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Ezetimibe for the Treatment of Nonalcoholic Steatohepatitis: Assessment by Novel Magnetic Resonance Imaging and Magnetic Resonance Elastography in a Randomized Trial (MOZART Trial)

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Ezetimibe inhibits intestinal cholesterol absorption and lowers low-density lipoprotein cholesterol. Uncontrolled studies have suggested that it reduces liver fat as estimated by ultrasound in nonalcoholic steatohepatitis (NASH). Therefore, we aimed to examine the efficacy of ezetimibe versus placebo in reducing liver fat by the magnetic resonance imaging-derived proton density-fat fraction (MRI-PDFF) and liver histology in patients with biopsy-proven NASH. In this randomized, double-blind, placebo-controlled trial, 50 patients with biopsy-proven NASH were randomized to either ezetimibe 10 mg orally daily or placebo for 24 weeks. The primary outcome was a change in liver fat as measured by MRI-PDFF in colocalized regions of interest within each of the nine liver segments. Novel assessment by two-dimensional and three-dimensional magnetic resonance elastography was also performed. Ezetimibe was not significantly better than placebo at reducing liver fat as measured by MRI-PDFF (mean difference between the ezetimibe and placebo arms -1.3%, $P = 0.4$). Compared to baseline, however, end-of-treatment MRI-PDFF was significantly lower in the ezetimibe arm (15%-11.6%, $P < 0.016$) but not in the placebo arm (18.5%-16.4%, $P = 0.15$). There were no significant differences in histologic response rates, serum alanine aminotransferase and aspartate aminotransferase levels, or longitudinal changes in two-dimensional and three-dimensional magnetic resonance elastography-derived liver stiffness between the ezetimibe and placebo arms. Compared to histologic nonresponders (25/35), histologic responders (10/35) had a significantly greater reduction in MRI-PDFF (-4.35 ± 4.9% versus -0.30 ± 4.1%, $P < 0.019$). *Conclusions:* Ezetimibe did not significantly reduce liver fat in NASH. This trial demonstrates the application of colocalization of MRI-PDFF-derived fat maps and magnetic resonance elastography-derived stiffness maps of the liver before and after treatment to noninvasively assess treatment response in NASH. (HEPATOLOGY 2015;61:1239-1250)

Nonalcoholic steatohepatitis (NASH) is the most common cause of chronic liver disease in the United States.[1,2] It can lead to advanced fibrosis, cirrhosis, and hepatocellular carcinoma.[3-6] It is associated with metabolic syndrome traits including insulin resistance, diabetes, and dyslipidemia.[7-9] Oxidative stress, insulin resistance, and lipotoxicity have been linked to the pathogenesis of

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDL, low-density lipoprotein; MRE, magnetic resonance elastography; 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; 2D/3D, two- and three-dimensional.

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NASH; and therapeutic agents that modify these pathways have been tried in the treatment of NASH with modest success.[1,10-12]

There are no Food and Drug Administration-approved pharmacologic therapies for the treatment of NASH.[1] Increased dietary cholesterol has been shown to be associated with NASH.[13] Ezetimibe inhibits the intestinal absorption of luminal cholesterol by binding to the Niemann-Pick C1-like 1 sterol transporter in the membrane of the enterocyte brush border. The concept that reducing delivery of cholesterol to the liver may be beneficial in NASH led to the consideration of testing the role of ezetimibe as a therapy for NASH.[14] Ezetimibe treatment improved diet-induced hepatic steatosis and fibrosis and ameliorated dyslipidemia in Zucker rats (an animal model of obesity and insulin resistance) fed a high-fat diet, suggesting a possible therapeutic role of ezetimibe in the treatment of human NASH.[15]

In recent uncontrolled, pilot studies in patients with nonalcoholic fatty liver disease (NAFLD) and NASH, ezetimibe was shown to improve hepatic steatosis on ultrasound, reduce serum alanine aminotransferase (ALT), and improve histologic features of NASH.[16,17] Therefore, randomized, placebo-controlled trials are needed to examine the efficacy of ezetimibe in the treatment of NASH.

Utilizing advanced magnetic resonance imaging (MRI) techniques, we examined the efficacy of ezetimibe versus placebo at reducing liver fat as measured by an accurate and validated MRI-derived biomarker, proton density-fat fraction (MRI-PDFF), in patients with biopsy-proven NASH. Secondary end points included reduction in serum ALT and aspartate aminotransferase (AST), improvement in liver histology, and reduction in low-density lipoprotein (LDL) cholesterol. In addition, we explored the value of advanced magnetic resonance elastography (MRE) methods including both two-dimensional (2D) as well as three-dimensional (3D) MRE.

Patients and Methods

Study Design and Population

The MOZART (Magnetic Resonance Imaging and Elastography in Ezetimibe Versus Placebo for the Assessment of Response to Treatment in NASH) trial was a randomized, double-blind, allocation-concealed, investigator-initiated, placebo-controlled clinical trial designed to test the efficacy of 24 weeks' treatment with ezetimibe at 10 mg daily orally versus placebo in the treatment of NASH. The study was designed and conducted according to the CONSORT Guidelines (Supporting Table 1). No interim analysis was performed. The MOZART trial patient population was derived from the San Diego Integrated NAFLD Research Consortium (SINC) cohort, which is a city-wide collaboration set up to study NAFLD led by the principal investigator (R.L.), including the University of California at San Diego Medical Center, the Sharp Health System, the Balboa Naval Medical Center, and Kaiser Permanente Southern California.[18,19] Patients with biopsy-proven NASH seen at any of these sites were referred to the University of California at San Diego NAFLD Translational Research Unit. The study was conducted at the Clinical and Translational Research Institute, University of California at San Diego. This study was registered at clinicaltrials.gov

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(registration number NCT01766713) and approved by the Food and Drug Administration under an investigational new drug application from the principal investigator (R.L.). The protocol was approved by the University of California at San Diego Institutional Review Board. Written informed consent was obtained from all patients.

Inclusion and Exclusion Criteria

Patients had to be age 18 years or older, have an ALT above the upper limits of normal (19 U/L for women and 30 U/L for men), and show presence of hepatic steatosis as defined by $\geq$5% on MRI-PDFF. All patients had liver biopsies showing evidence of NASH defined by the presence of the following features: steatosis, ballooning degeneration, and lobular inflammation with or without perisinusoidal fibrosis on biopsy performed within 6 months of enrollment. A score of 1 or more was required for steatosis, ballooning, and lobular inflammation on baseline biopsy.

Subjects were excluded if they had evidence of other forms of liver disease shown by the presence of serum hepatitis B surface antigen, hepatitis C viral RNA, positive autoimmune serologies with biopsy consistent with autoimmune hepatitis, hemochromatosis by 3+ or 4+ stainable iron on biopsy and homozygosity/heterozygosity on genetic analysis, low ceruloplasmin levels with biopsy suggestive of Wilson's disease, or low alpha-1-antitrypsin levels with biopsy suggestive of alpha-1-antitrypsin disease. Further exclusion criteria included alcohol intake of more than 30 g/day in the previous 10 years or greater than 10 g/day in the previous year, decompensated cirrhosis with Child-Pugh score greater than 7 points, active substance abuse, significant systemic illnesses, renal insufficiency, positive human immunodeficiency virus test, pregnancy, evidence of hepatocellular carcinoma, ingestion of drugs known to cause hepatic steatosis, ingestion of drugs known to improve NASH such as vitamin E or pioglitazone, contraindications to liver biopsy, or inability to undergo MRI.

Baseline Assessments

Baseline evaluation prior to treatment initiation included routine medical history, an alcohol history assessment by completing the AUDIT and Skinner Lifetime Drinking questionnaire, physical exam, height, weight measurements (performed by a trained investigator), and calculation of body mass index. Subjects underwent blood tests which included ALT, AST, alkaline phosphatase, gamma-glutamyl transferase, total bilirubin, albumin, hemoglobin A1c, fasting glucose and insulin, prothrombin time/international normalized ratio, and lipid panel.

Randomization and Allocation Concealment

Subjects were randomized to either an ezetimibe or a placebo group in blocks of four in a 1:1 ratio by the investigational drug services at the University of California at San Diego using computer-generated numbers. Independent investigational drug services pharmacists dispensed either active or placebo treatment pills, which were identical in appearance. Pills were prepackaged in identical bottles, labeled according to the computer-generated randomization numbers, and delivered to the research clinic. The allocation sequence was concealed from the research coordinators and all investigators including hepatologists, radiologists, and the pathologist who assessed and enrolled subjects in the trial. Treatment allocation was unblinded only after the completion of all procedures in the entire study including all posttreatment liver biopsy and MRI studies on all patients.

Study Visits

Upon meeting inclusion and exclusion criteria and completing baseline evaluation, patients were randomized at week 0 to receive either ezetimibe or placebo. Patients returned to the research clinic at weeks 4, 12, and 24 after randomization. At these clinic visits, routine blood tests were obtained, body weight and vital signs were recorded, and the number of pills was counted to document compliance. A physical exam and careful history of liver-related symptoms as well as possible side effects of ezetimibe were also obtained at each visit. At the completion of 24 weeks of therapy, patients underwent MRI, magnetic resonance spectroscopy (MRS, as an internal validation for MRI), MRE, biochemical testing, and a liver biopsy.

Primary and Secondary Outcomes

The primary outcome was a change in liver fat as measured by MRI-PDFF in colocalized regions of interest (ROI) within each of the nine liver segments (for details see below, "MRI and MRE Protocol"). Secondary end points included LDL reduction and histology-determined two-point reduction in NAFLD activity score without worsening fibrosis (for details see below, "Liver Histology Protocol"). Both 2D and 3D MRE-derived reductions in liver stiffness were also measured.

Liver Histology Protocol

The liver biopsies were scored using the Nonalcoholic Steatohepatitis Clinical Research Network histologic scoring system.[20] A single hepatopathologist (G.L.), who was blinded to clinical as well as radiologic data, the order of liver biopsy specimens, and

previous results of the biopsy, performed all liver biopsy assessments for this study. Blinding was not broken until after completion of all histologic as well as radiologic/clinical data collection procedures. The average (±SD) length of liver biopsy specimens was 17.0 mm (±6.6), and the average (±SD) number of portal tracts seen on the biopsy specimen was 14.9 (±7.9). These are similar to previously performed high-quality clinical trials in NASH and reflect good clinical practice at a large NAFLD clinical research site.[8,18]

A two-point drop in NAFLD activity score without worsening of hepatic fibrosis was considered improvement in liver histology and considered a secondary end point.[21] Liver fibrosis (stage 0-4) was scored using the NASH Clinical Research Network histologic staging system.[16]

MRI and MRE Protocol

MRI for Fat Quantification. Baseline and post-treatment MRI to assess changes in liver fat were performed utilizing a state-of-the-art MRI-PDFF method.[22] The PDFF is an objective, quantitative, and noninvasive imaging biomarker of liver fat content; the measurement is independent of scanner manufacturer, scanner platform, field strength, and other confounders that frequently confound fat content estimations made by conventional MRI techniques.[23,24] We have previously shown the utility of this method in the assessment of quantitative changes in liver fat in NASH trials.[18] The MRI-PDFF method is further elaborated in the Supporting Information. The mean ± SD interval between the baseline MRI and the initiation of the study drug or placebo was 22 ± 34 days.

Detailed Fat Mapping of the Whole Liver. Magnetic resonance examinations were performed by experienced research MR technologists with expertise in the procedures. All scans were analyzed, under the supervision of the radiology investigator (C.S.), by a single trained image analyst blinded to clinical data, treatment group allocation, histological data, and the order (baseline or follow-up) of each scan. Imaging PDFF was recorded in ROIs approximately 300-400 mm^2 in area placed on the PDFF parametric maps, avoiding blood vessels, bile ducts, and artifacts.

Colocalization Before and After Treatment. To assess longitudinal changes in fat content, one colocalized ROI was placed in each of the nine liver segments (nine separate ROIs) on the baseline and follow-up MR exams (see Fig. 2A for segment-by-segment colocalization shown for a representative patient before and after treatment).

Internal Validation Using MRS. For each patient MRS was performed in a single location (voxel) in the right lobe of the liver, and the PDFF in that voxel was measured as previously described.[23,25] In this study, the PDFF measured by MRS at that voxel was the reference for MRI-PDFF and provided internal validation for the accuracy of the MRI-PDFF measurements. To colocalize MRI-PDFF with the MRS voxel, three additional ROIs were placed on the PDFF maps in the same locations as the spectroscopic voxel (one through the middle third of the voxel, one through the superior third of the voxel, and one through the inferior third of the voxel), and these MRI-PDFF estimates were averaged.

Magnetic Resonance Elastography. Previous studies have shown that MRE is an accurate and robust, noninvasive, quantitative imaging biomarker for hepatic fibrosis.[26,27] In addition, Chen and colleagues[28] have shown that MRE may be helpful in early detection of NASH as an imaging biomarker. These data provided scientific justification for including MRE as an exploratory end point (see further details in the Supporting Information).

The MRE exams were performed with patients in the supine position, with a 19-cm-diameter acoustic pressure-activated passive driver placed over the right anterior chest wall with its center level with xiphoid process. The 2D gradient echo MRE acquisitions were performed with parameters as previously described.[28-31] In addition, 3D MRE was performed using spin-echo planer sequences with the following parameters: acquisition matrix = 72 × 72 × 40; TR/TE = 1400/64 (40Hz), 1333/52 (60Hz), one motion encoding gradient pair, three orthogonal motion directions, 3.5 mm slice thickness, and a parallel imaging factor of 3.

The data were processed to generate images depicting the complex shear stiffness of liver tissue, using direct inversion.[28,31]

Colocalization in Assessing Longitudinal Changes in MRE. To assess longitudinal changes in mechanical properties of the liver, multislice colocalized ROIs were manually specified on the baseline and follow-up 2D and 3D MRE exams. The criteria of ROI placement are (1) including liver parenchyma only, (2) excluding regions without adequate magnitude or shear wave amplitude, and (3) excluding the top and bottom five slices to obviate boundary effect.

Statistical Analysis

The chi-squared test was used for comparisons between categorical variables, and a paired *t* test was used to compare mean differences between continuous variables in the ezetimibe versus placebo groups.

Table 1. Baseline Demographic, Biochemical, and Histologic Characteristics of Subjects

	Ezetimibe (n = 25)	Placebo (n = 25)	*P* Value
Demographics			
Age (years)	49.0 ± 14.9	49.5 ± 13.7	0.9139
Female patients	14 (56%)	17 (68%)	0.3821
Weight (kg)	94.1 ± 18.1	91.8 ± 18.9	0.6662
Height (m)	1.7 ± 0.1	1.7 ± 0.1	0.9837
BMI (kg/m^2)	33.8 ± 5.2	32.9 ± 5.1	0.5432
White (vs. nonwhite)	19 (76%)	21 (84%)	0.4795
Hispanic (vs. non)	8 (32.0%)	9 (36%)	0.7653
Diabetes	7 (28%)	7 (28%)	1.000
Biochemical profile			
ALT (IU/L)	51.0 (29.0)	47.0 (26.0)	0.9615
AST (IU/L)	33.0 (23.0)	32.0 (28.0)	0.6572
AST:ALT	0.8 (0.5)	0.8 (0.4)	0.7284
Alkaline phosphatase (U/L)	72.0 (29.0)	72.0 (37.0)	0.4584
GGT (U/L)	49.0 (32.0)	32.5 (42.0)	0.4049
Total bilirubin (mg/dL)	0.5 (0.4)	0.4 (0.2)	0.7167
Glucose (mg/dL)	104.0 (25.0)	106.0 (41.0)	0.6504
Insulin (μU/mL)	23.0 (15.5)	26.5 (18.0)	0.2322
Hemoglobin A1C (%)	5.9 (0.7)	6.1 (1.0)	0.7015
FFA (mmol/L)	0.5 (0.3)	0.7 (0.3)	0.2140
HOMA-IR	6.4 (5.1)	6.5 (5.7)	0.2205
Triglycerides (mg/dL)	152.0 (58.0)	149.0 (104.0)	0.5567
Total cholesterol (mg/dL)	182.0 (25.0)	170.0 (54.0)	0.5001
LDL (mg/dL)	100.0 (32.0)	90.0 (50.5)	0.3831
Histology			
Steatosis	2.0 (2.0)	3.0 (1.0)	0.1419
Lobular inflammation	1.0 (1.0)	2.0 (1.0)	0.1658
Ballooning	1.0 (1.0)	1.0 (1.0)	0.6996
Fibrosis	1.0 (1.0)	1.0 (3.0)	0.6891
NAS	5.0 (2.0)	5.0 (2.0)	0.1775

Mean ± standard deviation is presented for normally distributed variables, median (interquartile range) for nonnormally distributed variables. The Wilcoxon-Mann-Whitney test was performed on all continuous/ordinal variables, and the chi-squared or Fisher's exact test was performed on all categorical variables.

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; FFA, free fatty acids; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance; NAS, NAFLD activity score.

The primary analysis was performed as an intention-to-treat analysis. Primary and secondary comparisons within treatment groups were calculated using paired *t* tests, two-tailed independent sample *t* tests, or nonparametric tests as appropriate. Pooled within-group Pearson's correlations were used to look at associations across groups. A two-tailed *P* value ≤ 0.05 was considered statistically significant. Statistical analyses were performed using the SAS statistical software package, version 9.4 (SAS, Cary, NC). All authors had access to the study data and reviewed and approved the final manuscript. A priori, we assumed that a 5% (net effect) difference would be the minimal appreciable difference that would be clinically relevant.[32] Based on the previous colesevelam trial[18] and uncontrolled studies using ezetimibe, the ezetimibe group was predicted to have at least a 6% reduction in liver fat compared to baseline and 1% or less in the placebo group. We also predicted less than a 10% dropout based upon previous studies conducted by the principal investigator in NASH.[18] To achieve a power of 90% with a β of 0.05, a sample size of 22 subjects in each arm was needed. Therefore, we planned to randomize a total of 50 patients to either the ezetimibe or the placebo group so that even with dropouts the study would be adequately powered to test the hypothesis.

Results

Between January 2013 and December 2013, 50 patients with biopsy-proven NASH were randomized to either ezetimibe or placebo. Of the 25 patients randomized into each arm, two patients in the treatment and two patients in the placebo arm discontinued treatment (Supporting Fig. 1). The study population included 62% women and was predominantly white (80%), with 34% of participants of Hispanic ethnicity. Both groups had similar baseline characteristics, as shown in Table 1.

Primary Outcome: Effect of Ezetimibe on Liver Fat as Assessed by MRI-PDFF. Ezetimibe was not significantly better than placebo at reducing liver fat content as measured by MRI-PDFF (mean difference between ezetimibe and placebo arms, -1.3%, $P = 0.48$, as shown in Table 2, and Fig. 1A,B).

Compared to baseline, end-of-treatment MRI-PDFF was significantly lower in the ezetimibe (15%-11.6%, $P < 0.016$) but not in the placebo (18.5%-16.4%, $P = 0.15$) arm. Hence, ezetimibe lowered liver fat by a small but clinically unimportant amount. Individual patient data on changes in liver fat by MRI-PDFF stratified by treatment group are shown in Fig. 1A, and differences between the baseline and end-of-treatment MRI-PDFF by the treatment group are shown in Fig. 1B. The detailed MRI-PDFF fat mapping protocol of the nine liver segments and the overall average at baseline and posttreatment at each level for a representative patient are shown in Fig. 2A. The panel also shows comprehensive MR assessment including MRS (Fig. 2B) as well as 2D and 3D MRE (Fig. 2C) of the same patient before and after treatment.

To demonstrate the internal validity of MRI-PDFF results, MRS (reference standard for hepatic fat quantification) was performed in colocalized ROIs with MRI-PDFF. This confirmed the results obtained by MRI-PDFF (Table 2). In both groups, MRI-PDFF and MRS correlated robustly for measurements of fat fraction at baseline and posttreatment, with the correlation coefficient ranging from $r^2 = 0.95$ to 0.99 ($P < 0.0001$).

Table 2. Ezetimibe Versus Placebo: Longitudinal Full Liver Fat Mapping Using MRI PDFF and MRS With Colocalized MRI Measurements

2A	Ezetimibe (n = 23)			Placebo (n = 22)			Difference
Liver Segments	Baseline	Posttreatment	*P* Value	Baseline	Posttreatment	*P* Value	(*P* Value)
1	15.1 (8.6)	11.9 (6.8)	**0.0249**	18.1 (7.5)	16.5 (5.9)	0.2298	−1.5 (0.4341)
2	13.9 (8.3)	10.8 (6.5)	**0.0336**	17.3 (7.9)	15.7 (5.9)	0.2458	−1.3 (0.4913)
3	14.8 (9.1)	11.9 (7.8)	0.0585	18.2 (7.7)	16.5 (6.1)	0.2803	−1.2 (0.5832)
4a	15.6 (8.8)	11.9 (6.9)	**0.0044**	18.6 (7.8)	16.7 (5.8)	0.1677	−1.8 (0.3028)
4b	15.1 (8.9)	12.0 (7.3)	**0.0326**	18.6 (7.7)	16.3 (6.6)	0.0712	−0.8 (0.6646)
5	15.0 (9.7)	11.3 (7.5)	**0.0148**	18.9 (9.3)	16.5 (7.1)	0.1119	−1.3 (0.5149)
6	14.8 (8.9)	11.2 (7.1)	**0.0170**	18.3 (8.6)	15.9 (6.3)	0.1232	−1.2 (0.5462)
7	15.2 (8.6)	11.5 (6.6)	**0.0067**	19.1 (8.8)	16.7 (6.5)	0.1526	−1.4 (0.4951)
8	15.4 (8.6)	11.7 (6.8)	**0.0098**	19.2 (8.6)	17.0 (6.5)	0.1562	−1.5 (0.4547)
MRI PDFF (%) average	15.0 (8.7)	11.6 (6.9)	**0.0158**	18.5 (8.0)	16.4 (6.1)	0.1512	−1.3 (0.4839)

2B	Ezetimibe (n = 19)			Placebo (n = 21)			Difference
	Baseline	Posttreatment	*P* Value	Baseline	Posttreatment	*P* Value	(*P* Value)
MRS (%)	16.4 (8.6)	13.1 (7.0)	**0.0178**	18.5 (7.8)	17.0 (6.2)	0.3024	−1.9 (0.3345)

2C	Ezetimibe (n = 19)			Placebo (n = 21)			Difference
MRI-level	Baseline	Posttreatment	*P* Value	Baseline	Posttreatment	*P* Value	(*P* Value)
MRI-s	15.8 (8.8)	12.3 (6.9)	**0.0142**	18.5 (8.2)	16.6 (6.9)	0.1828	−1.6 (0.3944)
MRI-m	15.7 (8.9)	12.3 (7.2)	**0.0163**	18.4 (8.3)	16.4 (6.7)	0.1549	−1.5 (0.4046)
MRI-i	15.5 (9.1)	12.1 (7.0)	**0.0257**	17.9 (8.4)	16.2 (6.6)	0.2175	−1.7 (0.3870)
MRI average	15.7 (8.9)	12.2 (7.0)	**0.0173**	18.2 (8.3)	16.4 (6.7)	0.1800	−1.6 (0.3906)
Pearson *r*	0.992 $P < 0.0001$	0.994 $P < 0.0001$		0.990 $P < 0.0001$	0.982 $P < 0.0001$		
Spearman ρ	0.989 $P < 0.0001$	0.982 $P < 0.0001$		0.977 $P < 0.0001$	0.952 $P < 0.0001$		

Data are expressed as means (SD) or mean difference with *P* values in parentheses. Associated *P* values are from *t* test. Correlation coefficient expressed as Pearson's *r* and nonparametric Spearman's rho ρ with corresponding *P* value. *P* values shown in bold are statistically significant.

In 2A, MRI PDFFs measured in all nine liver segments were used to calculate segmental and overall fat fraction averages at baseline and posttreatment between the ezetimibe and placebo groups. In 2B, longitudinal changes in MRS measurements are shown. In 2C, internal cross-validation is shown between MRI-PDFF and MRS-PDFF and their correlations.

Abbreviations: MRI-PDFF, magnetic resonance imaging proton density-fat fraction; MRS, magnetic resonance spectroscopy; MRI-s, magnetic resonance imaging superior; MRI-m, magnetic resonance imaging middle; MRI-i, magnetic resonance imaging inferior.

Effect of Ezetimibe on 2D and 3D MRE-Estimated Liver Stiffness. Nineteen patients in the treatment arm and 21 patients in the placebo arm underwent 2D MRE at 60 Hz before and after treatment. The mean (±SD) liver stiffness in the ezetimibe versus placebo group at baseline was 3.0 (±0.9) and 2.7 (±1.3), respectively (Table 3). There were no significant differences in longitudinal changes in 2D MRE-derived stiffness between the ezetimibe and the placebo arms (Table 3).

Sixteen patients in the treatment arm and 20 patients in the placebo arm had 3D MRE sequences before and after treatment (Table 3). The 3D MRE was done at two frequencies, 40 Hz and 60 Hz. The mean (±SD) liver stiffness values at 40 Hz in the ezetimibe and placebo groups at baseline were 1.9 (±0.5) and 1.7 (±1.1), respectively (Table 3). There were no significant differences in longitudinal changes in 3D MRE-derived stiffness at either 40 Hz or 60 Hz between the ezetimibe and the placebo arms (Table 3).

Effect of Ezetimibe on ALT, AST, and LDL. There were no significant decreases in serum ALT, AST, and gamma-glutamyl transferase between the ezetimibe and the placebo arms. Table 4 provides detailed data on the anthropometric and biochemical measures monitored during the trial.

As expected, ezetimibe was significantly better than placebo at reducing LDL and total cholesterol levels, confirming the lipid-lowering effect of ezetimibe in patients with NASH (Table 4). Due to the known LDL-lowering properties of ezetimibe, this reduction in LDL provides an indirect measurement of compliance in both groups.

Effect of Ezetimibe on Liver Histology. Ezetimibe did not have any significant effect on any of the histologic variables (Table 5). Among patients who underwent end-of-treatment liver biopsy, five of 17 patients in the ezetimibe arm and five of 18 patients in the placebo arm had a two-point reduction in NAFLD activity score without any worsening of fibrosis and were classified as histologic responders. Therefore, there was no significant difference in the histologic response between the groups.

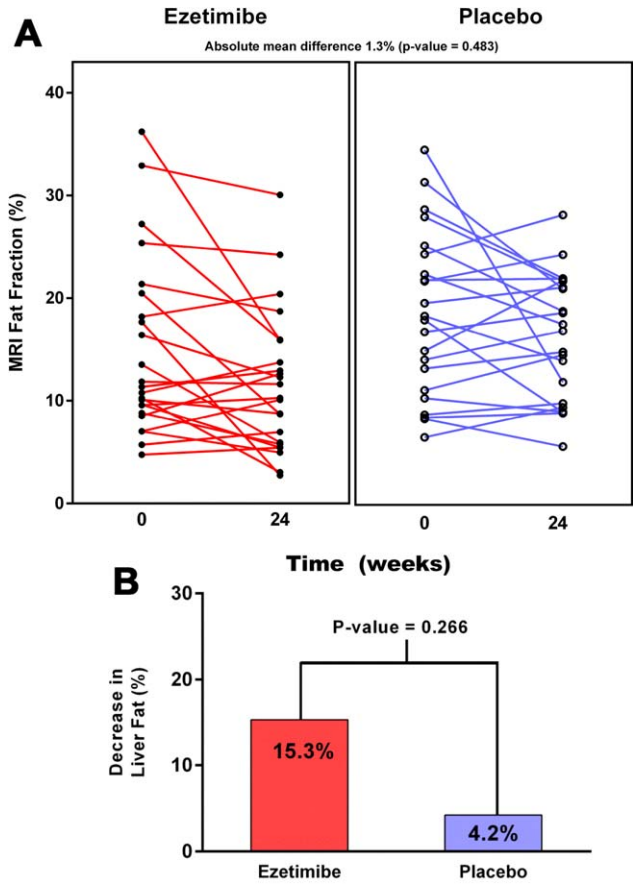

Fig. 1. (A) Individual patient data on liver fat content as assessed by MRI-PDFF before randomization and at the end of 24 weeks stratified by the treatment group assignment (ezetimibe-treated patients are to the left and shown by red lines, and placebo-treated patients are to the right and shown by blue lines). There was no significant difference in the changes in MRI-PDFF between the treatment groups (P = 0.483). (B) Percentage decline in liver fat relative to baseline by MRI-PDFF stratified by the treatment group assignment (ezetimibe-treated patients are to the left and shown by the red bar, and placebo-treated patients are to the right and shown by the blue bar). There was no significant difference in the changes in MRI-PDFF between the treatment groups (P = 0.266).

Compared to histologic nonresponders (25/35), histologic responders (10/35) had a significantly greater reduction in in MRI-PDFF (-4.35% ± 4.9 versus -0.30% ± 4.1, $P < 0.019$), suggesting that the histological assessments of change in steatosis were accurate. Changes in MRI-PDFF and changes in steatosis grade are shown in Supporting Fig. 2.

We conducted sensitivity analyses by dividing the cohort into those below and those above the median LDL cholesterol and found that the results remained consistent (see Supporting Fig. 3).

Adverse Events. Of the 25 patients in each treatment arm, two patients in the ezetimibe arm and two in the placebo arm dropped out of the study. In the ezetimibe arm, two patients discontinued therapy and follow-up due to changes in their job schedule. In the placebo arm, one patient stopped treatment due to muscle aches and one patient discontinued due to change in employment. Overall, there were no differences in side effects between the groups (Supporting Table 2).

Discussion

Main Findings

In this randomized, double-blind, placebo-controlled MOZART trial, ezetimibe was not better than placebo at reducing liver fat or improving liver histology in NASH. Ezetimibe lowered liver fat by a small but clinically unimportant amount. The trial, thus, provides compelling evidence that ezetimibe has no specific role in the treatment of NASH. The internal validity of the MRI-PDFF-derived changes in liver fat were confirmed by colocalized MRS-based measurements.

This is the first report of 2D and 3D MRE being utilized to examine changes in liver stiffness in a NASH trial demonstrating feasibility. Future studies, especially that target antifibrotic mechanisms, may utilize the MRE protocol used in this trial as a proof of concept for noninvasive and quantitative assessment of treatment response. The placebo-arm data on the changes (as well as the SD of the values before and after treatment) in 2D and 3D MRE-derived liver stiffness would be useful to extrapolate placebo response in antifibrotic trials, and these would be helpful in sample size estimation to see significant differences due to antifibrotic therapy in NASH.

In addition, we confirmed the findings from previous trials that ezetimibe can be safely used in patients with NAFLD as a lipid-lowering therapy and is effective at lowering serum LDL cholesterol.[33,34]

Rationale for Using MRI-PDFF for Assessment of Primary Outcome. We selected MRI-PDFF to measure the primary outcome because it is noninvasive, does not require exposure to ionizing radiation, and provides objective and quantitative estimates of fat content throughout the liver.[25,35] Also, MRI-PDFF is more objective than ultrasound, which is operator-dependent and interpreted qualitatively. It is more accurate than CT, which has limited grading accuracy and utilizes ionizing radiation. And MRI-PDFF is more practical to perform and provides greater spatial coverage than MRS, which is technically difficult to perform and analyze, can only be performed at select centers with expertise, and evaluates liver fat content in only a single 2 × 2 × 2 cm^3 cube (voxel) within the liver.[18] Finally, we have previously shown that MRI-PDFF is more sensitive than histology at assessing quantitative changes in liver fat in NASH trials.[18,19]

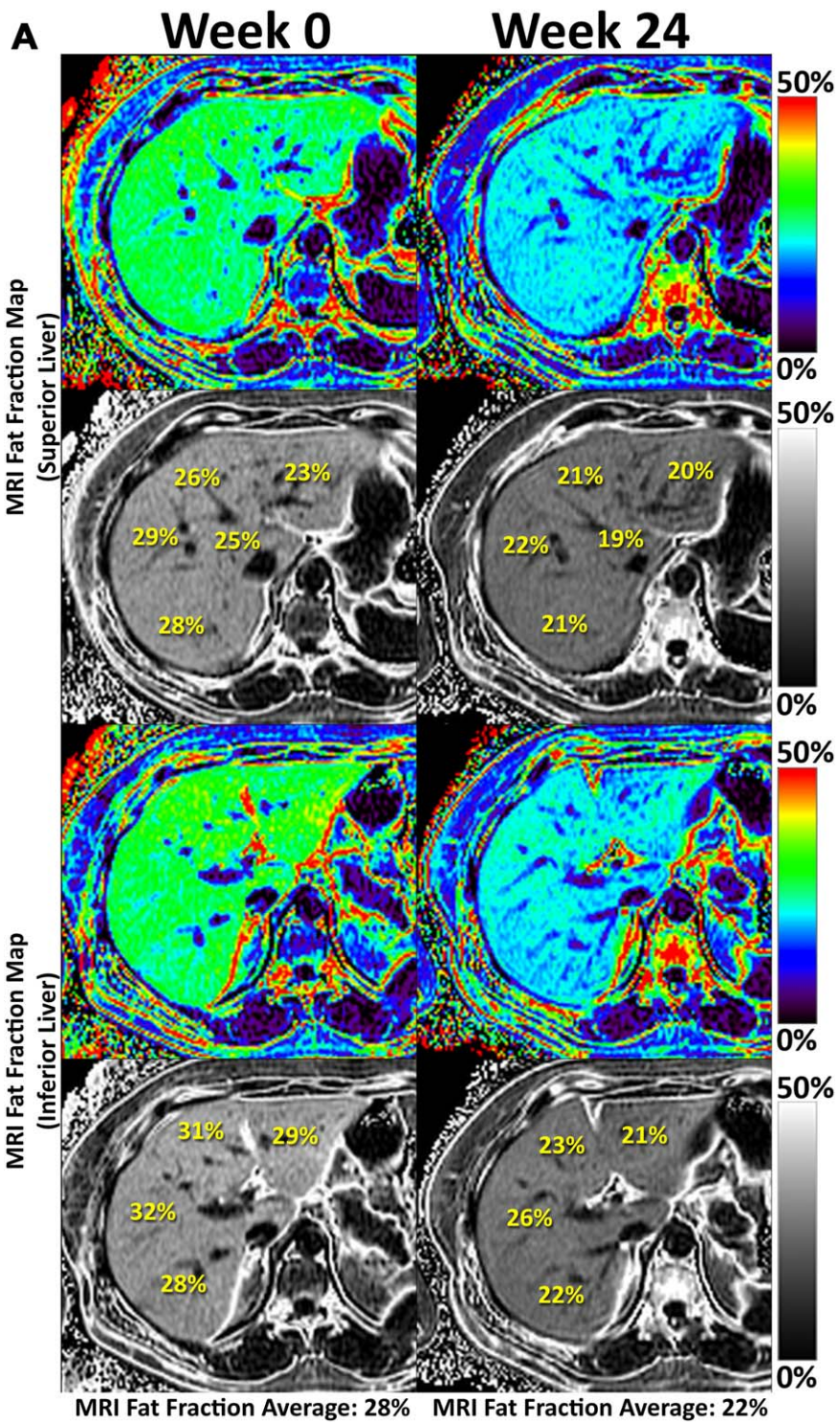

Fig. 2. (A) Whole-liver fat mapping with MRI-PDFF for a single patient. MRI-PDFF measurements of liver segments 1, 2, 4a, 7, and 8 in the superior plane (upper panel) and of liver segments 3, 4b, 5, and 6 in the inferior plane (lower panel) are shown at weeks 0 (left column) and 24 (right column) for a patient. The fat fraction in a single liver segment is estimated by MRI-PDFF. Using nine ROIs, one in each segment, the calculated total liver fat fraction average at week 0 was 28%, and this decreased to 22% at week 24. MRI-PDFF data from all nine liver segments gives a fat map for the entire liver where longitudinal within-segment changes of liver fat can be appreciated. (B) MRS measured fat fraction in the same patient. MRS measurements from a $2 \times 2 \times 2$ cm^3 cube (voxel) within the right liver lobe of the same patient in which MRI-PDFF was performed are shown at week 0 (left column) and week 24 (right column). The corresponding MRS fat fraction at week 0 was 28.8%, and this decreased to 22% at week 24. (C) Liver stiffness measured by 2D and 3D MRE in the same patient. The 2D MRE was done at 60 Hz, and the 3D MRE was done at 40 Hz and 60 Hz. The MR shear wave elastograms obtained at week 0 are shown to the left, and those at week 24 are shown to the right. All three shear wave elastograms showed a decrease in the elasticity of the liver.

Several preclinical studies from diverse groups provided a strong rationale for evaluating the role of ezetimibe in the treatment of NASH.[14,15] Small pilot uncontrolled studies showed beneficial effects of ezetimibe in improving serum ALT, AST, and liver fat by ultrasound and showed improvement in liver histology in a diverse group of patients with NAFLD as well as NASH.[16,17,34] However, these studies did not have a placebo group and were mainly conducted in Asian populations in an open label manner.[34] Therefore, the results seen in the prior studies indicated the need for a randomized placebo-controlled trial in well-characterized patients with biopsy-proven NASH.

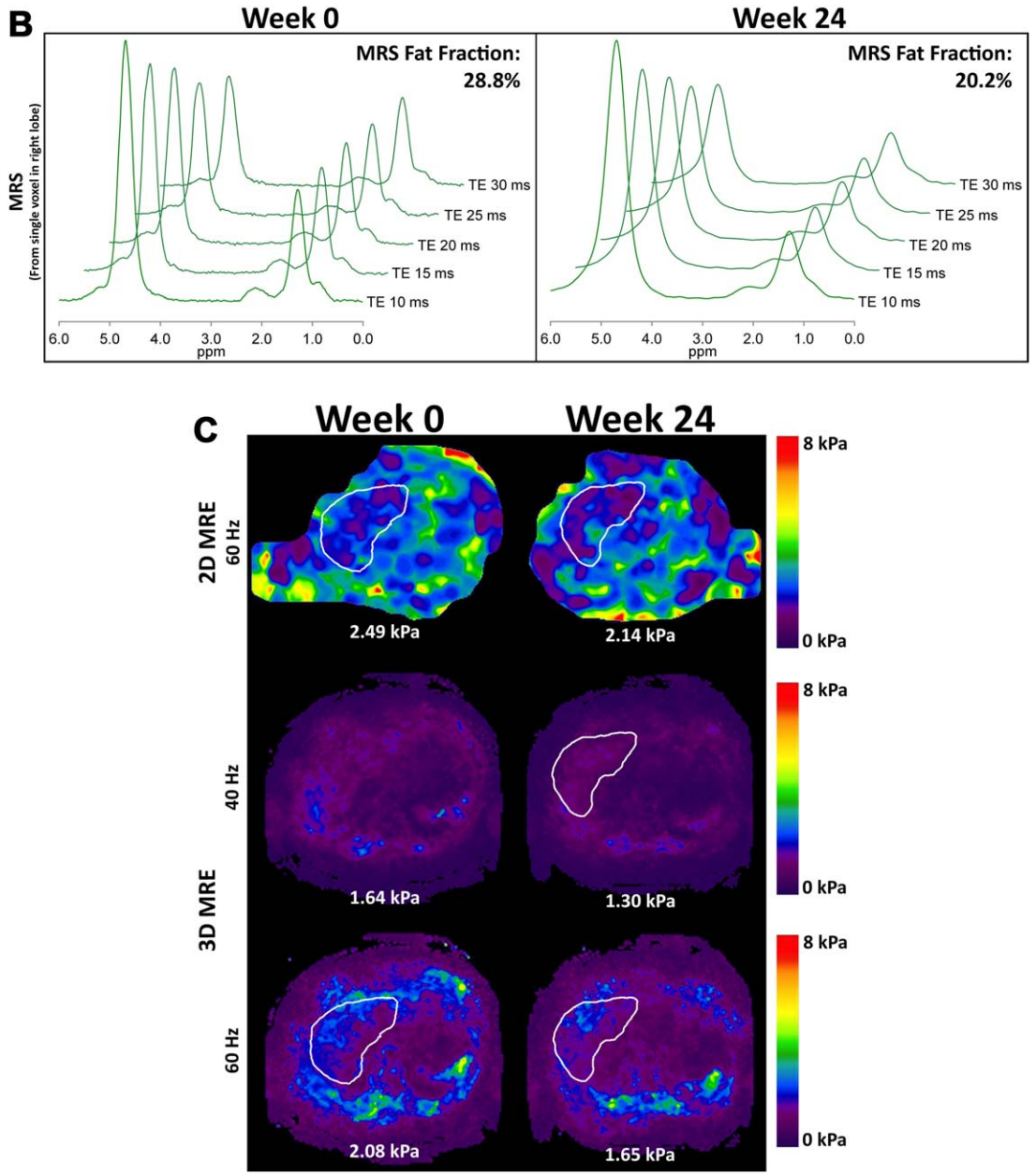

Fig. 2. (Continued)

Design and Innovation in Noninvasive MRI Assessment. Strengths of the trial were as follows. Using a randomized, allocation-concealed, double-blind, placebo-controlled study design, the MOZART trial provided a rigorous assessment of the efficacy of ezetimibe in the treatment of patients with biopsy-proven NASH. The trial utilized a novel, accurate, and precise noninvasive imaging biomarker, the MRI-PDFF, for assessment of treatment response and was conducted by an experienced multidisciplinary team of investigators with special expertise in advanced MR assessment of NASH. The assessment of quantitative changes in liver fat in colocalized ROIs within each of the nine liver segments provides a robust framework for utilization in future trials in NASH. The trial explored the role of advanced MR methods including 2D and 3D MRE in the setting of a clinical trial as an exploratory end point. This study describes the protocol that may be used for assessment of longitudinal changes in colocalized ROIs before and after treatment in MRE-derived liver stiffness in clinical trials underscoring the novelty and innovation in study design.

However, we acknowledge the following limitations of this study. All study visits and MR assessments were

Table 3. Ezetimibe Versus Placebo: Longitudinal Changes in Liver Stiffness Values Using 2D MRE and 3D MRE

	Ezetimibe (n = 19)			Placebo (n = 21)			
2D MRE	**Baseline**	**Posttreatment**	***P* Value**	**Baseline**	**Posttreatment**	***P* Value**	**Difference (*P* Value)**
60 Hz	3.3 (1.1)	3.4 (1.3)	0.7374	3.2 (1.1)	3.3 (1.3)	0.6769	0.0 (0.9560)
	Ezetimibe (n = 16)			**Placebo (n = 20 [40 Hz], n = 19 [60 Hz])**			
3D MRE	**Baseline**	**Posttreatment**	***P* Value**	**Baseline**	**Posttreatment**	***P* Value**	**Difference (*P* Value)**
40 Hz	2.1 (0.9)	2.0 (0.6)	0.7556	2.2 (1.0)	2.1 (1.0)	0.2448	0.1 (0.6233)
60 Hz	2.7 (1.3)	2.5 (1.0)	0.1344	2.8 (1.2)	2.7 (1.2)	0.4207	−0.1 (0.4895)

Data are expressed as means (SD). Associated *P* values are from *t* test.

Table 4. Changes in Anthropometric and Biochemical Variables Between Ezetimibe- and Placebo-Treated Patients

	Ezetimibe (n = 23)			Placebo (n = 22)			
	Baseline	**Posttreatment**	***P* Value**	**Baseline**	**Posttreatment**	***P* Value**	**Difference (*P* Value)**
Weight (kg)	93.0 ± 17.8	92.9 ± 18.7	0.8281	92.6 ± 19.6	92.0 ± 19.8	0.2808	0.2 (0.8079)
BMI (kg/m^2)	33.6 ± 5.2	33.2 ± 5.5	0.2225	33.6 ± 5.1	33.4 ± 5.0	0.2969	−0.3 (0.4839)
ALT (IU/L)	47.0 (29.0)	48.0 (43.0)	0.7682	45.5 (32.0)	42.0 (14.0)	0.5110	2.0 (0.6702)
AST (IU/L)	33.0 (23.0)	33.0 (36.0)	0.9332	31.0 (34.0)	32.0 (33.0)	0.2124	1.0 (0.6004)
AST/ALT	0.8 (0.6)	0.8 (0.4)	0.4065	0.8 (0.4)	0.7 (0.4)	0.4584	0.0 (0.9304)
Glucose (mg/dL)	104.0 (25.0)	99.0 (24.0)	0.9500	108.5 (40.0)	106.5 (24.0)	0.6598	−5.0 (0.8101)
Insulin (μU/mL)	22.5 (13.0)	26.5 (15.0)	0.3787	24.5 (18.5)	33.0 (19.0)	0.0889	−3.0 (0.5177)
Hemoglobin A1C (%)	5.9 (0.6)	5.9 (0.9)	0.1699	6.1 (1.0)	6.0 (0.8)	0.5538	0.2 (0.1663)
Triglycerides (mg/dl)	152.0 (63.0)	125.0 (59.0)	0.2139	144.5 (110.0)	142.0 (107.0)	0.3883	−8.5 (0.0977)
Total Cholesterol (mg/dl)	182.0 (26.0)	152.0 (46.0)	**0.0003**	169.0 (56.0)	175.0 (37.0)	0.3344	**−24.0 (0.0024)**
LDL (mg/dL)	99.0 (37.0)	76.0 (30.0)	**<0.0001**	89.0 (53.0)	90.5 (39.0)	0.8048	**−20.0 (0.0019)**
FFA (mmol/L)	0.5 (0.3)	0.5 (0.4)	0.9765	0.7 (0.3)	0.5 (0.3)	**0.0314**	0.1 (0.1216)
Alkaline phosphatase (IU/L)	74.0 (34.0)	89.0 (45.0)	**0.0284**	71.0 (39.0)	70.5 (35.0)	0.6139	6.0 (0.1255)
GGT (IU/L)	44.0 (36.0)	41.5 (38.0)	0.5286	33.0 (38.0)	36.5 (31.0)	0.5523	3.0 (0.5069)
Total bilirubin (mg/dl)	0.4 (0.4)	0.4 (0.3)	0.1088	0.4 (0.3)	0.4 (0.2)	0.8865	0.1 (0.1993)
Direct bilirubin (mg/dl)	0.1 (0.0)	0.1 (0.1)	0.0547	0.1 (0.1)	0.1 (0.0)	0.6875	0.0 (0.1235)
HOMA-IR	6.4 (4.5)	6.4 (6.2)	0.6502	6.5 (5.4)	9.1 (5.2)	0.6215	0.7 (0.8257)

Data are expressed as median (interquartile range) with *P* values from Wilcoxon signed rank test or mean difference with *P* value in parentheses.

Abbreviations: BMI, body mass index; LDL, low-density lipoprotein; FFA, free fatty acids; GGT, gamma-glutamyl transferase; HOMA-IR, homeostatic model assessment of insulin resistance.

Table 5. Changes in Liver Histology in Ezetimibe- Versus Placebo-Treated Patients

	Ezetimibe (n = 17)			Placebo (n = 18)			
	Baseline	**Posttreatment**	***P* Value**	**Baseline**	**Posttreatment**	***P* Value**	**Difference (*P* Value)**
Steatosis							
Median	2.0 (1.0)	1.0 (1.0)	0.2500	3.0 (1.0)	2.0 (1.0)	0.1133	0.0 (0.692)
N grade 0/1/2/3	0/7/6/4	0/5/3/2		0/2/5/11	0/4/8/6		
Lobular inflammation							
Median	1.0 (1.0)	2.0 (1.0)	0.2891	2.0 (1.0)	2.0 (1.0)	0.7266	0.0 (0.142)
N grade 0/1/2/3	0/10/7/0	0/7/9/1		0/5/13/0	0/7/11/0		
Ballooning							
Median	1.0 (1.0)	1.0 (2.0)	0.2734	1.5 (1.0)	1.0 (1.0)	0.2891	0.0 (0.552)
N grade 0/1/2	1/10/6	5/7/5		1/8/9	1/12/5		
Fibrosis							
Median	1.0 (2.0)	1.0 (3.0)	1.000	1.0 (3.0)	1.0 (2.0)	0.3750	0.0 (0.222)
N grade 0/1/2/3/4	5/6/2/3/1	7/3/2/5/0		6/5/1/5/1	4/6/2/5/1		
NAFLD activity score							
Median	5.0 (2.0)	4.0 (2.0)	0.2910	5.0 (1.0)	5.0 (2.0)	0.1372	0.0 (0.927)
		Ezetimibe n **(%)**			Placebo n (%)		Fisher's *P* value
2+ point improvement in NAS		5 (33.3%)			5 (27.8%)		1.000

Data are expressed as median (interquartile range) with *P* values from Wilcoxon's signed rank test or as n (%) with *P* values from Fisher's exact test.

performed at the University of California at San Diego's NAFLD Translational Research Unit and Liver Imaging Group, respectively. It is possible that the reduction in steatosis could be more significant if the baseline MRI-PDFF was higher. However, the baseline liver fat content was similar to that in previous treatment trials in NASH.[11,18] Hence, if the therapy was effective, we would have seen an effect. Therefore, the generalizability and, perhaps more importantly, availability of these advanced MR methods for assessment of treatment response at other clinical centers require further validation in the setting of a multicenter trial. At this stage, the use of MRE-derived treatment response assessment may be considered as an adjunct or as complementary to conventional methods of fibrosis assessment in NASH.

In conclusion, histologic responder versus nonresponder comparative analyses and placebo-arm changes with MRI-PDFF and both 2D and 3D MRE-derived liver stiffness may help in the design of future clinical trials by providing more comprehensive assessment of treatment and placebo effects. Further secondary analyses of the placebo-arm changes in 2D and 3D MRE-derived liver stiffness would help establish the expected changes in these parameters on placebo and the variance in liver stiffness over a 24-week period. These would help inform the sample-size estimation of clinical trials with a desired effect size, especially when assessing antifibrotic treatment response in a NASH trial. Noninvasive quantitative assessment of liver fat has been increasingly utilized in clinical trials in NASH. This trial provides pilot data on feasibility as well as a protocol for performing 2D and 3D MRE in the setting of a randomized control trial and sets the stage for noninvasive MRE-based quantitative assessment of liver fibrosis and stiffness in colocalized ROIs in future clinical trials in NASH-related fibrosis.

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Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.27647/suppinfo.