Role of Vitamin E for Nonalcoholic Steatohepatitis in Patients With Type 2 Diabetes: A Randomized Controlled Trial

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OBJECTIVE

While vitamin E has shown to improve nonalcoholic steatohepatitis (NASH) in patients without diabetes, information on patients with type 2 diabetes mellitus (T2DM) is lacking. The aim of this study was to determine whether vitamin E, alone or combined with pioglitazone, improves histology in patients with T2DM and NASH.

RESEARCH DESIGN AND METHODS

This was a proof-of-concept, randomized, double-blind, placebo-controlled trial conducted from 2010 to 2016. Patients with T2DM and biopsy-proven NASH (n = 105) were randomized to vitamin E 400 IU b.i.d., vitamin E 400 IU b.i.d. plus pioglitazone 45 mg/day, or placebo. Eighty-six patients completed the 18-month study. The primary end point was a two-point reduction in the nonalcoholic fatty liver disease activity score from two different parameters, without worsening of fibrosis. Secondary outcomes were resolution of NASH without worsening of fibrosis, individual histological scores, and metabolic parameters.

RESULTS

More patients on combination therapy achieved the primary outcome versus placebo (54% vs. 19%, P = 0.003) but not with vitamin E alone (31% vs. 19%, P = 0.26). Both groups showed improvements in resolution of NASH compared with placebo (combination group: 43% vs. 12%, P = 0.005; vitamin E alone: 33% vs. 12%, P = 0.04). While steatosis assessed by histology improved with combination therapy (P < 0.001) and vitamin E alone (P = 0.018), inflammation (P = 0.018) and ballooning (P = 0.022) only improved with combination therapy. No improvement in fibrosis was observed in any group.

CONCLUSIONS

In this proof-of-concept study, combination therapy was better than placebo in improving liver histology in patients with NASH and T2DM. Vitamin E alone did not significantly change the primary histological outcome.

Nonalcoholic fatty liver disease (NAFLD) has become a silent epidemic worldwide. It is estimated to affect 25–44% of the overall population, and it is significantly more common in patients with type 2 diabetes mellitus (T2DM) (1). Moreover, patients with T2DM have the highest risk of developing cirrhosis and hepatocellular carcinoma.

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The mechanisms involved remain incompletely understood but include insulin resistance, subclinical inflammation, abnormal bile acid metabolism, and a dysregulated microbiome, among others (2). A key factor in the development of NAFLD is an increase in free fatty acid (FFA) flux to the liver from dysfunctional, insulin-resistant adipose tissue as well as increased rates of de novo lipogenesis (3). Increased flux of FFA to the liver and hepatocyte lipotoxicity may result in mitochondrial dysfunction and incomplete FFA oxidation, with the consequent generation of reactive oxygen species and inflammatory lipid intermediates (3,4). In turn, these can trigger the more severe and progressive form of the disease known as nonalcoholic steatohepatitis (NASH), which is characterized by inflammation and hepatocyte ballooning that set off fibrosis and pose a risk of developing cirrhosis and hepatocellular carcinoma (5,6).

Unfortunately, treatment options remain limited. Currently, there are no U.S. Food and Drug Administration (FDA)-approved pharmacological agents available to treat NASH. Lifestyle interventions that achieve ~10% weight loss (7) and bariatric surgery (8) are effective but require a multidisciplinary approach not available to most patients and a difficult-to-sustain, long-term effort. Vitamin E (9,10), pentoxifylline (11), pioglitazone (9,12), and liraglutide (13) have proven to be safe and effective overall in randomized controlled trials (RCTs) of 6–24 months predominantly in patients without diabetes. Pioglitazone is the only clinically available agent shown to improve liver histology in patients with T2DM and NASH (14,15). As recently reported by our group (16), pioglitazone may offer greater benefit to patients with T2DM largely because of more rapid disease progression in these patients, but this has not been carefully explored with other existing agents, such as vitamin E.

We performed a proof-of-concept combination therapy in patients with T2DM and NASH for a number of reasons. First, patients with diabetes have the highest risk of disease progression and, as such, are the natural target population for a new and more aggressive approach. Second, vitamin E has been effective in patients without T2DM, but there is a lack of evidence from an RCT in T2DM that has precluded the use of this potentially valuable medication in this high-risk population (5,6). Third, we wanted to test for an additional or a synergistic benefit from two agents with acceptable safety and efficacy (although from relatively small cohorts of patients), but with seemingly different mechanisms of action, in a similar way ursodeoxycholic acid was assessed in combination with vitamin E in the past (17). This responds to a growing consensus within the field that successful treatment of NASH will require combination therapy, as is routinely done for diabetes, hypertension, and dyslipidemia (18). Finally, both agents are relatively inexpensive, and even as novel and more effective/safer agents will likely become available in the near future, this combination could become an approach to build upon or at least an alternative for patients who will not be able to afford the newer and likely more expensive treatments. In this context, the aim of the study was to assess the safety and efficacy of vitamin E, either alone or in combination with pioglitazone, in patients with NASH and T2DM.

RESEARCH DESIGN AND METHODS

Design Overview

This was an investigator-initiated, multicenter, parallel-group, double-blind, randomized (1:1:1 allocation), placebo-controlled trial conducted between June 2010 and September 2016 in a U.S. Department of Veterans Affairs (VA) population. The institutional review boards of the University of Florida and the University of Texas Health Science Center at San Antonio approved the study, and all subjects provided written informed consent before participation. The study always included three arms as described in the original protocol. This was supported by procurement of study medication and independent randomization schedule by the VA research pharmacy, as well as supervision by an external and independent committee (VA Clinical Science Research and Development Data Monitoring Committee), which convened quarterly to assess patient recruitment and adverse event reports and ensure the safety of study participants. However, this was not clear in the original ClinicalTrials.gov registration because of clerical errors, which led to inaccurate study registration in 2009–2010 regarding the three arms of the study.

Patients

Subjects were recruited from endocrinology and hepatology clinics at two VA medical centers (i.e., Audie L. Murphy in San Antonio and Malcom Randall in Gainesville). Patients were eligible for the trial if they had a diagnosis of T2DM and histologically confirmed NASH. Exclusion criteria were use of thiazolidinediones, glucagon-like peptide 1 agonists, sodium–glucose cotransporter 2 inhibitors, or vitamin E; other etiologies of liver disease (or abnormal laboratory findings, e.g., AST or ALT threefold or greater than the upper limit of normal); drugs that can produce hepatic steatosis (amiodarone, tamoxifen, methotrexate, etc.); type 1 diabetes mellitus; or severe heart, pulmonary, or renal disease. Detailed inclusion/exclusion criteria are included in the Supplementary Data.

Randomization, Masking, and Interventions

After initial screening (medical history, physical examination, laboratory studies, 75-g oral glucose tolerance test [OGTT]), subjects were instructed to keep physical activity and diet constant during the run-in phase (mean duration 1 month) and were educated on lifestyle modification as described in prior studies (14, 15). After completion of baseline metabolic measurements, subjects were individually randomized (1:1:1) to either *1*) vitamin E 400 IU b.i.d. alone plus placebo, *2*) vitamin E 400 IU b.i.d. plus pioglitazone (Actos; Takeda Pharmaceuticals, Tokyo, Japan) 30 mg/day titrated after 2 months to 45 mg/day, or *3*) placebo of both. The computer-generated randomization and patient allocation were performed by the research pharmacist without any stratification and using a block factor of 4, which was unknown to investigators. Vitamin E was provided by Nature Made (Pharmavite, Northridge, CA). Placebo tablets with identical physical characteristics were made at the Cooperative Studies Program Clinical Research Pharmacy Coordinating Center at Albuquerque VA Medical Center in New Mexico. All medications were stored at the research pharmacy and dispensed in identical bottles.

Outcomes and Follow-up

The primary outcome in this report was defined as a reduction in NAFLD activity score (NAS) of ≥2 (from two different histological categories) without any worsening of fibrosis after 18 months of therapy, as previously used (15). Secondary liver histological outcomes included *1*) resolution of NASH without worsening of fibrosis, *2*) improvement in individual histological scores, and *3*) steatosis, activity, and fibrosis (SAF) score (not prespecified in the protocol).

Baseline liver biopsies were read by a team of experienced clinical pathologists just to establish (or exclude) the presence of NASH, thus determining the inclusion/exclusion of patients in the trial. However, all analyses presented in the article were based on central biopsy readings. At the end of the study, all biopsies were reread by two experienced research pathologists who were blinded to patient identity, intervention assignment, or pre- or posttreatment sequence (0 or 18 months). For any discrepancies, an agreement between both reads was achieved by having the slides blindly reassessed. Mean biopsy length was 26.6 mm, with <6% of biopsies ≤10 mm. Diagnosis of definite NASH was defined as zone 3 accentuation of macrovesicular steatosis (any grade), hepatocellular ballooning (any degree), and lobular inflammatory infiltrates (any amount). The NAS was calculated as the sum of steatosis, inflammation, and ballooning grades in the liver biopsy, and histopathological changes were determined using standard criteria (19). Resolution of NASH was defined as absence of ballooning (0) and inflammation (0–1) after 18 months of therapy in patients with a diagnosis of definite NASH at baseline.

Additional secondary outcomes included *1*) fasting plasma glucose and insulin, hemoglobin A_{1c}, lipid profile, adiponectin, and ALT and AST concentrations; *2*) total body fat by DEXA; *3*) hepatic triglycerides by magnetic resonance proton spectroscopy (^{1}H-MRS), as previously described (20); and *4*) insulin resistance and insulin secretion during an OGTT, including indexes of insulin resistance, such as the Matsuda index, as previously validated (21).

Follow-ups were scheduled every month for the first 4 months and then every other month and included vital signs, review of self-monitoring of blood glucose results, and safety laboratory studies. At each visit, presence of adverse events and study drug compliance by pill counting (percent of pills taken in relation to the number of pills that should have been taken in this period of time) were assessed. Adverse events were classified by the principal investigator as mild (asymptomatic or mild symptoms, no intervention required), moderate (not fulfilling criteria for mild or severe), or severe (medically significant and requiring hospitalization or prolongation of hospitalization). After 18 months of treatment, the OGTT, DEXA, ^{1}H-MRS, and percutaneous liver biopsy were repeated. At this point, the medication code was disclosed to investigators and patients.

Statistical Analysis

Data were summarized as number (percent) for categorical variables and as mean ± SD for numeric variables. Categorical (dichotomous) variables were compared using χ^2 or Fisher exact test. Comparisons between groups were performed by Kruskal-Wallis test or ANOVA for numeric variables, depending on variable distribution.

The primary analysis was an intention-to-treat comparison between the proportion of patients with histological improvement in each active treatment group versus the proportion of patients with improvement in the placebo group. All randomized patients were included in the final analysis. On the basis of the prespecified primary analysis, subjects who did not complete 18 months were classified as not having improvement as previously done by other groups (9,22). Analyses were also done while restricting the sample to patients with definite NASH at baseline on the basis of final biopsy readings. In addition, multiple imputation was used to impute values of histological outcomes for patients not having a second liver biopsy. Treatment group, age, sex, and baseline histologic parameters were used to impute missing histologic parameters at month 18; missing values were considered to be missing at random. Forty data sets were created. Calculated proportions for the different histologic outcomes in each data set were combined according to Rubin's rules. Because there were two planned primary comparisons, Bonferroni-adjusted P values <0.025 were considered to indicate statistical significance. Considering an expected histological improvement of 17% and 60% for the placebo and each active treatment group, respectively (on the basis of results with pioglitazone alone as previously reported by our group [15]); an α-error of 0.025; a power of 0.80; and a dropout rate of 20%, we calculated that 101 patients were needed for this study. Analyses were performed using Stata 11.0 (StataCorp, College Station, TX) statistical software, and graphs were done with GraphPad Prism 6.0 (GraphPad Software, La Jolla, CA).

RESULTS

Baseline Clinical Characteristics, Compliance, and Adverse Events

A total of 105 patients with T2DM and NASH were randomized to one of three groups: placebo, vitamin E alone, or vitamin E with pioglitazone (Fig. 1). Baseline clinical characteristics, including diabetes, dyslipidemia, and hypertension medication use, were similar in all groups (Table 1). Twenty-six patients were recruited in San Antonio, Texas, and 79 in Gainesville, Florida. No differences were observed on the basis of place of recruitment. Nineteen patients did not complete the 18-month study (Fig. 1). In addition, two patients completing 18 months of therapy refused to have a second liver biopsy. We observed no clinical differences at baseline between patients prematurely discontinued from the study and those completing 18 months. Reasons for early discontinuation are reported in Fig. 1. Overall compliance with study medication during the 18 months was 92% (only five patients completing 18 months had a compliance <80%, with three in the placebo group, one in the vitamin E alone group, and one in the combination therapy group). Four patients died during the study for cardiovascular reasons (two in the vitamin E alone group [ischemic and hemorrhagic stroke] and two in the combination therapy group [acute coronary syndrome and sudden death]). In addition, one patient in the placebo group was discontinued as a result of ALT/AST elevation. Oral vitamin E was well-tolerated overall, without significant adverse events. Combination therapy was associated with more peripheral

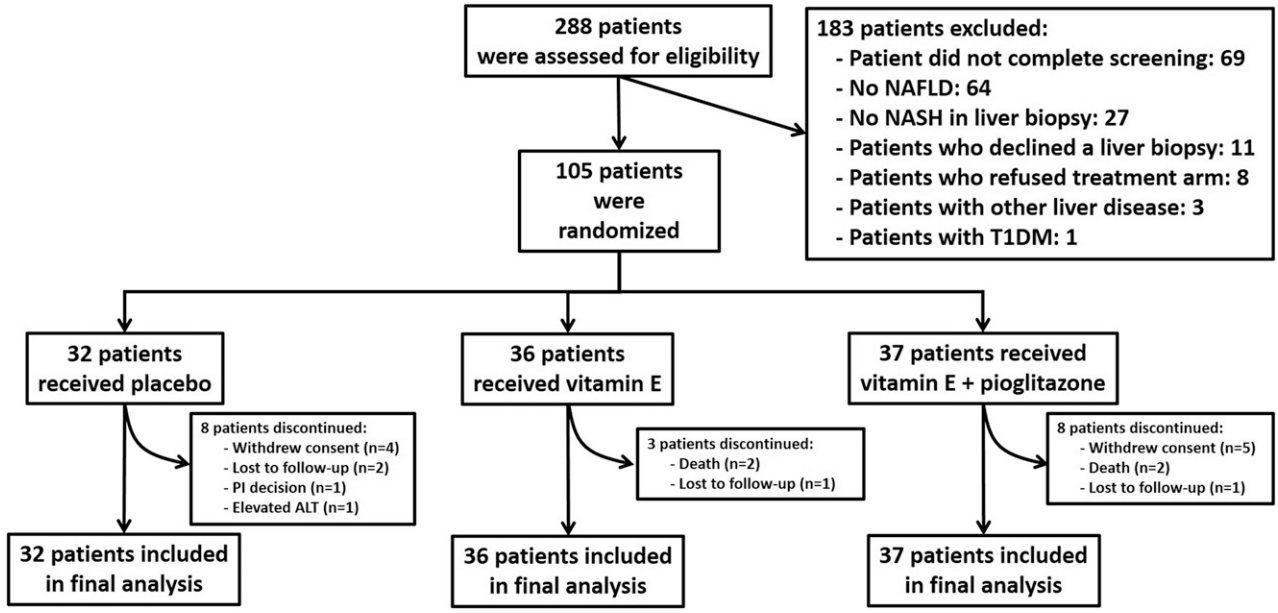

Figure 1—Study flowchart. PI, principal investigator; T1DM, type 1 diabetes mellitus.

edema and weight gain than placebo. Episodes of hypoglycemia were also more frequent in the combination therapy group but usually associated with the use of sulfonylureas, insulin, or both. No patient developed bladder cancer, osteoporosis, or osteoporotic bone fractures. See Supplementary Table 1 for a detailed description of adverse events.

Liver Histology

Primary Outcome

The severity of liver disease was similar among the three groups at baseline (Table 1). For the prespecified analysis of the primary outcome, patients who did not have a second liver biopsy were considered as treatment failures. Response to vitamin E alone was not significantly different from placebo (31% vs. 19%, treatment difference 12% [−1% to 32%], $P = 0.26$), whereas combination therapy with vitamin E and pioglitazone was associated with a higher response than placebo (54% vs. 19%, treatment difference 35% [14–56%], $P = 0.003$).

Primary and secondary histological outcomes on the basis of the multiple imputation analysis are available in Table 2. As can be observed, multiple imputation–based analysis of the primary outcome showed similar overall results compared with the prespecified analysis, only reaching significance compared with placebo in the combination therapy group. When a similar approach was used (i.e., multiple imputation) in only patients with definite NASH at baseline on the basis of the final central readings (21 in the placebo group, 23 in the vitamin E alone group, and 24 in the combination therapy group), 57% achieved the primary outcome with vitamin E alone vs. 27% with placebo (treatment difference 30% [0–58%], $P = 0.05$), and this only reached significance in the combination group, where 81% of patients improved on the basis of the primary outcome (treatment difference 54% [29–79%], $P < 0.001$).

Secondary Outcomes

On the basis of the prespecified analysis, resolution of NASH was higher in the vitamin E group than in the placebo group (33% vs. 12%, treatment difference 21% [2–40%], $P = 0.04$; not reaching the significance threshold of 0.025) and even higher with combination therapy (43% vs. 12%, treatment difference 31% [11–50%], $P = 0.005$). After multiple imputation of missing data, resolution of NASH occurred in 42% of patients receiving vitamin E alone vs. 18% receiving placebo (treatment difference 23% [2–44%], $P = 0.04$) (Table 2). Significantly better results were observed in the combination therapy group (57% vs. 18%, treatment difference 39% [18–60%], $P < 0.001$). Mean scores for steatosis, inflammation, and ballooning were reduced with vitamin E and pioglitazone ($P < 0.001$, $P = 0.018$, and $P = 0.022$, respectively, vs. placebo), but only steatosis showed a significant reduction with vitamin E ($P = 0.018$ vs. placebo). More patients on vitamin E and pioglitazone compared with placebo had improved steatosis ($P < 0.001$), inflammation ($P = 0.05$), and ballooning ($P = 0.03$), with negative results for the vitamin E alone group compared with placebo ($P = 0.07$, $P = 0.54$, and $P = 0.21$, respectively). The SAF score significantly improved with combination therapy ($P = 0.011$), but not with vitamin E alone ($P = 0.27$). No significant changes were observed in the mean score of fibrosis, but both active groups showed a trend toward a higher number of patients having improved fibrosis stage compared with placebo (50% with vitamin E vs. 30% in placebo, $P = 0.09$; 52% with vitamin E and pioglitazone, $P = 0.07$ vs. placebo).

Effect on Weight, Plasma Aminotransferases and Other Biomarkers

Metabolic changes after 18 months of therapy are described in Table 3. Of note, changes in diabetes, dyslipidemia, and hypertension medications were kept at a minimum during the 18 months of the trial and were not different among the groups. As can be observed, treatment with vitamin E alone was not associated with any significant weight change after 18 months of therapy (0.5 ± 5.6 vs. −0.8 ± 4.2 kg in the placebo group, $P = 0.32$) (Supplementary Fig. 1). On the contrary, vitamin E and pioglitazone

Table 1—Baseline clinical and demographic characteristics

	Placebo (n = 32)	Vitamin E (n = 36)	Vitamin E + pioglitazone (n = 37)
Age (years)	57 ± 11	60 ± 9	60 ± 6
Male/female sex, n	30/2	33/3	30/7
Race			
White	23 (72)	27 (75)	26 (70)
African American	2 (6)	4 (11)	3 (8)
Hispanic	7 (22)	5 (14)	8 (22)
BMI (kg/m^2)	33.6 ± 4.0	33.8 ± 4.6	35.2 ± 4.3
Total body fat (%)	36 ± 5	37 ± 6	38 ± 6
Fasting plasma glucose (mg/dL)	153 ± 37	158 ± 41	144 ± 43
Hemoglobin A_{1c} (%)	7.2 ± 1.2	7.5 ± 1.3	7.3 ± 1.1
Diabetes medications			
Metformin	27 (84)	29 (81)	29 (78)
Sulfonylureas	13 (41)	15 (42)	14 (38)
Insulin	8 (25)	10 (28)	10 (27)
Fasting plasma insulin (μU/mL)	18 ± 13	22 ± 14	16 ± 10
Fasting plasma FFA (mmol/L)	0.41 ± 0.15	0.39 ± 0.14	0.41 ± 0.15
Intrahepatic triglyceride content# (%)	10.5 ± 5.8	11.7 ± 5.7	13.8 ± 8.4
Plasma AST (units/L)	40 ± 23	41 ± 22	32 ± 18
Plasma ALT (units/L)	53 ± 33	53 ± 32	40 ± 25
Total cholesterol (mg/dL)	171 ± 40	174 ± 44	170 ± 53
LDL cholesterol (mg/dL)	94 ± 33	98 ± 39	91 ± 44
HDL cholesterol (mg/dL)	39 ± 10	39 ± 9	38 ± 10
Triglycerides (mg/dL)	154 (117–255)	156 (126–210)	163 (108–274)
Patients on statins	25 (78)	26 (72)	29 (78)
Patients on blood pressure medications	29 (91)	29 (81)	31 (84)
NAS	4.2 ± 1.6	3.9 ± 1.6	3.7 ± 1.3
Steatosis grade	1.8 ± 0.7	1.7 ± 0.8	1.6 ± 0.8
Inflammation grade	1.6 ± 0.6	1.3 ± 0.5	1.4 ± 0.5
Ballooning grade	0.9 ± 0.8	0.9 ± 0.8	0.7 ± 0.6
Fibrosis stage	1.5 ± 1.0	1.6 ± 1.2	1.4 ± 1.1

Data are n (%), mean ± SD, or median (range), unless otherwise indicated. #On the basis of data from 52 patients who underwent liver ^{1}H-MRS to quantify intrahepatic triglyceride content at baseline. All histological data correspond to the final central readings.

combination therapy was associated with significant weight gain (5.7 ± 5.4 kg after 18 months, $P < 0.001$), which became statistically significant after 4 months but reached a plateau at 14 months (Supplementary Fig. 1). Only the group receiving pioglitazone showed a significant change in glycemic control as assessed by hemoglobin A_{1c}, but improvement in fasting plasma glucose did not reach statistical significance (Table 3). Vitamin E alone was not associated with changes in glycemic control. However, both active arms were associated with a significant reduction in intrahepatic triglyceride content as assessed by ^{1}H-MRS compared with placebo (data based on only 33 subjects with complete ^{1}H-MRS data). Both treatment arms were associated with reductions of plasma ALT and AST concentrations (Supplementary Fig. 1). Reductions in plasma ALT levels became significant after only 2 months of therapy in both active groups compared with placebo, and these improvements persisted until the end of the study. Regarding plasma AST, changes were more remarkable with vitamin E alone than with combination therapy mainly because of higher baseline levels. No significant changes were observed in the lipoprotein profile after 18 months, except for a modest increase of plasma HDL cholesterol in patients receiving vitamin E with pioglitazone.

Metabolic Effects Assessed During the OGTTs

As can be observed in Supplementary Fig. 2, glucose excursion during the OGTT was significantly lower after 18 months of vitamin E and pioglitazone, with no changes in the other two groups. Of note, improvements in glucose levels in the combination group occurred together with a reduction in plasma insulin levels during the OGTT (patients on insulin were excluded from this analysis), which suggests improved insulin sensitivity as can be observed in Supplementary Fig. 3. Patients receiving vitamin E alone did not have a significant change in insulin sensitivity as estimated by the Matsuda index.

CONCLUSIONS

The key finding of our study is that vitamin E alone was not different from placebo in achieving improvement in the primary liver histological outcome, and that was less effective than the combination of vitamin E and pioglitazone. Treatment with vitamin E was not different from placebo regarding the primary outcome or the proportion of patients achieving improvement in steatosis, inflammation, ballooning, or fibrosis. However, while all the secondary end points were negative, the placebo-

Table 2—Primary and secondary histological outcomes

	Placebo (n = 32)	Vitamin E (n = 36)	P value vs. placebo	Vitamin E + pioglitazone (n = 37)	P value vs. placebo
Primary outcome: reduction of ≥2 points in NAS (from two different parameters), without worsening of fibrosis					
Prespecified analysis (noncompleters as failures)	6 (19)	11 (31)	0.26	20 (54)	0.003
Multiple imputation of missing data	7 (22)	13 (36)	0.18	24 (65)	<0.001
Resolution of NASH without worsening of fibrosis					
Prespecified analysis (noncompleters as failures)	4 (12)	12 (33)	0.04	16 (43)	0.005
Multiple imputation of missing data	5 (17)	14 (40)	0.04	20 (54)	0.002
Change in SAF score	−0.17 (0.75)	−0.36 (0.69)	0.27	−0.63 (0.72)	0.011
Steatosis					
≥1-point improvement	15 (46)	24 (68)	0.07	32 (87)	<0.001
Mean change in score	−0.4 (0.9)	−1.0 (1.0)	0.018	−1.3 (1.0)	<0.001
Inflammation					
≥1-point improvement	14 (43)	13 (36)	0.54	25 (66)	0.05
Mean change in score	−0.2 (0.8)	−0.4 (0.7)	0.29	−0.6 (0.7)	0.018
Ballooning					
≥1-point improvement	11 (35)	18 (50)	0.21	23 (61)	0.03
Mean change in score	−0.1 (0.9)	−0.5 (0.9)	0.10	−0.6 (0.9)	0.022
Fibrosis					
≥1-point improvement*	10 (30)	19 (50)	0.09	19 (52)	0.07
Mean change in score	−0.3 (1.1)	−0.6 (1.0)	0.39	−0.6 (0.9)	0.22

Data are n (%) unless otherwise indicated. Multiple imputation was used to impute missing histological data for patients who did not complete 18 months of therapy, unless otherwise specified. Numbers of patients may not always seem to match the proportion because they were estimated from the combination of 40 imputed data sets. *Defined as any improvement in fibrosis, without worsening of NASH. All histological data correspond to the final central readings.

subtracted effect of some histological features (e.g., ∼22% for steatosis, ∼15% for ballooning) was within the magnitude observed in patients without diabetes in the PIVENS (Pioglitazone vs. Vitamin E vs. Placebo for Treatment of Non-Diabetic Patients With Nonalcoholic Steatohepatitis) trial (∼23% and ∼21%, respectively) (9). Resolution of NASH occurred in 42% with vitamin E vs. 18% with placebo (P = 0.04) and was similar to the 36% vs. 21% (P = 0.05) reported in PIVENS (9). Therefore, vitamin E remained relevant to the management of, at least some, patients with diabetes and NASH in whom histology improved. The benefit from vitamin E appeared to be lower than that of combined therapy, which had a net (placebo-subtracted) benefit of 43% for the prespecified primary histological outcome (i.e., NAS ≥2 without worsening of fibrosis). Whether the combination of vitamin E and pioglitazone may offer more benefit than pioglitazone monotherapy was not directly assessed in the current study, but the histological results were almost identical to that reported with pioglitazone alone in two prior RCTs in patients with prediabetes or T2DM (∼40% placebo-subtracted improvement in NAS ≥2 without worsening of fibrosis and in resolution of NASH)

Table 3—Changes in metabolic and biochemical parameters in patients completing 18 months of follow-up

Change after 18 months	Placebo (n = 24)	Vitamin E (n = 33)	P value	Vitamin E + pioglitazone (n = 29)	P value
Weight (kg)	−0.8 ± 4.2	0.5 ± 5.6	0.29	5.7 ± 5.4	<0.001
BMI (kg/m^2)	−0.6 ± 1.6	0.1 ± 2.3	0.24	1.4 ± 1.6	<0.001
Total body fat (%)	0 ± 3	0 ± 3	0.66	2 ± 3	0.007
Fasting plasma glucose (mg/dL)	6 ± 53	−3 ± 39	0.43	−16 ± 36	0.08
Hemoglobin A_{1c} (%)	0.3 ± 1.6	−0.3 ± 1.2	0.10	−0.9 ± 1.0	0.002
Fasting plasma insulin (μU/mL)	3 ± 12	−3 ± 6	0.02	−3 ± 6	0.03
Intrahepatic triglyceride content# (%)	1 ± 7	−6 ± 6	0.03	−10 ± 6	<0.001
Total cholesterol (mg/dL)	−11 ± 31	5 ± 29	0.05	1 ± 43	0.23
LDL cholesterol (mg/dL)	−12 ± 31	0 ± 30	0.12	−4 ± 31	0.45
HDL cholesterol (mg/dL)	−1 ± 4	1 ± 4	0.05	3 ± 7	0.009
Triglycerides (mg/dL)	13 (−2 to 46)	14 (−28 to 74)	0.73	−2 (−48 to 31)	0.40

Data are mean ± SD or median (range). #On the basis of data from 33 patients who underwent liver ^{1}H-MRS to quantify intrahepatic triglyceride content at baseline and after 18 months.

(14,15) (Supplementary Figs. 4 and 5). The reasons for lack of an additive effect for two agents believed to be targeting different metabolic/molecular pathways are unclear. It may be that once pioglitazone mitigates hepatocyte mitochondrial dysfunction and inflammation by reversing adipose tissue insulin resistance, reducing fatty acid flux to the liver and tricarboxylic acid cycle activity (23), inflammation/oxidative stress targeted by vitamin E can improve no further. Another possible explanation is that in T2DM, there are unique mechanisms for the development of NASH that are unaffected by vitamin E, such as worse insulin resistance or others inherent to hyperglycemia.

The current study confirms and expands prior reports on the role of pioglitazone in NASH (9,12,14,15), but unlike those prior works, this study was dedicated exclusively to patients with T2DM. The magnitude of the histological benefit was comparable to our prior studies where ~50% of patients had T2DM: About two-thirds of patients had resolution of NASH, and a similar number had an improvement in NAS ≥2 without worsening of fibrosis (~40% placebo-subtracted net effect) (14,15). These findings, combined with well-established cardiovascular benefits (24), make thiazolidinediones the first-line therapy for NASH in patients with T2DM. On the other hand, while treatment response was encouraging, it also fell short of expectations in a number of patients, calling for the urgent discovery of novel, more potent and specific therapeutic agents. About 40% of patients were nonresponders to combination therapy (i.e., resolution of NASH or fibrosis). Moreover, neither arm had a significant effect on hepatic fibrosis, although both active arms showed a trend toward a higher rate of patients achieving improvement in fibrosis without worsening of NASH. Results from studies assessing the effect of pioglitazone on fibrosis have been inconsistent, with some, but not all, reporting promising results (9,12,14,15). Several meta-analyses have also addressed this issue, but their results have also been conflicting (25,26). Of note, while pioglitazone alone did not substantially improve fibrosis, one may envision that affecting resolution of NASH per se may contribute to delayed fibrosis progression. Indeed, prior reports have observed that pioglitazone was associated with a reduction in fibrosis progression compared with placebo (15).

Use of pioglitazone was associated with significant weight gain and lower-limb edema, which were more pronounced in patients taking insulin and/or sulfonylureas. No cases of bladder cancer or osteoporotic fractures were observed in patients taking pioglitazone. Vitamin E has not been widely adopted by health care providers (27). This appears to be, at least in part, due to concerns related to prostate cancer and cardiovascular disease with long-term use (28,29). During the trial, no patient on vitamin E developed prostate cancer during the 18-month study period. However, four patients died of cardiovascular disease: two in the vitamin E arm and two in the combination arm. All had preexisting cardiovascular disease but were stable for at least 6 months before enrollment. No patients died of cardiovascular disease while on pioglitazone during previous NASH RCTs or in the vitamin E arm of PIVENS. Of note, this study used the same vitamin E manufacturer and dose as PIVENS (9). The relatively small number of patients included in this and prior studies with vitamin E does not allow us to establish a solid conclusion on the role vitamin E plays in cardiovascular disease in patients with NASH. In addition to sample size, a limitation of the study is the absence of a group receiving pioglitazone monotherapy. However, a four-arm study was not feasible for an investigator-initiated study, and prior information on pioglitazone monotherapy was already available from our prior study (15), which was carried out using the exact same design as the current study and, therefore, allowed for an informal head-to-head comparison (Supplementary Figs. 4 and 5).

Because the study was carried out in a VA hospital, it included predominantly male subjects (only 12 women out of 105 patients, well distributed in the three groups). Therefore, specific analyses to assess response by sex could not be done. This is important because the role of sex and reproductive status on treatment response in patients with NASH has not been carefully considered in the past (30). Nineteen patients did not complete 18 months of follow-up. This dropout rate is within the range of prior RCTs in patients with biopsy-proven NASH: 13% in PIVENS (9) and the GOLDEN-505 trials (31). To minimize any potential selection bias as a result of dropouts, different approaches were used to handle missing data (i.e., single and multiple imputation) with similar results.

There is extensive debate in the field regarding the most appropriate histological outcome to use in NASH clinical trials (32). Recently, the FDA has endorsed two major outcomes to be used in clinical trials: *1*) resolution of NASH without worsening of fibrosis and *2*) improvement in liver fibrosis of one or more stages with no worsening of steatohepatitis. However, we decided to maintain the original primary outcome set in the initial conception of the study. Of note, a similar primary outcome has also been reported in other recent large RCTs (22,33). While fibrosis has emerged as the most important predictor of liver-related complications, there are several clinically important aspects that make disease activity/steatohepatitis still very relevant. These include: *1*) fibrosis progression is accelerated in the setting of worse steatohepatitis; *2*) early diagnosis and treatment provide the best opportunity to prevent fibrosis development and liver-related complications; and *3*) cardiovascular disease remains the major cause of death in patients with NAFLD, and early intervention may reduce this risk independently of changes in liver fibrosis.

In conclusion, 18 months of vitamin E therapy was not associated with a significant response in patients with T2DM and NASH. Together with some uncertainties regarding the safety of vitamin E, the overall results suggest that routine use of vitamin E in patients with T2DM and NASH should not be recommended. Pioglitazone in combination with vitamin E was effective in this population but to a similar degree as previously reported by our group (15) for pioglitazone alone, suggesting only a minor benefit on resolution of NASH, if any, from adding vitamin E to pioglitazone treatment. In this context, pioglitazone remains the pharmacological treatment of choice for patients with NASH and T2DM, which agrees with current guidelines (5,6), at least until a safer or more effective insulin sensitizer becomes available. There is

also considerable opportunity for improvement by adding a second agent that could lead more patients to resolution of NASH and, particularly, affect fibrosis. However, we are just at the dawn of combination therapy for the management of patients with NASH, and further evidence is needed until a formal recommendation can be made in this regard.

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