Effect of Vitamin E or Metformin for Treatment of Nonalcoholic Fatty Liver Disease in Children and Adolescents

The TONIC Randomized Controlled Trial

Joel E. Lavine, MD, PhD
Jeffrey B. Schwimmer, MD
Mark L. Van Natta, MHS
Jean P. Molleston, MD
Karen F. Murray, MD
Philip Rosenthal, MD
Stephanie H. Abrams, MD, MS
Ann O. Scheimann, MD, MBA
Arun J. Sanyal, MD
Naga Chalasani, MBBS
James Tonascia, PhD
Aynur Ünalp, MD, PhD
Jeanne M. Clark, MD, MPH
Elizabeth M. Brunt, MD
David E. Kleiner, MD, PhD
Jay H. Hoofnagle, MD
Patricia R. Robuck, PhD, MPH
for the Nonalcoholic Steatohepatitis Clinical Research Network

Context Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in US children and adolescents and can present with advanced fibrosis or nonalcoholic steatohepatitis (NASH). No treatment has been established.

Objective To determine whether children with NAFLD would improve from therapeutic intervention with vitamin E or metformin.

Design, Setting, and Patients Randomized, double-blind, double-dummy, placebo-controlled clinical trial conducted at 10 university clinical research centers in 173 patients (aged 8-17 years) with biopsy-confirmed NAFLD conducted between September 2005 and March 2010.

Interventions Daily dosing of 800 IU of vitamin E (58 patients), 1000 mg of metformin (57 patients), or placebo (58 patients) for 96 weeks.

Main Outcome Measures The primary outcome was sustained reduction in alanine aminotransferase (ALT) defined as 50% or less of the baseline level or 40 U/L or less at visits every 12 weeks from 48 to 96 weeks of treatment. Improvements in histological features of NAFLD and resolution of NASH were secondary outcome measures.

Results Sustained reduction in ALT level was similar to placebo (10/58; 17%; 95% CI, 9% to 29%) in both the vitamin E (15/58; 26%; 95% CI, 15% to 39%; $P=.26$) and metformin treatment groups (9/57; 16%; 95% CI, 7% to 28%; $P=.83$). The mean change in ALT level from baseline to 96 weeks was –35.2 U/L (95% CI, –56.9 to –13.5) with placebo vs –48.3 U/L (95% CI, –66.8 to –29.8) with vitamin E ($P=.07$) and –41.7 U/L (95% CI, –62.9 to –20.5) with metformin ($P=.40$). The mean change at 96 weeks in hepatocellular ballooning scores was 0.1 with placebo (95% CI, –0.2 to 0.3) vs –0.5 with vitamin E (95% CI, –0.8 to –0.3; $P=.006$) and –0.3 with metformin (95% CI, –0.6 to –0.0; $P=.04$); and in NAFLD activity score, –0.7 with placebo (95% CI, –1.3 to –0.2) vs –1.8 with vitamin E (95% CI, –2.4 to –1.2; $P=.02$) and –1.1 with metformin (95% CI, –1.7 to –0.5; $P=.25$). Among children with NASH, the proportion who resolved at 96 weeks was 28% with placebo (95% CI, 15% to 45%; 11/39) vs 58% with vitamin E (95% CI, 42% to 73%; 25/43; $P=.006$) and 41% with metformin (95% CI, 26% to 58%; 16/39; $P=.23$). Compared with placebo, neither therapy demonstrated significant improvements in other histological features.

Conclusion Neither vitamin E nor metformin was superior to placebo in attaining the primary outcome of sustained reduction in ALT level in patients with pediatric NAFLD.

Trial Registration clinicaltrials.gov Identifier: NCT00063635

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COINCIDENT WITH THE RISE IN prevalence of childhood and adolescent obesity over the past few decades, nonalcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease in children in the United States.[1,2] NAFLD encompasses a range of severity from bland steatosis to nonalcoholic steatohepatitis (NASH) that may ultimately result in advanced fibrosis, cirrhosis, and hepatocellular carcinoma.[3] Cirrhosis due to NAFLD has been described in children.[4] Presence of NAFLD in children independently increases risks for cardiovascular disease.[5]

Author Affiliations and **Members of the Nonalcoholic Steatohepatitis Clinical Research Network** are listed at the end of this article.
Corresponding Author: Joel E. Lavine, MD, PhD, Columbia University Medical Center, 3959 Broadway, CHN 7-702, New York, NY 10032 (jl3553@columbia.edu).

NAFLD is a clinicopathological diagnosis in which more than 5% of hepatocytes demonstrate macrovesicular steatosis in an individual without significant history of alcohol intake. NASH is characterized by steatosis with hepatocellular ballooning and lobular inflammation, often accompanied by perisinusoidal fibrosis.[6] Although many children exhibit a histologic pattern of NAFLD or NASH consistent with the features reported in adults,[7] a significant subset demonstrate unique features regarding location or presence of fat, inflammation, and fibrosis.[8] Age- or duration-specific differences likely contribute to the etiopathogenesis of NAFLD. NAFLD, as opposed to NASH, was selected as the histologic entry criterion for this study because of a lack of knowledge about the natural history of this pediatric subset.

Insulin resistance is frequently identified in both adults and children with NAFLD[9]; oxidative stress is considered a contributor to progression.[10] Treatment approaches to NAFLD in adults and children target reduction in insulin resistance and oxidative stress. The only recognized management strategies use dietary modification and exercise.[12,13] No randomized controlled trials have been performed in children using histology, which is regarded as the gold-standard assessment.[11] Based on pediatric pilot data demonstrating potential efficacy of metformin[14] or vitamin E,[15] the NASH Clinical Research Network (NASH CRN), which is supported by the National Institutes of Health, initiated a phase 3, multicenter, randomized, double-blinded, placebo-controlled trial evaluating vitamin E or metformin for the Treatment of NAFLD in Children (TONIC).

METHODS

The TONIC protocol and consent and assent forms were developed by members of the treatment trial committee of the NASH CRN with approval by the steering committee. The study was approved by the institutional review boards at each center. Investigational new drug approval was obtained from the Food and Drug Administration. Each patient's parent or guardian provided written consent and each patient gave written assent. Data quality and patient safety assessments were reviewed regularly by an independent data and safety monitoring board. There were no formal stopping rules. A single interim efficacy analysis was performed when 50% of participants completed the 96-week course of treatment and had their follow-up liver biopsies. The manuscript was prepared by the writing committee and reviewed and approved by all steering committee members.

Full details of the trial design have been published previously.[16] The TONIC trial design conformed to the revised CONSORT standards for reporting randomized trials.[17] Eligible children were identified and recruited from unsolicited referrals to the 10 participating clinical centers starting in September 2005 and ending September 2007. Patients aged 8 to 17 years with NAFLD and persistently elevated levels of alanine aminotransferase (ALT) were eligible. All patients and parents were provided uniform standard-of-care advice on diet and exercise at each visit by physicians and dieticians. NAFLD was defined by a liver biopsy demonstrating more than 5% steatosis within a 6-month period before randomization. Persistent elevation of ALT was defined by a value greater than 60 U/L for 1 to 6 months before and at the time of randomization. Patients with diabetes mellitus or cirrhosis were excluded. The rationale for their exclusion was to avoid potential decompensation in either condition. Children younger than 8 years were excluded to avoid problems with swallowing pills. Other exclusion factors were monogenic inborn errors of metabolism, pregnancy, viral hepatitis, alcohol use, and other causes of chronic liver disease.

Biopsies were interpreted by a site pathologist to determine eligibility and later read centrally by NASH CRN pathologists masked to treatment assignment. Histological activity was assessed using the NAFLD activity score on a scale of 0 to 8, with higher scores indicating more severe disease; the components of this measure include steatosis (0-3), lobular inflammation (0-3), and hepatocellular ballooning (0-2).[7] Biopsies also were assessed for the presence or absence of NASH. These determinations were made independently of the lesion scores and were based on pattern of injury as well as the presence and degree of individual lesions.

Randomization and Treatment Assignment

Eligible patients were randomized in permuted blocks of treatments stratified by clinical center. Patients were assigned in a 1:1:1 ratio to 1 of 3 groups for 96 weeks of treatment, either (1) oral metformin (500 mg twice daily) and vitamin E placebo twice daily, (2) vitamin E (400 IU twice daily) and metformin placebo twice daily, or (3) vitamin E placebo and metformin placebo, each twice daily. The natural form of vitamin E (RRR-α-tocopherol, Nature Made 400-IU soft gel) and identical-appearing placebo were supplied by Pharmavite (Northridge, California) under a clinical trial agreement with the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Metformin 500-mg tablets and identical-appearing placebo were overencapsulated with the dose selected based on prior pilot study results in pediatric NAFLD[15] and considerations of tolerable pill size and quantity. Adherence was assessed with pill counts confirmed at each visit. Subgroup analyses were performed to assess that treatment group results did not vary with adherence by stratifying those with 80% or greater adherence vs those with less than 80% adherence.

Follow-up Visits

Visits were scheduled 4 weeks and 12 weeks after randomization and every 12 weeks thereafter through 96 weeks of treatment. Another visit 24 weeks after treatment cessation (120 weeks postrandomization) was performed to assess durability of response and further

safety evaluation. The final patient follow-up was March 2010. Each visit through the end of treatment included taking a standardized medical history; serum collection; and recording interim safety-related events, adherence, and pill counts. Any serious adverse events identified from spontaneous reports that were deemed unexpected and related were sent to the data coordinating center and NIDDK within 7 days of the event becoming known to the clinic staff. Urine pregnancy tests were performed for female participants of child-bearing potential at each visit. At baseline and 96-week end-of-treatment visits, patients underwent anthropometric assessments of weight, height, waist and hip circumference, triceps skin fold, and Tanner stage; laboratory evaluations, including fasting insulin and serum glucose, ALT, aspartate aminotransferase (AST), alkaline phosphatase, total bilirubin, α-tocopherol, γ-glutamyltransferase (GGT), cholesterol, and triglycerides; body composition assessment with dual-energy x-ray absorptiometry (DXA); and a Pediatric Quality of Life Inventory (version 4.0) (physical health functioning and psychosocial health summary), completed by both the child and the parent or guardian. At 96 weeks, a percutaneous liver biopsy was performed immediately after treatment was ended. At weeks 48, 96, and 120, the following assessments were added if not already included: physical activity, liver symptom, pediatric quality of life, and nutrition questionnaires; hemoglobin, hematocrit, white blood cell, and platelet counts; fasting lipids; homeostatis model assessment of insulin resistance (HOMA-IR); serum glucose; glucose tolerance testing with insulin; C-peptide; free fatty acids; leptin; and C-reactive protein. Sociodemographic variables, including race and ethnicity with categorization similar to the 2010 US Census, were reported by the patient or the patient's parent or guardian at baseline. These data were collected to test for subgroup effects because NASH varies by race and ethnicity.

Primary and Secondary Outcomes and Primary Analysis

The primary outcome was sustained reduction in ALT level, defined as 50% or less of the baseline level or 40 U/L or less at each visit from 48 to 96 weeks of treatment. The primary analysis was intention to treat with all patients analyzed according to assigned treatment. Patients missing a 96-week ALT measurement were imputed as not achieving a sustained reduction. Differences between groups were analyzed using the Mantel-Haenszel χ^2 test stratified by clinic.

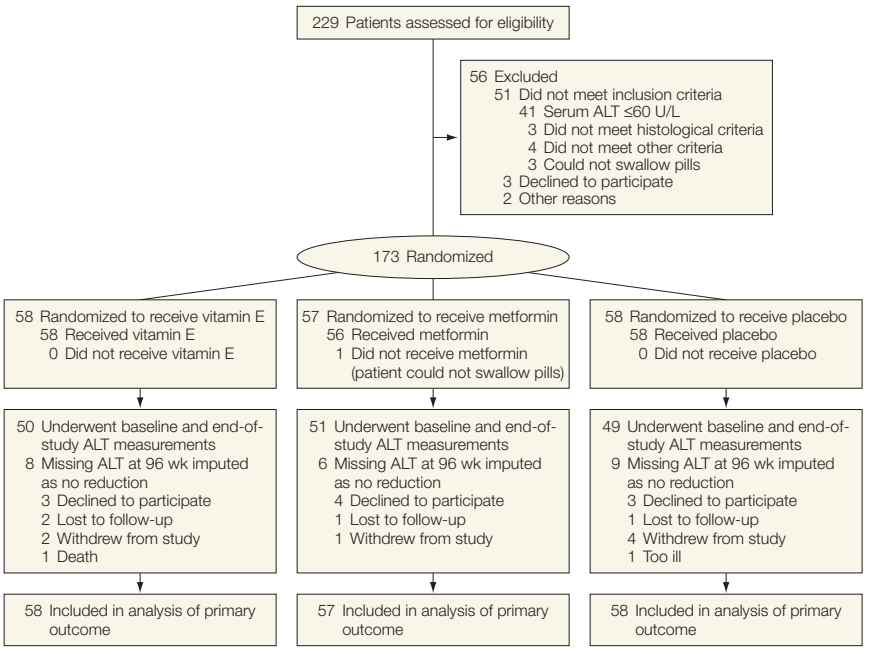
There were no planned subgroup analyses; however, post hoc subgroup analyses were done to explore whether the overall primary outcome results differed in particular groups of patients. Logistic regression models were used for the subgroup analyses and included terms for treatment group, subgroup, and the interaction of treatment group and subgroup. The interaction terms represent potential subgroup differences in treatment effects and were considered statistically significant if the interaction $P < .01$. The subgroups evaluated were sex, age, race, Hispanic ethnicity, Tanner stage, elevated ALT, presence of NASH, body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared), weight, vitamin E levels, and adherence.

To explore the emergence of any time-related patterns of response to treatment, means and 95% confidence intervals (CIs) for changes relative to baseline in ALT, AST, alkaline phosphatase, weight, BMI *z* score, and HOMA-IR were plotted vs time from randomization until end of treatment at 96 weeks and until 24 weeks after treatment (at 120 weeks). Mean and 95% CIs for changes from baseline in ALT were compared at 24, 48, 72, and 96 weeks using analysis-of-covariance (ANCOVA) models for ALT at each time point related to treatment group and the baseline ALT measure. Changes in liver histology scores from the baseline biopsy to the end-of-treatment biopsy were compared using either a χ^2 test for binary outcomes related to improvement in histology or an ANCOVA model for changes in scores. ANCOVA models were also used to compare changes from baseline to the end of treatment in serum biochemistry tests; lipids; metabolic measures; and quality-of-life scores reported by child and parent/guardian. Frequencies of adverse events were compared across the treatment groups using Fisher exact test.

Secondary outcome measures were assessed using change from baseline to week 96 and included the following: those with NASH and borderline NASH improving to "not NASH"; NAFLD activity score; individual histological scores, including hepatocellular ballooning, fibrosis, steatosis, and lobular inflammation; health-related quality of life; anthropometric variables; insulin resistance and serum lipid profiles; and levels of AST, GGT, and alkaline phosphatase. Secondary outcome measures were assessed for completers and were analyzed using ANCOVA adjusting for the baseline measure of the outcome for continuous measures, χ^2 tests for unordered categorical measures, and Cochran χ^2 tests for trend for ordered categorical measures. *P* values were nominal and not adjusted for multiple comparisons or multiple looks. Analyses were performed using SAS version 9.2 (SAS Institute, Cary, North Carolina) and Stata version 11.1 (StataCorp, College Station, Texas).

Although improvement in histology is a more desirable primary outcome measure than improvement in ALT, lack of any prior histology-based NAFLD trials in children precluded sample size calculations for adequate power. The intended sample size was 180 patients with equal allocation to the 3 treatment groups, which was calculated to provide 90% power with a 2-sided type I error of .025 (because of the 2 planned comparisons) and an estimated response rate of 50% vs 20% in sustained reduction in ALT level between the active treatment groups and placebo, respectively. The

Figure 1. CONSORT Flow Diagram of TONIC Trial Participants



All patients were evaluated on an intention-to-treat basis. ALT indicates alanine aminotransferase.

expected response rate of 50% for metformin was estimated using pilot data from a 48-week metformin study, the same response rate was assumed for vitamin E, and the response rate of 20% in the placebo group was consensus estimate from the investigators. The study was planned to stop at a fixed calendar time (September 2007) because of budgeting and drug supply logistics, so the achieved sample size was 173 (96% of target), which corresponds to 88% power rather than 90%.

RESULTS

Study Patients

Patient screening, enrollment, and retention by treatment group are detailed in FIGURE 1. One hundred seventy-three patients were randomized to receive vitamin E (n=58), metformin (n=57), or placebo (n=58). Ages ranged from 8 to 17 years (mean, 13.1 years), and the majority were male (81%) and Hispanic (61%). Most patients were peripubertal, obese, and insulin resistant. Baseline characteristics were similar for all 3 treatment groups with respect to demographics, anthropometrics, quality-of-life assessments, pertinent laboratory data, and liver histology (TABLE 1).

Primary Outcome

Eighty-seven percent of patients completed 96 weeks of treatment and had end-of-study clinical parameters and liver biopsy obtained. All enrolled patients were included in analysis of the primary outcome, sustained reduction in ALT level. The attainment of sustained reduction in ALT level was similar to placebo (10/58; 17%; 95% CI, 9% to 29%) in both the vitamin E (15/58; 26%; 95% CI, 15% to 39%; $P=.26$) and metformin treatment groups (9/57; 16%; 95% CI, 7% to 28%; $P=.83$) (TABLE 2). The mean change in ALT level from baseline to 96 weeks was –35.2 U/L (95% CI, –56.9 to –13.5) in the placebo group vs –48.3 U/L (95% CI, –66.8 to –29.8) in the vitamin E group ($P=.07$) and –41.7 U/L (95% CI, –62.9 to –20.5) in the metformin group ($P=.40$).

Although 100% (70/70) of patients at the largest clinic had complete data at the end of treatment vs 78% (80/103) at the other clinics, sensitivity analysis by completer status showed results consistent with the negative primary outcome findings for vitamin E and metformin (eTable 1, available at http://www.jama.com). Subgroup analyses evaluating sex, age, race, Hispanic ethnicity, Tanner stage, degree of ALT elevation, presence of NASH, BMI, weight, vitamin E level, and adherence to medications were also consistent with negative primary outcome findings for vitamin E and metformin (eTable 1). Change in ALT level at 24, 48, 72, and 96 weeks (Table 2 and FIGURE 2) showed a significant difference between those treated with vitamin E and placebo at week 24 (at week 48, $P=.04$), but this difference diminished at weeks 72 and 96, primarily because of continued reductions in ALT levels in the placebo group.

Secondary Outcomes

Histology. Among the 121 patients who had either NASH or borderline NASH at baseline, the resolution of NASH was significantly greater in children treated with vitamin E than with placebo (58%; 95% CI, 42% to 73%; 25/43; vs 28%; 95% CI, 15% to 45%; 11/39; $P=.006$). This was mainly attributable to significant improvement in hepatocellular ballooning by vitamin E treatment (change in mean score, –0.5; 95% CI, –0.8 to –0.3, vs +0.1; 95% CI, –0.2 to 0.3; $P=.006$). Forty-four percent of patients taking vitamin E (95% CI, 30% to 59%; 22/50) had improvement in hepatocellular ballooning compared with 21% taking placebo (95% CI, 11% to 36%; 10/47; $P=.02$). Vitamin E treatment also significantly improved NAFLD activity score (change in mean score, –1.8; 95% CI, –2.4 to –1.2, vs –0.7; 95% CI, –1.3 to –0.2; $P=.02$). However, vitamin E did not have significant effects on steatosis, inflammation, or fibrosis as individual components. Forty-four percent of patients treated with metformin (95%

CI, 30% to 59%; 22/50) had improvement in hepatocellular ballooning compared with 21% of patients treated with placebo (95% CI, 11% to 36%; 10/47; $P=.02$). No other significant improvement was found in those treated with metformin compared with placebo regarding steatosis, inflammation, change in NAFLD

Table 1. Baseline Characteristics by Treatment Group

	Mean (SD)[a]			
	Vitamin E (n = 58)	Metformin (n = 57)	Placebo (n = 58)	Total (N = 173)
Age, y	13.4 (2.3)	13.1 (2.4)	12.9 (2.6)	13.1 (2.4)
Female sex, No. (%)	11 (19.0)	10 (17.5)	12 (20.7)	33 (19.1)
Hispanic ethnicity, No. (%)	36 (62.1)	31 (54.4)	39 (67.2)	106 (61.3)
Race, No. (%)				
American Indian or Alaska Native	7 (12.1)	6 (10.5)	10 (17.2)	23 (13.3)
Asian	1 (1.7)	2 (3.5)	0	3 (1.7)
Black or African American	3 (5.2)	1 (1.8)	0	4 (2.3)
Native Hawaiian or other Pacific Islander	0	0	1 (1.7)	1 (0.6)
White	40 (69.0)	43 (75.4)	45 (77.6)	128 (74.0)
≥2 Races	1 (1.7)	1 (1.8)	0	2 (1.2)
Refusal/not stated	6 (10.3)	4 (7.0)	2 (3.4)	12 (6.9)
Self-reported QOL score[b]				
Physical health	78 (17)	77 (18)	76 (21)	77 (19)
Psychosocial health	70 (19)	71 (16)	68 (19)	70 (18)
Parent/guardian-reported QOL score[b]				
Physical health	66 (23)	64 (23)	65 (24)	65 (23)
Psychosocial health	64 (18)	62 (19)	61 (21)	62 (19)
Serum biochemistry tests				
AST, U/L	70 (37)	69 (45)	74 (42)	71 (41)
ALT, U/L	121 (65)	121 (68)	126 (62)	123 (65)
GGT, U/L	50 (25)	52 (51)	50 (32)	51 (37)
Alkaline phosphatase, U/L	220 (94)	237 (99)	229 (93)	228 (95)
Total bilirubin, mg/dL	0.68 (0.33)	0.64 (0.25)	0.63 (0.32)	0.65 (0.30)
α-Tocopherol, mg/L	9.5 (4.9)	8.4 (2.7)	9.3 (4.8)	9.1 (4.3)
Lipids				
Triglycerides, mg/dL	154 (107)	151 (103)	153 (92)	153 (100)
Total cholesterol, mg/dL	179 (42)	174 (45)	176 (35)	176 (40)
HDL, mg/dL	37 (9)	38 (7)	38 (10)	38 (9)
LDL, mg/dL	114 (34)	105 (30)	108 (27)	109 (30)
Metabolic characteristics				
HOMA-IR, mg/dL × μU/mL/405	8.6 (7.8)	7.9 (5.4)	11.0 (17.6)	9.2 (11.6)
Fasting serum glucose, mg/dL	87 (8)	90 (10)	90 (9)	89 (9)
Weight, kg	91 (28)	88 (23)	86 (24)	88 (25)
Waist circumference, cm	108 (18)	104 (13)	105 (12)	105 (15)
BMI[c]	34 (7)	34 (5)	33 (6)	34 (6)
BMI *z* score	2.33 (0.34)	2.35 (0.30)	2.35 (0.26)	2.35 (0.30)
Body composition, % total fat[d]	44 (6)	44 (7)	43 (7)	43 (6)
Tanner stage[e]	2.6 (1.5)	2.6 (1.4)	2.5 (1.5)	2.6 (1.4)
Liver histology				
Fibrosis stage	1.2 (1.0)	1.3 (1.0)	1.2 (1.0)	1.2 (1.0)
Definite NASH, No. (%)	27 (46.6)	24 (42.1)	22 (37.9)	73 (42.2)
Steatosis score	2.3 (0.8)	2.1 (0.8)	2.1 (0.8)	2.2 (0.8)
Lobular inflammation score	1.6 (0.6)	1.6 (0.6)	1.7 (0.6)	1.6 (0.6)
Ballooning degeneration score	1.0 (0.8)	0.8 (0.8)	0.8 (0.8)	0.8 (0.8)
NAFLD activity score	4.8 (1.6)	4.5 (1.2)	4.6 (1.3)	4.6 (1.4)

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; QOL, quality of life.

SI conversion factors: To convert AST, ALT, GGT, and alkaline phosphatase to μkat/L, multiply by 0.0167; to convert total bilirubin to μmol/L, multiply by 17.104; to convert α-tocopherol to μmol/L, multiply by 23.22; to convert triglycerides to mmol/L, multiply by 0.0113; to convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259; and to convert glucose to mmol/L, multiply by 0.0555.

[a] Except where otherwise noted.

[b] Pediatric Quality of Life Inventory (version 4.0) scores were recoded to range from 0 to 100, with increasing scores indicating better quality of life.

[c] Calculated as weight in kilograms divided by height in meters squared.

[d] Three patients, 1 assigned to each group, were too obese to be assessed. Their values were imputed with the 95th percentile value.

[e] Tanner stage was defined using genital stage for boys and breast stage for girls.

activity score, or resolution of NASH (TABLE 3).

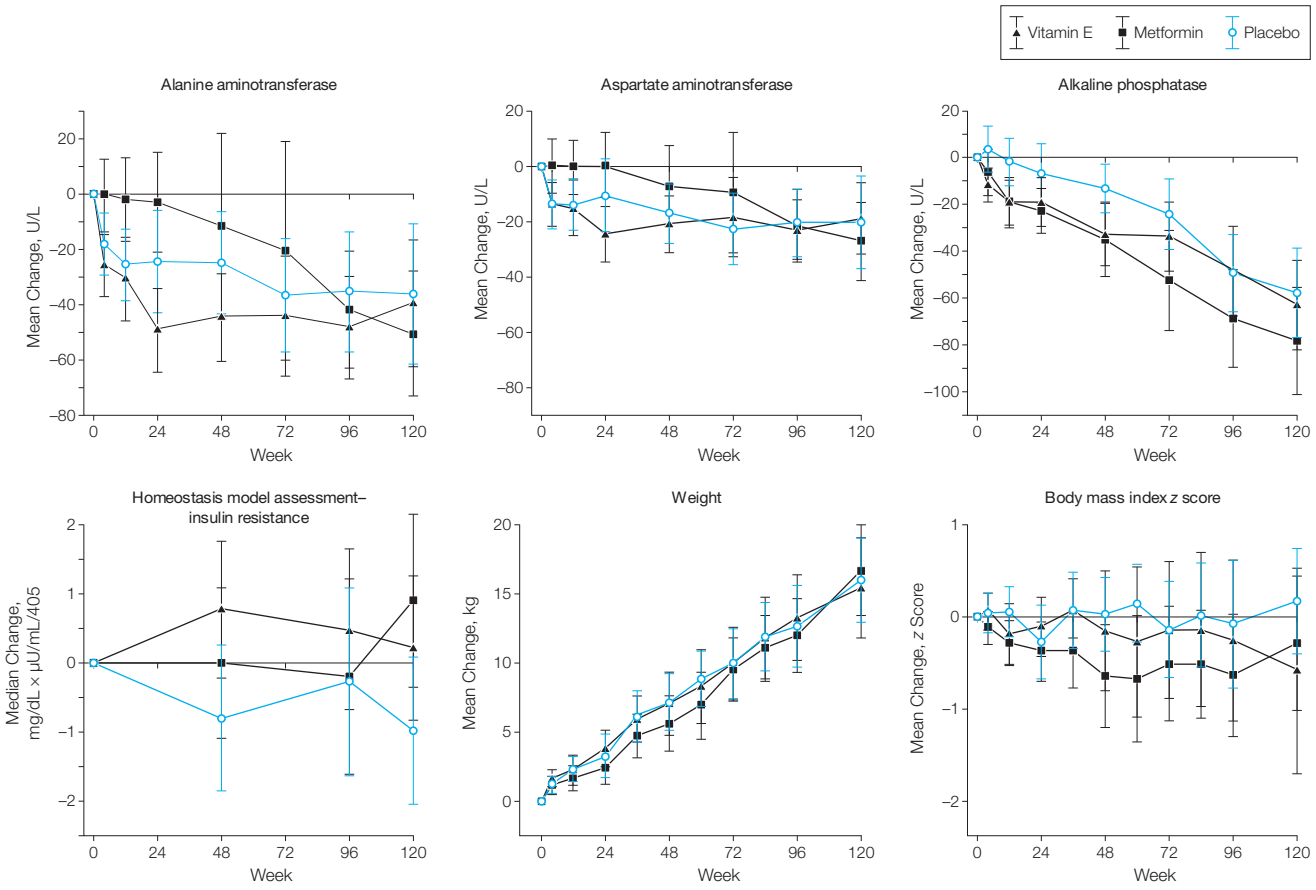
Serum Biochemistry Tests. All 3 groups demonstrated a decrease in ALT, AST, GGT, and alkaline phosphatase levels over 96 weeks of treatment, but no decrease was significant comparing baseline and week-96 vitamin E or metformin and placebo (TABLE 4 and Figure 2). Serum triglyceride levels increased, and levels of total cholesterol, low-density lipoprotein

Table 2. Primary Outcome: Sustained Reduction in ALT Level by Treatment Group

	Vitamin E (n = 58)	Metformin (n = 57)	Placebo (n = 58)	*P* Value[a] Vitamin E vs Placebo	*P* Value[a] Metformin vs Placebo
Sustained reduction in ALT level, No. (%) [95% CI][b]	15 (26) [15 to 39]	9 (16) [7 to 28]	10 (17) [9 to 29]	.26	.83
Relative efficacy vs placebo, % (95% CI)[c]	50 (–36 to 206)	–9 (–149 to 109)			
Change in ALT level from baseline, mean (95% CI), U/L[d]					
Week 24	–49.2 (–64.4 to –33.9)	–3.0 (–21.1 to 15.0)	–24.5 (–43.0 to –5.9)	.005	.09
Week 48	–44.5 (–60.3 to –28.7)	–11.7 (–45.3 to 22.0)	–25.0 (–43.7 to –6.4)	.04	.52
Week 72	–44.2 (–65.9 to –22.5)	–20.5 (–59.8 to 18.8)	–36.4 (–57.1 to –15.8)	.29	.51
Week 96	–48.3 (–66.8 to –29.8)	–41.7 (–62.9 to –20.5)	–35.2 (–56.9 to –13.5)	.07	.40

Abbreviations: ALT, alanine aminotransferase; CI, confidence interval.
[a] Based on χ^2 test for binary outcomes or analysis-of-covariance model regressing change from baseline on treatment group and baseline value of the outcome for continuous outcomes.
[b] Sustained reduction defined as ALT ≤40 U/L or ≤0.5 × baseline ALT level at 48-, 60-, 72-, 84-, and 96-week visits. Primary outcome was imputed as no sustained reduction if data were missing either at week 96 or at all 4 visits from week 48 to week 84; numbers imputed were 8, 6, and 9 for the vitamin E, metformin, and placebo groups, respectively.
[c] Relative efficacy vs placebo = (1 – relative risk) × 100.
[d] Number of patients with complete data was similar across treatment groups at each follow-up visit and ranged from 45 to 52.

Figure 2. Changes in Secondary Outcome During Treatment and Follow-up



Values for those completing each visit are expressed as changes relative to baseline over time from randomization until end of treatment. The number of patients at each visit within each treatment group was nearly constant with a mean of 49 and range of 44 to 53. Error bars indicate 95% confidence intervals.

Table 3. Change From Baseline to End of Treatment in Liver Histology by Treatment Group

	Vitamin E (n = 50)	Metformin (n = 50)	Placebo (n = 47)	*P* Value[a] Vitamin E vs Placebo	*P* Value[a] Metformin vs Placebo
Fibrosis score					
No. (%) improved [95% CI]	18 (37) [23 to 52]	22 (44) [30 to 59]	19 (40) [26 to 56]	.71	.72
Mean change (95% CI)	–0.3 (–0.6 to 0.0)	–0.4 (–0.7 to –0.0)	–0.2 (–0.6 to 0.1)	.48	.60
Steatosis score					
No. (%) improved [95% CI]	27 (54) [39 to 68]	26 (52) [37 to 66]	19 (40) [26 to 56]	.18	.25
Mean change (95% CI)	–0.8 (–1.1 to –0.5)	–0.6 (–0.9 to –0.2)	–0.4 (–0.8 to –0.1)	.24	.50
Lobular inflammation score					
No. (%) improved [95% CI]	22 (44) [30 to 59]	23 (46) [32 to 61]	20 (43) [28 to 59]	.89	.73
Mean change (95% CI)	–0.4 (–0.6 to –0.2)	–0.3 (–0.5 to –0.0)	–0.3 (–0.6 to –0.1)	.14	.97
Ballooning degeneration score					
No. (%) improved [95% CI]	22 (44) [30 to 59]	22 (44) [30 to 59]	10 (21) [11 to 36]	.02	.02
Mean change (95% CI)	–0.5 (–0.8 to –0.3)	–0.3 (–0.6 to –0.0)	0.1 (–0.2 to 0.3)	.006	.04
Change in NAFLD activity score, mean (95% CI)	–1.8 (–2.4 to –1.2)	–1.1 (–1.7 to –0.5)	–0.7 (–1.3 to –0.2)	.02	.25
Resolution of NASH, No. (%) [95% CI][b]	25 (58) [42 to 73]	16 (41) [26 to 58]	11 (28) [15 to 45]	.006	.23

Abbreviations: CI, confidence interval; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis.
[a] *P* values derived from either χ^2 test for binary outcomes or analysis-of-covariance model regressing change from baseline to 96 weeks on treatment group and baseline value of the outcome for continuous outcomes.
[b] Defined as number of patients with no NASH at week 96 among patients with borderline or definite NASH at baseline. Excludes 7, 11, and 8 patients with no NASH at baseline in vitamin E, metformin, and placebo groups, respectively.

cholesterol, and high-density lipoprotein cholesterol decreased among all groups. These changes were not statistically significant between either the vitamin E or metformin groups vs placebo. Similarly, no significant differences between groups were evident in fasting serum glucose levels or HOMA-IR changes (Table 4 and Figure 2).

Anthropometric Variables. The overall mean increase in weight, BMI, and waist circumference per patient were 12.6 kg (95% CI, 11.0 to 14.3), 1.8 (95% CI, 1.3 to 2.2), and 5.1 cm (95% CI, 3.6 to 6.5) during treatment, respectively, despite recommended standard of care on diet and exercise. Although weight gain was slightly less in those patients receiving metformin, there were no significant differences between week 96 and baseline comparing vitamin E or metformin with placebo (Table 4 and Figure 2). BMI *z* score also decreased minimally in the patients treated with metformin, but remained essentially unchanged throughout the study across all treatment groups. Truncal fat values, measured by DXA, decreased slightly in all groups but showed no significant differences between groups. There were uniform increases over 96 weeks in physician-documented Tanner stage (Table 4).

Quality of Life and Safety. There were improvements in all groups (≥4.0 points) in self-reported physical and psychosocial parameters that may be of clinical consequence (Table 4), but there were no statistically significant differences in changes for physical or emotional functioning in quality of life comparing treatment groups for self-reported and parent/guardian-reported surveys.

Differences between treatment groups in terms of frequency or severity of adverse events were not significant (eTable 2). Particularly, no changes were evident comparing development of diabetes, change in BMI, elevations in ALT level 2-fold or greater from baseline, or need for hospitalization or surgery (eTable 2). Four children (1 receiving vitamin E, 2 receiving metformin, 1 receiving placebo) had life-threatening or disabling depression or mood alteration, and 1 in the vitamin E treatment group committed suicide. Five children receiving placebo, 1 receiving metformin, and none receiving vitamin E developed diabetes. No complications resulted from percutaneous liver biopsies.

COMMENT

To our knowledge, this is the first multicenter randomized controlled trial for evaluating treatment of pediatric NAFLD and the only trial evaluating treatment of any pediatric liver disease using a designated histologic outcome. Trials in adults with NASH have used changes in liver histology as primary outcomes primarily because alternative noninvasive biomarkers lack validation. For this trial, sample size determination would have required extrapolation from adult data if histology were chosen as the primary end point. However, children exhibit differences from adults in key histologic features such as frequent portal inflammation and fibrosis but less frequent hepatocellular ballooning injury, lobular inflammation, and perisinusoidal fibrosis.[8] There are also likely to be differences in etiopathogenesis and natural history based on early penetrance, pubertal changes, and relative or complete lack of ethanol intake. Thus, the primary end point was selected based on prior pediatric experience using reduction in ALT level.[14,15] ALT level is significantly associated with NAFLD activity score and fibrosis stage in children, albeit insufficiently predictive for individual purposes of diagnosis, staging, or grading.[11] Sustained reduction in ALT level was selected as the primary end point while recognizing that analyses of changes in histological features were essential.

A significant change in the predefined primary outcome in this trial was not achieved, although the reductions in ALT levels markedly differed between those receiving vitamin E and placebo through week 48. This difference diminished over subsequent visits, primarily due to continued improvement in ALT level in the placebo group. The percentage and extent of ALT reduction over the initial 24 weeks mimics that found in a previously reported randomized controlled trial (PIVENS) using the same vitamin E dose in adults with NASH.[18] The improvement in ALT level over a longer interval in the placebo group may be due to the frequency of reinforced adherence to diet and exercise recommendations, increased motivation associated with study participation, or advancing puberty. Sensitivity analyses showed results did not vary based on age, body weight, or BMI, indicating that results were unrelated to dose effects.

Children treated with vitamin E demonstrated significant improvements in terms of resolution of NASH in those with NASH or borderline NASH at baseline compared with placebo. There were also significant improvements in hepatocellular ballooning and NAFLD activity score. Improvements in the histologic features are interrelated to a certain extent, as the diagnosis of NASH typically requires the presence of ballooning, and the NAFLD activity score uses ballooning as 1 of the 3 scored components.[7] Ballooning is a major distinguishing feature of NASH, conferring a greater risk of disease progression in longitudinal studies, and therefore, a significant decrease in the severity of ballooning is a clinically important finding. This feature reflects injury to the cytoskeleton and associates with other signs of cellular injury, including increased Mallory bodies and increased fragments of cytokeratin 18.[19] Apparently, as reported pre-

Table 4. Change From Baseline to End of Treatment in Serum Biochemistry Test Results, Lipid Levels, Metabolic Characteristics, and Quality of Life by Treatment Group

	Mean (95% CI)			*P* Value[a]	
	Vitamin E (n = 50)	Metformin (n = 51)	Placebo (n = 49)	Vitamin E vs Placebo	Metformin vs Placebo
Change in self-reported QOL[b]					
Physical health	7.6 (2.7 to 12.5)	5.4 (0.8 to 10.0)	5.4 (–0.7 to 11.5)	.08	.63
Psychosocial health	6.0 (1.4 to 10.6)	4.0 (–0.4 to 8.4)	5.6 (–0.0 to 11.2)	.15	.96
Change in parent/guardian-reported QOL[b]					
Physical health	1.5 (–7.9 to 11.0)	4.1 (–3.8 to 12.0)	4.8 (–1.5 to 11.0)	.96	.87
Psychosocial health	4.3 (–1.6 to 10.1)	1.9 (–4.0 to 7.8)	6.1 (0.1 to 12.2)	.48	.39
Change in serum biochemistry tests					
ALT, U/L	–48.3 (–66.8 to –29.8)	–41.7 (–62.9 to –20.5)	–35.2 (–56.9 to –13.5)	.07	.40
AST, U/L	–22.8 (–33.3 to –12.3)	–21.5 (–34.6 to –8.4)	–20.4 (–32.7 to –8.0)	.32	.29
GGT, U/L	–7.4 (–33.3 to –12.3)	–14.3 (–24.9 to –3.7)	–4.4 (–11.6 to 2.8)	.37	.09
Alkaline phosphatase, U/L	–49.2 (–68.5 to –29.9)	–70.0 (–91.2 to –48.8)	–50.2 (–66.7 to –33.6)	.92	.20
Total bilirubin, mg/dL	0.05 (–0.05 to 0.15)	0.03 (–0.05 to 0.10)	0.12 (0.04 to 0.20)	.38	.11
α-Tocopherol, mg/L	9.4 (6.2 to 12.6)	–0.5 (–1.1 to 0.2)	–0.9 (–2.1 to 0.4)	<.001	.44
Changes in lipids					
Triglycerides, mg/dL	35.2 (9.8 to 60.6)	2.1 (–21.3 to 25.5)	18.9 (1.3 to 36.5)	.30	.24
Total cholesterol, mg/dL	–2.5 (–10.6 to 5.7)	–6.7 (–14.7 to 1.3)	–7.5 (–15.2 to 0.3)	.29	.96
HDL, mg/dL	–3.7 (–5.3 to –2.2)	–0.8 (–2.6 to 1.0)	–2.6 (–4.6 to –0.6)	.09	.25
LDL, mg/dL	–5.2 (–13.0 to 2.6)	–6.1 (–11.9 to –0.3)	–6.2 (–13.2 to 0.7)	.48	.93
Change in metabolic characteristics					
HOMA-IR, mg/dL × μU/mL/405	0.6 (–2.7 to 3.9)	–0.0 (–1.9 to 1.8)	–1.4 (–8.3 to 5.6)	.71	.42
Fasting serum glucose, mg/dL	1.1 (–2.6 to 4.7)	–1.0 (–4.3 to 2.2)	4.2 (–2.7 to 11.2)	.10	.14
Weight, kg	13.3 (10.2 to 16.4)	12.0 (9.3 to 14.6)	12.7 (9.7 to 15.6)	.65	.78
Waist circumference, cm	5.7 (3.5 to 7.9)	3.9 (1.7 to 6.2)	5.6 (2.5 to 8.6)	.81	.50
BMI[c]	2.1 (1.2 to 3.0)	1.3 (0.6 to 2.0)	1.9 (1.1 to 2.7)	.77	.25
BMI *z* score	–0.03 (–0.11 to 0.06)	–0.06 (–0.13 to 0.00)	–0.01 (–0.08 to 0.06)	.78	.25
Body composition, % total fat[d]	–1.1 (–2.6 to 0.4)	–2.4 (–3.6 to –1.2)	–1.9 (–3.5 to –0.3)	.43	.67
Tanner stage	1.3 (1.0 to 1.6)	1.3 (1.0 to 1.7)	1.2 (1.0 to 1.5)	.79	.33

Abbreviations: ALT, alanine aminotransferase; AST aspartate aminotransferase; BMI, body mass index; CI, confidence interval; GGT, γ-glutamyltransferase; HDL, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; LDL, low-density lipoprotein cholesterol; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; QOL, quality of life.

SI conversion factors: To convert AST, ALT, GGT, and alkaline phosphatase to μkat/L, multiply by 0.0167; to convert total bilirubin to μmol/L, multiply by 17.104; to convert α-tocopherol to μmol/L, multiply by 23.22; to convert triglycerides to mmol/L, multiply by 0.0113; to convert total, HDL, and LDL cholesterol to mmol/L, multiply by 0.0259; and to convert glucose to mmol/L, multiply by 0.0555.

[a] *P* values derived from analysis-of-covariance model regressing change from baseline to 96 weeks on treatment group and baseline value of the outcome.

[b] Pediatric Quality of Life Inventory (version 4.0) scores were recoded to range from 0 to 100 with increasing scores indicating better quality of life.

[c] Calculated as weight in kilograms divided by height in meters squared.

[d] Two patients assigned to vitamin E and 1 patient assigned to metformin were too obese to be assessed at week 96. Their values at 96 weeks were imputed with the 95th percentile value at baseline.

viously, serum ALT level is too variable a measure to reflect changes in histology of the magnitude achieved in this trial.[11] There were no significant changes in fibrosis, degree of inflammation, or steatosis independent of the clinical center. The improvements in resolution of NASH and hepatocellular ballooning mirror the results found in the PIVENS trial, which evaluated the same formulation, dose, and duration of treatment with vitamin E in adults with NASH.[18] Unlike the PIVENS trial, this trial did not demonstrate significant comparative improvement in lobular inflammation or steatosis found with vitamin E treatment. Study differences may have arisen because of differences between PIVENS and TONIC inclusion criteria and the lesser number of patients assigned to each treatment in TONIC. Another explanation for differences between PIVENS and TONIC histology outcomes could be attributable to differences between children and adults in terms of distribution and severity of fat and inflammation,[8] or potential differences in etiopathogenesis between the predominantly white adults in PIVENS[18] and Hispanic children in TONIC.

Other than hepatocellular ballooning, metformin did not result in any significant histological improvements compared with placebo over 96 weeks. Given that metformin is known to improve insulin sensitivity,[20] it is possible the metformin dose was inadequate, adherence was poor, or both. Choice of dose was based on a prior pilot trial showing improvement in insulin sensitivity, liver fat fraction, and ALT level in children with biopsy-proven NAFLD.[14] The need for overencapsulation required to make a matching placebo and concerns over children's capabilities to consume large pills precluded consideration of larger doses. For those taking metformin, adherence may have been diminished because of adverse effects, which can include dose-dependent nausea, vomiting, and diarrhea.

Some patients in the placebo group demonstrated improvements in particular histologic features at week 96 in comparison with baseline. Histologic features, including steatosis, lobular inflammation, and fibrosis, improved among placebo-group patients, but hepatocellular ballooning improved less often. Although resolution of NASH in the placebo group was significantly less frequent than in those treated with vitamin E, resolution in some suggests that changes associated with study participation, including adoption of lifestyle advice, could be beneficial. Intensive therapeutic lifestyle interventions involving diet, exercise, and behavioral modification have been shown to improve NASH histology in adult randomized controlled trials.[12]

No adverse events were attributable to treatment. Retention of research patients and early termination of study patients by study physicians were similar between all 3 groups. However, this study was not designed to evaluate potential infrequent adverse effects such as increased risk of bleeding with vitamin E. Although 5 children in the placebo group and none in the vitamin E group developed diabetes, this difference was not statistically significant. No evidence exists that vitamin E prevents development of insulin-resistant diabetes. There were no treatment-related changes in serum lipid levels, body size, or body composition.

Limitations of this study include the possibility of a false-negative primary outcome due to underenrollment from the prespecified target sample size. However, the enrollment was only 7 patients short of the target (173/180); thus, full enrollment would not have substantially increased power. Enrolling children with NAFLD but not requiring NASH may have limited the amount of improvement that could be achieved with treatment. Also, inclusion criteria required persistent elevation of serum ALT, so conclusions cannot be made about treatment efficacy in children who have NASH with lesser elevations. Third, children with cirrhosis or diabetes were excluded, so potential benefit or harm in treating these groups was not assessed. Fourth, no attempt was made to combine vitamin E and metformin to determine if there could be increased benefit from dual therapy. Last, the secondary outcome analyses were based on completers rather than intention to treat.

In summary, this double-blind, placebo-controlled, randomized trial of metformin or vitamin E for the treatment of NAFLD in children without diabetes or cirrhosis had a negative primary outcome. The data suggest that children treated with vitamin E who had biopsy-proven NASH or borderline NASH had significant improvement in secondary histologic outcomes with vitamin E. Those children who showed an improvement over placebo were those with initial hepatocellular ballooning degeneration. However, risk of biopsy might outweigh the benefits of therapy, so development of noninvasive markers for identification and monitoring of those who may benefit is desirable. Lifestyle modification is warranted for all children with NAFLD. The role of treatment with vitamin E in those who have a biopsy demonstrating borderline or definite NASH remains to be determined.

Author Affiliations: Department of Pediatrics, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Columbia University, New York, New York (Dr Lavine); Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, University of California, San Diego (Dr Schwimmer); Departments of Epidemiology (Mr Van Natta and Dr Ünalp) and Medicine (Dr Clark); Department of Pediatrics, Division of Gastroenterology and Nutrition (Dr Scheimann); Department of Biostatistics (Dr Tonascia); Johns Hopkins University, Baltimore, Maryland; Department of Pediatrics, Section of Pediatric Gastroenterology, Indiana University, James Whitcomb Riley Hospital for Children, Indianapolis (Dr Molleston); Department of Pediatrics, University of Washington School of Medicine, Seattle Children's Hospital, Seattle (Dr Murray); Department of Pediatrics, Division of Gastroenterology, Hepatology, and Nutrition, University of California, San Francisco (Dr Rosenthal); Department of Pediatrics, Section of Gastroenterology, Hepatology, and Nutrition, Texas Children's Hospital, Baylor College of Medicine, Houston (Dr Abrams); Department of Medicine, Division of Pediatric Gastroenterology, Hepatology, and Nutrition, Virginia Commonwealth Medical Center, Virginia Commonwealth University, Richmond (Dr Sanyal); Department of Medicine, Division of Gastroenterology/Hepatology, Indiana University, Indianapolis (Dr Chalasani); Department of Pathology and Immunology, Washington University, St Louis, Missouri (Dr Brunt); and Department of Laboratories, National Cancer Institute (Dr Kleiner), National Institute of Diabetes and Digestive and Kidney Diseases (Drs Hoofnagle and Robuck), National Institutes of Health, Bethesda, Maryland.

Author Contributions: Dr Lavine had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Lavine, Schwimmer, Van Natta, Molleston, Murray, Rosenthal, Sanyal, Chalasani, Tonascia, Ünalp, Clark, Kleiner, Hoofnagle, Robuck.
Acquisition of data: Lavine, Schwimmer, Murray, Rosenthal, Abrams, Scheimann, Sanyal, Kleiner.
Analysis and interpretation of data: Lavine, Schwimmer, Van Natta, Murray, Rosenthal, Scheimann, Sanyal, Chalasani, Tonascia, Ünalp, Clark, Brunt, Kleiner, Hoofnagle, Robuck.
Drafting of the manuscript: Lavine, Van Natta, Molleston, Murray, Rosenthal, Sanyal, Chalasani, Tonascia, Ünalp, Brunt, Hoofnagle.
Critical revision of the manuscript for important intellectual content: Lavine, Schwimmer, Van Natta, Murray, Rosenthal, Abrams, Scheimann, Sanyal, Chalasani, Tonascia, Ünalp, Clark, Kleiner, Hoofnagle, Robuck.
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Members of the Nonalcoholic Steatohepatitis Clinical Research Network: *Steering committee*: Joel E. Lavine, MD, PhD (Co-chair), Columbia University; Arun J. Sanyal, MD (Co-chair), Virginia Commonwealth University; Stephanie H. Abrams, MD, MS, Baylor College of Medicine; Nathan M. Bass, MD, PhD, University of California, San Francisco; Naga Chalasani, MBBS, Indiana University; Anna Mae Diehl, MD, Duke University; Kris V. Kowdley, MD, Virginia Mason Medical Center; Rohit Loomba, MD, MHSc, University of California, San Diego; Arthur J. McCullough, MD, Case Western Reserve University; Jean P. Molleston, MD, Indiana University; Karen F. Murray, MD, University of Washington; Patricia R. Robuck, PhD, MPH, National Institute of Diabetes and Digestive and Kidney Diseases; Philip Rosenthal, MD, University of California, San Francisco; Brent A. Neuschwander-Tetri, MD, St Louis University; James Tonascia, PhD, Johns Hopkins University, Data Coordinating Center. *Protocol subcommittee:* Joel E. Lavine, MD, PhD; Jeffrey B. Schwimmer, MD; Aynur Ünalp, MD, PhD. *Clinical centers of the NASH CRN*: Case Western Reserve University and Cleveland Clinic Foundation, Cleveland: Arthur J. McCullough, MD; Margaret Stager, MD; Srinivasan Dasarathy, MD; Carol Hawkins, RN; Yao-Chang Liu, MD; Ruth Sargent, LPN; Judy Whitwell; Duke University Medical Center, Durham: Anna Mae Diehl, MD; Manal F. Abdelmalek, MD; Cynthia Guy, MD; Dawn Piercy, FNP; Melissa Smith; Yi-Ping Pan; Johns Hopkins University School of Medicine, Baltimore: Ann O. Scheimann, MD, MBA; Michael Torbenson, MD; Kimberly Pfeifer, RN; Indiana University School of Medicine, Indianapolis: Naga Chalasani, MBBS; Jean P. Molleston, MD; Oscar W. Cummings, MD; Ann Klipsch, RN; Linda Ragozzino, RN; Girish Subbarao, MD; Raj Vuppalanchi, MD; St Louis University School of Medicine, St Louis: Brent A. Neuschwander-Tetri, MD; Jose Derdoy, MD; Elizabeth M. Brunt, MD; Sarah Barlow, MD; Susan Stewart, RN; Joan Siegner, RN; Joyce A. Hoffmann, CCRC; Judy Thompson, RN; Baylor College of Medicine, Houston: Stephanie H. Abrams, MD, MS; Leanel A. Fairly, RN; University of California, San Diego: Joel E. Lavine, MD, PhD (now at Columbia University); Jeffrey B. Schwimmer, MD; Cynthia Behling, MD, PhD; Janis Durelle; Steven Rose, MD; Tanya Stein, MD; University of California, San Francisco: Nathan M. Bass, MD, PhD; Philip Rosenthal, MD; Linda D. Ferrell, MD; Raphael Merriman, MD; Mark Pabst; Danuta Filipowski, MD; Virginia Commonwealth University, Richmond: Arun J. Sanyal, MD; Daphne Bryan, MD; Melissa J. Contos, MD; Velimir A. C. Luketic, MD; Michael Fuchs, MD; Bimalijit Sandhu, MD; Carol Sargeant, RN, BSN; Sherry Boyett, RN, BSN; Melanie White, RN, BSN; Amy Jones; Children's National Medical Center, Washington, DC: Parvathi Mohan, MD; Kavita Nair; Virginia Mason Medical Center, Seattle: Kris V. Kowdley, MD; James Nelson, PhD; Sarah Ackermann; Cheryl Shaw, MPH; University of Washington, Seattle: Karen F. Murray, MD; Matthew Yeh, MD, PhD; Melissa Young. *Resource centers*: National Cancer Institute, Bethesda: David E. Kleiner, MD, PhD; National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda: Edward C. Doo, MD; Jay H. Hoofnagle, MD; Patricia R. Robuck, PhD, MPH; Eunice Kennedy Shriver National Institute of Child Health and Human Development, Bethesda: Terry T.-K. Huang, PhD, MPH; Johns Hopkins University, Bloomberg School of Public Health (Data Coordinating Center), Baltimore: James Tonascia, PhD; Patricia Belt, BS; Jeanne M. Clark, MD, MPH; Ryan Colvin, MPH; Michele Donithan, MHS; Mika Green, MA; Milana Isaacson, BS; Wana K. Jin, BS; Laura A. Miriel, BS; Alice Sternberg, ScM; Aynur Ünalp, MD, PhD; Mark L. Van Natta, MHS; Ivana Vaughn, MPH; Laura A. Wilson, ScM; Katherine P. Yates, ScM. Data and safety monitoring board: Timothy Morgan, MD; Maureen Jonas, MD; Elizabeth Rand, MD; John Buse, MD; PhD; David M. Reboussin, PhD; William Lee, MD.

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Online-Only Material: eTables 1 and 2 are available at http://www.jama.com.

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