liver volume and one could consider the blood vessels to be part of the liver. Moreover, in longitudinal comparisons, the underestimation is likely to be similar between baseline and follow-up, and between treatments groups. Hence, no bias is anticipated if this biomarker is applied in clinical trials.

To assess the total liver fat burden, we developed and evaluated a novel metric, TLFI, defined as the product of the segmented liver volume by the average PDFF within the

segmented volume. It should be emphasized TLFI is not an exact measure of total hepatic fat volume or total hepatic fat mass, but rather an *index* of total liver fat burden. The reason is that PDFF, from which the index is derived, reflects the proportion of fat within the mobile, i.e. MR visible, pool of protons in liver but does not provide information on the non-mobile, i.e., MR invisible, pool. With current MR techniques about 25% of liver tissue by volume is thought to be MR invisible [20], including bound water, membrane lipids, macromolecules, and minerals. The PDFF estimate does not take into account these MR invisible components. If the exact proportion of MR-visible and MR-invisible components in each voxel were known, it would be possible to convert PDFF in each voxel to volumetric and mass fat fractions [51–53], from which the total liver fat volume or mass could be calculated, but this is not yet possible with current knowledge and MR technology.

We found significant cross-sectional correlations between fractional liver fat content, liver volume, and TLFI. These results are consistent with those of Bora et al. who found a cross-sectional correlation between liver volume and degree of steatosis as determined by CT densitometry [33]. In our study, the correlation between liver mean PDFF and liver volume was weak, both at baseline and at follow-up, suggesting that multiple factors other than fractional fat content determine the liver volume. One would expect the liver of a large individual to be larger than that of a small individual, for example. Age, sex, and as-yet unidentified genetic, developmental, and environmental factors also could be contributory. In light of the many potential factors, the weak positive correlation observed in our study may be related to the incremental volume attributable to accumulation of fat in hepatocytes. Further research is needed to explore these possibilities. By comparison, the correlations between TLFI and liver volume and between TLFI and mean PDFF were moderate or strong; these are expected findings since TLFI is derived from liver mean PDFF and liver volume. Nevertheless, these correlations were not perfect, which suggests that TLFI may contribute new information not conveyed by either of the other imaging biomarkers in isolation.

Although the cross-sectional relationships between liver mean PDFF and liver volume were weak, the longitudinal correlations between changes in these imaging biomarkers were moderate to strong. We found that an increase in liver fat fraction was associated with liver hypertrophy while a decrease in liver fat fraction was associated with volume reduction. For every 10% change in PDFF, we observed a concomitant volume change of 263 mL on average. These findings are consistent with those of Kiki et al [54] who observed liver volume increase in rats exposed to a high fat diet, as compared with a control group. Similarly, human studies have shown liver volume reduction accompanying a decrease in liver fat as assessed by computed tomography attenuation values after 3 months of a low-calorie diet in patients with NAFLD [38], or after a 1 to 3 months exercise and diet program in potential living liver donors [39]. To our knowledge, however, ours is the first study to evaluate relationships between liver volume and fractional liver fat content in a cohort of human subjects in which both steatosis progression and regression are represented. Thus, ours is the first study to show that in humans, an increase in liver fractional fat content is associated with liver hypertrophy. Also, this is the first study to assess longitudinal changes in liver volume and fractional liver fat using PDFF as the biomarker. This is important

because our results can be directly comparable to future studies that use PDFF as a biomarker.

Since liver fat fraction and liver volume tended to change longitudinally in the same direction, we also found that an increase in liver fat fraction was associated with progression in total fat burden while a decrease in fat fraction was associated with reduction in burden. For every 10% change in PDFF, we observed a concomitant TLFI change of 233 %•mL on average. The exact relationship between PDFF change and TLFI change may depend on multiple factors, including but not limited to the population and intervention. The type of intervention, such as lifestyle changes, dietary changes, or drug, may affect the relative change in liver volume and PDFF. Hence, TLFI may even be more interesting since it could be related to the mechanism of change of liver volume and fat content if these are affected differently by different interventions. Further studies are needed in independent cohorts with different types of interventions to more precisely define the exact relationship between changes in these variables. While we proposed a TLFI metric of the entire liver fat burden, it is also conceivable to calculate a similar fat liver index on a lobar or segmental basis. This may become a predictor of functional liver remnant of interest to liver transplant surgeons harvesting a liver lobe as part of a living-donor liver transplant.

In this study, we estimated the intra- and inter-reader agreement of the three potential biomarkers proposed by independently repeating segmentation on a random sample of five cases. The intra- and inter-reader agreement on liver volume were modest, whereas the agreement on PDFF and TLFI were high. Hence, some of the volume changes should be interpreted with caution, a limitation of our study. We believe that the variability in liver volume estimation was attributable in part to the difficulty in segmenting the liver dome and the portions of the liver adjacent to the heart or stomach. It is possible that acquiring source images with thinner slices may permit more reliable segmentation and more precise estimation of liver volume. A related limitation is that we could not confirm the accuracy of the liver volume estimation using an independent reference standard such as CT since this was not part of the original clinical trial. Verification of the liver volume estimation will require further study. Another limitation is that we could not validate TLFI against a reference standard. Such validation will require phantom or animal models.

The exclusion of patients in whom the MRI-PDFF estimation sequence could not provide whole-liver coverage may have reduced the number of subjects with large livers. However, the number of subjects excluded for this technical reason remains small ($2/43 = 4.7\%$), and is therefore unlikely to modify the overall trend and invalidate our interpretation of the results.

A few additional technical issues merit discussion. In this study, we used a magnitude-based method to measure PDFF [23,24,55,30]. In principle, a complex-based method could be used to measure PDFF instead [25,48]. It is likely that similar results would be obtained with such a method, although further study will be needed to verify this supposition. Our acquisition times ranged from 12 to 34 seconds. Not all patients can hold their breaths comfortably for this duration, and it may be necessary in future studies to apply parallel imaging or other methods to reduce acquisition time while still providing whole-liver

coverage. Considering that the chemical shift was 3.5 pixels with our acquisition settings and that we only applied a one-pixel contraction around the periphery of the liver to exclude the chemical-shift artifact after segmentation, it is possible that we may have introduced a systematic bias by including pixels with 0% PDFF. In this study we assessed absolute change; an assessment of fractional change was outside our scope but may be considered in future studies. Finally, the manual segmentation method described here was time consuming and laborious. With current technology, whole liver segmentation represents a logistical challenge and is not feasible in most clinical settings. If the liver is segmented anyway, either in a clinical or research setting, then the additional effort required for TLFI estimation is negligible. Future work is needed to incorporate semi- or fully-automated segmentation methods to accelerate the post-processing and improve the feasibility of implementing these imaging biomarkers routinely.

In conclusion, our study suggests that the combination of liver segmentation and MRI-based fat estimation may be used to monitor fractional fat content (as estimated by PDFF), liver volume, and total liver fat burden (as assessed by TLFI) as quantitative imaging biomarkers in clinical trials. Measurement of these biomarkers may provide a more complete picture of liver fat content and its longitudinal change than traditional measurements based only on fractional fat content. Moreover, these biomarkers may permit new insight into longitudinal fat mobilization in and out of the liver by showing how changes in fractional liver fat content are accompanied by changes in liver volume and total liver fat burden. Importantly, these MRI-based measurements are non-destructive and can be recorded repeatedly. For these reasons, we believe that clinical trials in NASH may benefit by including imaging-based endpoints – such as liver volume, PDFF, and TLFI – in addition to traditional liver biopsy assessment. We speculate that TLFI may be a better marker of liver fat than PDFF because it denotes total hepatic fat burden rather than just fractional fat content. Future research is needed to refine the acquisition and post-processing methodology, validate the liver volume and TLFI estimations, and compare the three biomarkers for prediction of metabolic, cardiovascular, and other clinically significant outcomes.

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Abbreviations and acronyms

MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
PDFF	proton density fat fraction
ROI	region of interest
TLFI	total liver fat index

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Fig. 1.

(a) Manual segmentation of liver contour in the upper half of the liver performed on the first echo. (b) The segmentation was propagated to co-registered slices at five other echo times. (c) The corresponding segmented images were generated for the 6 echoes. (d) The PDFF was calculated pixel by pixel from the segmented source images acquired at the six echo times to generate a segmented PDFF map at each slice location. This process was repeated for each slice through the liver (not shown) to permit calculation of liver volume, liver mean PDFF, and TLFI.

Fig. 2. Longitudinal gray-scale MRI-PDFF maps (expressed in % with dynamic range from 0%–50%) through representative slices in the upper liver of two patients with biopsy-proven NASH. Sixty-year-old man randomized to the placebo group at (a) baseline and (b) 24 weeks follow-up. Forty-eight-year-old woman randomized to the colesevelam treatment group at (c) baseline and (d) 24 weeks follow-up. Notice that in the patient randomized to the placebo group there was minimal change in liver volume and a small decrease in liver mean PDFF and total liver fat index (TLFI). In contrast, in the patient randomized to the colesevelam group, there was marked increase in liver volume and liver mean PDFF, and even larger increase in TLFI.

Fig. 3.

Scatterplots show the cross-sectional relationships at baseline between (a) liver mean PDFF and volume, (b) liver mean PDFF and TLFI, and (c) liver volume and TLFI. Linear regression line, as well as corresponding equation, R^2 value, and significance level, are also shown.

Fig. 4.

Scatterplots show the cross-sectional relationships at follow-up between (a) liver mean PDFF and volume, (b) liver mean PDFF and TLFI, and (c) liver volume and TLFI. Linear regression line, as well as corresponding equation, R^2 value, and significance level, are also shown.

Fig. 5.

Scatterplots show the longitudinal relationships over an interval of 24 weeks between (a) liver mean PDFF and volume, (b) liver mean PDFF and TLFI, and (c) liver volume and TLFI. Linear regression line, as well as corresponding equation, R^2 value, and significance level, are also shown.

Table 1

Baseline Characteristics by Treatment Group.

	Colesevelam (n = 21)	Placebo (n = 22)	P value
Demographics			
Male gender, No. (%)	13 (62%)	11 (50%)	0.543
Age (years)	46.3 (13.0)	50.3 (10.8)	0.278
Weight (kg)	90.7 (19.7)	89.4 (21.2)	0.840
Height (m)	1.7 (0.1)	1.7 (0.2)	0.249
Body mass index (kg/m ²)	31.1 (4.8)	31.7 (5.1)	0.710
Ethnic origin			
White	10 (48%)	8 (36%)	0.543
Black	0 (0%)	0 (0%)	—
Asian	3 (14%)	5 (23%)	0.698
Hispanic	6 (29%)	6 (27%)	1.000
Multi-racial	1 (5%)	2 (9%)	1.000
Diabetes	7 (33%)	9 (41%)	0.755
Serum laboratory values			
ALT (U/L)	89.5 (72.8)	77.2 (50.5)	0.520
AST (U/L)	56.4 (49.7)	50.3 (34.3)	0.640
GGT (U/L)	61.3 (31.9)	85.1 (85.5)	0.239
Liver histology			
Steatosis score	2.0 (0.7)	2.1 (0.8)	0.685
Lobular inflammation score	1.7 (0.7)	1.5 (0.7)	0.440
Ballooning degeneration score	1.0 (0.7)	1.0 (0.7)	0.828
Fibrosis stage	1.2 (1.4)	1.2 (1.4)	0.931
NAFLD activity score	4.7 (1.3)	4.6 (1.3)	0.939
Liver imaging			
Liver volume (mL)	1882.1 (491.4)	2027.4 (629.7)	0.406
Liver mean PDFF (%)	15.1% (6.0%)	17.8% (7.3%)	0.200
TLFI (%•mL)	292.9 (161.5)	352.9 (162.8)	0.232

Note: Data are expressed as means or percentage mean with standard errors in parenthesis or as number with percent total in parenthesis where indicated. All labs were measured while fasting. NASH-CRN histologic scoring system was used for histologic grading and staging of liver biopsy. ALT = alanine aminotransferase. AST = aspartate aminotransferase. GGT = gamma-glutamyl transpeptidase. PDFF = proton density fat fraction. NAFLD = nonalcoholic fatty liver disease. TLFI = total liver fat index.

Table 2
Longitudinal assessment of liver volume, fractional fat, and total liver fat index using MRI.

MRI	Colesevelam (n = 21)				Placebo (n = 22)				Colesevelam vs placebo	
	Baseline	Post-treatment	Change (proportional change)	P value	Baseline	Post-treatment	Change (proportional change)	P value	Difference	P value
Liver volume (mL)	1882.1 (491.4)	2012.6 (531.3)	130.5 (6.9%)	0.007	2027.4 (629.7)	1964.3 (602.7)	-63.1 (-3.1%)	0.149	193.5	0.003
Liver mean PDFF (%)	15.1 (6.1)	17.1 (7.2)	2.0 (13.2%)	0.067	17.8 (7.3)	14.9 (5.7)	-2.9 (-16.3%)	0.044	4.8	0.007
TLFI (%•mL)	292.9 (161.5)	360.0 (210.1)	67.1 (41.5%)	0.016	352.9 (162.8)	293.7 (141.6)	-59.2 (-16.8%)	0.068	126.3	0.003

Note: Data are expressed as mean with standard errors in parenthesis, percentage mean with percent standard errors in parenthesis, or change with proportional change in parenthesis. *PDFF* = proton density fat fraction. *TLFI* = total liver fat index.

Table 3

Bland-Altman Analysis of Intra and Inter-Reader Repeatability

	Intra-observer (R1–R1')	Intra-observer (R2–R2')	Inter-observer (R1–R2)
Liver volume			
Baseline and follow-up (mL)	56.7 ± 183.9; (–127.2, 240.6)	–27.3 ± 110.5; (–137.7, 83.2)	5.1 ± 174.5; (–169.4, 179.6)
Change (mL)	45.1 ± 96.8; (–51.6, 142.0)	–39.0 ± 100.1; (–139.1, 61.1)	–35.1 ± 338; (–373.3, 303.0)
Liver mean PDFF			
Baseline and follow-up (%)	0.07% ± 0.23%; (–0.16%, 0.30%)	–0.05% ± 0.28%; (–0.33%, 0.23%)	–0.26% ± 0.36%; (–0.62%, 0.10%)
Change (%)	–0.09% ± 0.12%; (–0.22%, 0.03%)	–0.09% ± 0.38%; (–0.47%, 0.28%)	0.03% ± 0.55%; (–0.51%, 0.58%)
TLFI			
Baseline and follow-up (%•mL)	13.4 ± 36.9; (–23.5, 50.2)	–6.7 ± 23.6; (–30.3, 16.9)	9.2 ± 41.5; (–32.3, 50.7)
Change (%•mL)	6.2 ± 22.9; (–16.7, 29.1)	–10.3 ± 18.5; (–28.8, 8.2)	1.8 ± 45.1; (–43.2, 46.9)

Note: Results reported as bias ± repeatability coefficient (1.96 SD); (95% limits of agreement interval). *PDFF* = proton density fat fraction. *TLFI* = total liver fat index