Annals of Internal Medicine ORIGINAL RESEARCH

Long-Term Pioglitazone Treatment for Patients With Nonalcoholic Steatohepatitis and Prediabetes or Type 2 Diabetes Mellitus

A Randomized, Controlled Trial

Kenneth Cusi, MD; Beverly Orsak, RN; Fernando Bril, MD; Romina Lomonaco, MD; Joan Hecht, RN; Carolina Ortiz-Lopez, MD; Fermin Tio, MD; Jean Hardies, PhD; Celia Darland, RD; Nicolas Musi, MD; Amy Webb, MD; and Paola Portillo-Sanchez, MD

Background: The metabolic defects of nonalcoholic steatohepatitis (NASH) and prediabetes or type 2 diabetes mellitus (T2DM) seem to be specifically targeted by pioglitazone. However, information about its long-term use in this population is limited.

Objective: To determine the efficacy and safety of long-term pioglitazone treatment in patients with NASH and prediabetes or T2DM.

Design: Randomized, double-blind, placebo-controlled trial. (ClinicalTrials.gov: NCT00994682)

Setting: University hospital.

Participants: Patients (n = 101) with prediabetes or T2DM and biopsy-proven NASH were recruited from the general population and outpatient clinics.

Intervention: All patients were prescribed a hypocaloric diet (500–kcal/d deficit from weight-maintaining caloric intake) and then randomly assigned to pioglitazone, 45 mg/d, or placebo for 18 months, followed by an 18-month open-label phase with pioglitazone treatment.

Measurements: The primary outcome was a reduction of at least 2 points in the nonalcoholic fatty liver disease activity score (NAS) (in 2 histologic categories) without worsening of fibrosis. Secondary outcomes included other histologic outcomes, hepatic triglyceride content measured by magnetic resonance and proton spectroscopy, and metabolic parameters.

Results: Among patients randomly assigned to pioglitazone, 58% achieved the primary outcome (treatment difference, 41 percentage points [95% CI, 23 to 59 percentage points]) and 51% had resolution of NASH (treatment difference, 32 percentage points [CI, 13 to 51 percentage points]) ($P < 0.001$ for each). Pioglitazone treatment also was associated with improvement in individual histologic scores, including the fibrosis score (treatment difference, −0.5 [CI, −0.9 to 0.0]; $P = 0.039$); reduced hepatic triglyceride content from 19% to 7% (treatment difference, −7 percentage points [CI, −10 to −4 percentage points]; $P < 0.001$); and improved adipose tissue, hepatic, and muscle insulin sensitivity ($P < 0.001$ vs. placebo for all). All 18-month metabolic and histologic improvements persisted over 36 months of therapy. The overall rate of adverse events did not differ between groups, although weight gain was greater with pioglitazone (2.5 kg vs. placebo).

Limitation: Single-center study.

Conclusion: Long-term pioglitazone treatment is safe and effective in patients with prediabetes or T2DM and NASH.

Primary Funding Source: Burroughs Wellcome Fund and American Diabetes Association.

Ann Intern Med. doi:10.7326/M15-1774 **www.annals.org**

For author affiliations, see end of text.

This article was published at www.annals.org on 21 June 2016.

Nonalcoholic fatty liver disease (NAFLD) is reaching epidemic proportions worldwide (1) and is the most common chronic liver condition in obese patients with prediabetes or type 2 diabetes mellitus (T2DM). Histologic findings range from isolated steatosis (with no or minimal inflammation) to severe nonalcoholic steatohepatitis (NASH) and variable perisinusoidal or perivenular fibrosis (2). Patients with T2DM and NASH have the highest risk for cirrhosis and hepatocellular carcinoma (3, 4), and the presence of NAFLD seems to worsen microvascular and macrovascular complications of diabetes (5–7).

Given that most patients with T2DM have NAFLD (8–12) and many are at risk for NASH even if they have normal liver aminotransferase levels (6, 9, 13, 14), it is surprising that few trials have focused on this population. This distinction (patients with NASH with vs. without T2DM) is relevant because additional metabolic factors, such as hyperglycemia (15, 16), lower adiponectin levels (17, 18), worse dyslipidemia (19, 20), and more severe insulin resistance and hepatic steatosis (10, 16, 18–21), may account for the higher rates of severe liver disease observed in patients with T2DM (22).

Although the cause of NASH is multifactorial and treatment remains challenging (23), a major factor is the increase in liver triglyceride content caused by chronic release of free fatty acids (FFAs) from insulin-resistant dysfunctional adipose tissue (7, 24–27). Because thiazolidinediones target insulin resistance and adipose tissue dysfunction or inflammation that promotes hepatic "lipotoxicity" in NASH (7, 22, 28) (which is also a prominent feature of T2DM [15]), they may be more helpful for treating steatohepatitis in this population. In predominantly nondiabetic patients with NASH, several studies have reported variable degrees of his-

See also:

Editorial comment 1
Summary for Patients 2

tologic benefit with thiazolidinediones (29–33). In the largest study to date in patients without T2DM (34), pioglitazone was no better than placebo for the primary outcome but was beneficial for secondary outcomes, such as resolution of NASH. However, in patients with prediabetes or T2DM, the only available randomized, controlled trial is a relatively small proof-of-concept study (35). This is disappointing given that there are 29.1 million adults with diabetes (>90% with T2DM) and 86 million with prediabetes (36) in the United States, many of whom are at risk for cirrhosis from NASH. Moreover, because pioglitazone may also halt the progression of prediabetes to T2DM (37), defining its role in patients with prediabetes and NASH is critical. Finally, safety concerns about the long-term use of thiazolidinediones remain (38, 39); therefore, studies with extended thiazolidinedione exposure are needed before a pioglitazone-based approach can be embraced in this population.

The aim of our study was to assess the efficacy and safety of long-term pioglitazone treatment in improving liver histologic outcomes in patients with NASH and prediabetes or T2DM.

METHODS

Design Overview

This was a single-center, parallel-group, randomized (1:1 allocation), placebo-controlled study, conducted between December 2008 (first patient enrolled) and December 2014 (final data collection). Participants, investigators, and health care providers were blinded to treatment assignment throughout the study. The Institutional Review Board at the University of Texas Health Science Center at San Antonio (UTHSCSA) approved the study, and all participants provided written informed consent before enrollment.

In October 2009, while updating registry data for another study, investigators discovered that this trial, which they thought had been registered by other study personnel, was not registered. At the time of registration (ClinicalTrials.gov: NCT00994682), 29 patients (of 97 anticipated) were enrolled in the study. None of these patients had had the follow-up metabolic measurements or liver biopsies (primary outcome) that were to be performed at 18 months, and no interim analyses were done before the trial was registered. A recent review of ClinicalTrials.gov (November 2015) revealed that the initial trial registration data erroneously stated that patients with normal glucose tolerance would be randomly assigned to treatment or placebo. Given that the trial's eligibility criteria required patients to have an abnormal oral glucose tolerance test (OGTT) result (that is, prediabetes or T2DM), the investigators never planned to enroll patients with normal glucose tolerance. This error in trial registration was corrected by the principal investigator. The trial registry states that the primary end point is liver histologic outcomes (Kleiner criteria [40]) at 18 months, and these data are presented in **Appendix Table 1** (available at www.annals.org). In this article, the primary end point is defined as a reduction of at least 2 points in 2 categories of the NAFLD activity score (NAS) without worsening of fibrosis, an outcome that was not specified in the original registration. This end point has been accepted by investigators in this field as representing significant change in liver histologic outcomes in clinical trials involving patients with NASH (34, 41–43). Some secondary outcomes that were assessed, such as insulin secretion, prevention of the onset of T2DM or reversal of glucose intolerance, measurement of visceral fat by magnetic resonance imaging, bone density measurement via dual-energy x-ray absorptiometry (DXA), plasma measurements of bone metabolism, and molecular metabolic pathways, are not reported in this article.

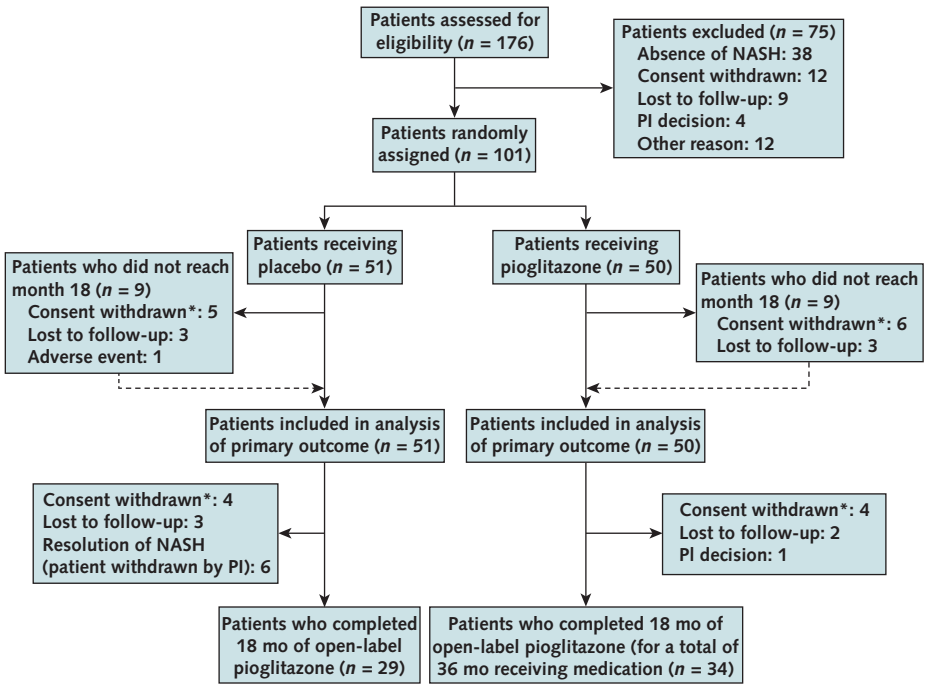
Setting and Participants

Participants were recruited from the general population of San Antonio, Texas, via newspaper advertisements and from the endocrinology and hepatology clinics at UTHSCSA and the Veterans Affairs Medical Center. Persons were eligible for the trial if they had histologically confirmed NASH and either prediabetes or T2DM. All patients had a screening 2-hour OGTT to diagnose or confirm a diagnosis of prediabetes or T2DM. Prediabetes was defined as impaired fasting glucose (5.6 to 6.9 mmol/L [100 to 125 mg/dL]), impaired glucose tolerance (7.8 to 11.1 mmol/L [140 to 199 mg/dL] on an OGTT), or a hemoglobin A_{1c} level of 5.7% to 6.4%. Exclusion criteria included use of thiazolidinediones or vitamin E; other causes of liver disease (22) or abnormal laboratory results (such as an aspartate aminotransferase [AST] or alanine aminotransferase [ALT] level ≥3 times the upper limit of normal [ULN]); type 1 diabetes mellitus; or severe heart, hepatic, or renal disease. Detailed inclusion and exclusion criteria are provided in the **Appendix** (available at www.annals.org).

Randomization and Interventions

After initial screening (medical history, physical examination, laboratory tests, and 75-g OGTT), patients began receiving placebo and were instructed by the research dietician (C.D.) to keep physical activity and diet constant during the run-in phase (mean duration, 1 month). After completion of baseline metabolic measurements, participants were prescribed a hypocaloric diet (500–kcal/d deficit from the calculated weight-maintaining diet) and were randomly assigned in a 1:1 ratio to either pioglitazone (Actos [Takeda Pharmaceuticals]), 30 mg/d (titrated after 2 months to 45 mg/d), or placebo. Randomization (computer-generated) and patient allocation were performed by the research pharmacist without stratification and using a block factor of 4, which was unknown to investigators. Takeda Pharmaceuticals provided pioglitazone and placebo pills with identical physical characteristics, which were stored at the research pharmacy and dispensed in identical bottles.

Figure 1. Study flow diagram.



NASH = nonalcoholic steatohepatitis; PI = principal investigator.
* Withdrew after being informed of the U.S. Food and Drug Administration's Drug Safety Communication in 2011 about the potential association between pioglitazone and bladder cancer.

Outcomes and Follow-up

The primary outcome was a reduction of at least 2 points in 2 histologic categories of the NAS without worsening of fibrosis after 18 months of therapy. Secondary liver histologic outcomes included resolution of NASH; improvement in individual histologic scores; or improvement in a combined histologic outcome, defined as a reduction in ballooning with at least a 2-point improvement in the NAS or an absolute NAS of 3 or lower (with improvement in steatosis or inflammation) without worsening of fibrosis.

Baseline liver biopsy specimens were read by a team of experienced clinical pathologists to establish or rule out the presence of NASH and thus determine whether patients were included or excluded. At the end of the study, all biopsy specimens were reread by an experienced research pathologist (F.T.), who was blinded to patient identity, intervention assignment, and pretreatment or posttreatment sequence (0, 18, or 36 months). Biopsy specimens were read by the research pathologist 2 times, with good to excellent intraobserver variability (agreement >75% for all histologic parameters). 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 the steatosis, inflammation, and ballooning grades from the liver biopsy, and histopathologic changes were determined by using standard criteria (44).

Additional secondary outcomes included the following: 1) fasting plasma glucose, fasting plasma insulin, FFA, hemoglobin A_{1c}, fasting plasma lipid profile, adiponectin, and cytokeratin-18 concentrations; 2) total body fat percentage, measured by DXA; 3) hepatic triglyceride content, measured by magnetic resonance and proton spectroscopy (^{1}H-MRS) as previously described (14, 16, 35, 45) (baseline and 18 months only); 4) glucose tolerance and insulin secretion on an OGTT; 5) endogenous glucose production (EGP), rate of glucose disappearance (R_d), and insulin-induced suppression of EGP and plasma FFA concentration, all measured during a euglycemic insulin clamp with tritiated glucose and indirect calorimetry (baseline and 18 months only) as previously reported (16) (**Appendix**); and 6) several indexes of fasting insulin resistance, such as the homeostatic model assessment of insulin resistance (HOMA-IR) score, hepatic insulin resistance index (calculated as fasting plasma insulin level × EGP), and adipose tissue insulin resistance index (calculated as fasting plasma insulin level × FFA), as previously validated (14, 16–19, 35) (**Appendix**).

Follow-up visits were scheduled every month for the first 4 months and then every other month and included measurement of vital signs, physical examination, review of self-monitoring of blood glucose results, and laboratory tests to assess safety. At each visit, presence of adverse events and study drug adherence were assessed, the latter by pill counting (percentage of pills taken in relation to the number that should have been

taken). Adverse events were classified by the principal investigator as mild (asymptomatic or mild symptoms, with 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, metabolic measurements (OGTT and euglycemic insulin clamp), DXA, ^{1}H-MRS, and liver biopsy were repeated, at which point the medication code was disclosed to investigators and patients. Patients initially assigned to pioglitazone were asked to continue at the same dose. In the placebo group, patients whose NASH resolved after 18 months were instructed to discontinue the study because pioglitazone treatment or a repeated liver biopsy were considered unethical and were not indicated, whereas those with persistent disease were invited to start pioglitazone therapy, titrated as described earlier. Patients had follow-up visits every 2 months, and the aforementioned metabolic measurements, DXA, ^{1}H-MRS, and liver biopsy were repeated at 36 months.

Statistical Analysis

Given expected histologic improvements of 15% and 50% in the placebo and pioglitazone groups, respectively; an α error of 0.05; a power of 0.90; and a dropout rate of 15%, we calculated that 97 patients were needed for this study. All randomly assigned patients were included in the final analysis. For histologic outcomes, multiple imputation was used to impute values for missing data (**Appendix**). Analyses were also done restricting the sample to patients with definite NASH at baseline (based on final biopsy readings) and counting patients who did not reach month 18 as not having histologic improvement (prespecified data analysis). Histologic outcomes and other categorical and dichotomous data were analyzed using the chi-square test or the Fisher exact test. Continuous variables were analyzed using mixed-effects linear regression under the assumption that data were missing at random. For the randomized phase, both 0- and 18-month data were considered for outcomes. The interaction term for time and treatment group was used as an independent variable to determine whether the change from 0 to 18 months differed between groups (fixed effect), and we included intercepts for participants as random effects. A similar approach (mixed-effects linear regression) was performed for within-group comparisons for the 18- to 36–month data, with assessment of only the effect of time on secondary outcomes (all patients received pioglitazone during this phase). Analyses were performed using Stata 11.0 (StataCorp).

Role of the Funding Source

This work was an investigator-initiated study that was financially supported by the Burroughs Wellcome Fund and the American Diabetes Association. Takeda Pharmaceuticals provided pioglitazone and placebo tablets. The funding sources had no role in the study design; the collection, analysis, or interpretation of data; or the writing of the manuscript.

Table 1. Baseline Patient Characteristics*

Characteristic	Placebo (n = 51)	Pioglitazone (n = 50)
Mean age (SD), *y*	49 (11)	52 (10)
Male, *n (%)*	35 (69)	36 (72)
T2DM, *n (%)*	28 (55)	24 (48)
Ethnicity, *n (%)*		
White	11 (22)	14 (28)
Hispanic	37 (73)	31 (62)
Other	3 (6)	5 (10)
Mean weight (SD), *kg*	99.2 (17.0)	98.2 (16.5)
Mean body mass index (SD), kg/m^2	34.5 (4.8)	34.3 (4.8)
Mean total body fat by DXA (SD), %	34 (8)	33 (7)
Mean fasting plasma glucose level (SD)		
mmol/L	6.7 (1.5)	6.9 (1.6)
mg/dL	121 (27)	124 (29)
Mean 2-h plasma glucose level (SD)		
mmol/L	11.3 (3.6)	11.7 (4.3)
mg/dL	203 (64)	211 (78)
Mean hemoglobin A_{1c} level (SD), %		
Patients without T2DM	5.7 (0.5)	5.7 (0.5)
Patients with T2DM	6.8 (1.0)	7.1 (0.9)
Mean fasting plasma insulin level (SD)		
pmol/L	96 (72)	90 (66)
μU/mL	16 (12)	15 (11)
Mean free fatty acid level (SD), *mmol/L*	0.54 (0.19)	0.49 (0.18)
Use of T2DM medications, *n (%)*		
Metformin	17 (33)	19 (38)
Sulfonylureas	16 (31)	12 (24)
Insulin	6 (12)	5 (10)
Use of statins, *n (%)*	19 (37)	19 (38)
Mean triglyceride level (SD)		
mmol/L	2.0 (1.2)	2.5 (1.9)
mg/dL	179 (109)	224 (171)
Mean total cholesterol level (SD)		
mmol/L	4.7 (1.1)	4.8 (1.2)
mg/dL	182 (42)	187 (46)
Mean LDL cholesterol level (SD)		
mmol/L	2.8 (0.9)	2.8 (1.1)
mg/dL	109 (33)	109 (44)
Mean HDL cholesterol level (SD)		
mmol/L	1.0 (0.2)	0.9 (0.2)
mg/dL	37 (9)	36 (9)
Mean aspartate aminotransferase level (SD), *U/L*	43 (22)	47 (21)
Mean alanine aminotransferase level (SD), *U/L*	57 (33)	62 (33)
Mean NAS (SD)	4.5 (1.2)	4.5 (1.5)
Steatosis grade	1.9 (0.8)	2.0 (0.8)
Inflammation grade	1.7 (0.5)	1.7 (0.6)
Ballooning grade	0.9 (0.4)	0.8 (0.4)
Mean fibrosis stage (SD)	0.9 (0.9)	1.1 (1.1)
Diagnosis of definite NASH based on final biopsy reading, *n (%)*	45 (88)	42 (84)

DXA = dual-energy x-ray absorptiometry; HDL = high-density lipoprotein; LDL = low-density lipoprotein; NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis; T2DM = type 2 diabetes mellitus.
* Percentages may not sum to 100 due to rounding.

RESULTS

Baseline Clinical Characteristics, Adherence to Treatment, and Adverse Events

A total of 101 patients with prediabetes or T2DM and NASH were randomly assigned to pioglitazone or placebo (**Figure 1**). Baseline clinical characteristics were similar between groups, as shown in **Table 1**.

Eighteen patients (9 in each group) did not complete the first 18 months of the study (**Figure 1**), mainly

Table 2. Effect of 18 mo of Pioglitazone Treatment on Primary and Secondary Liver Histologic Outcomes*

Outcome	Placebo (n = 51)	Pioglitazone (n = 50)	Treatment Difference (95% CI)	P Value
Primary outcome				
≥2-point reduction in NAS (in 2 categories) without worsening of fibrosis, *n (%)*	9 (17)	29 (58)	41 (23 to 59)	<0.001
Secondary outcomes				
Resolution of NASH, *n (%)*†	10 (19)	26 (51)	32 (13 to 51)	<0.001
Steatosis				
≥1-point improvement, *n (%)*	13 (26)	35 (71)	44 (25 to 63)	<0.001
Mean change in score (SD)	−0.2 (0.8)	−1.1 (1.0)	−0.9 (−1.3 to −0.5)	<0.001
Inflammation				
≥1-point improvement, *n (%)*	11 (22)	25 (49)	27 (8 to 46)	0.004
Mean change in score (SD)	−0.1 (0.8)	−0.6 (0.9)	−0.6 (−0.9 to −0.2)	<0.001
Ballooning				
≥1-point improvement, *n (%)*	12 (24)	25 (51)	27 (7 to 47)	0.004
Mean change in score (SD)	−0.2 (0.7)	−0.6 (0.6)	−0.4 (−0.7 to −0.2)	0.001
Fibrosis				
≥1-point improvement, *n (%)*	13 (25)	20 (39)	14 (−6 to 34)	0.130
Mean change in score (SD)	0 (1.2)	−0.5 (1.0)	−0.5 (−0.9 to 0)	0.039

NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis.
* Multiple imputation was used to impute missing histologic data for patients who did not complete 18 mo of therapy (**Appendix**). 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 absence of NASH after 18 mo of therapy in patients with definite NASH at baseline.

due to withdrawal of their consent (6 in the pioglitazone group and 5 in the placebo group) after being informed in 2011 about a potential risk for bladder cancer with pioglitazone (46). Of the 77 eligible patients for the 18- to 36–month open-label phase, 4 in each group withdrew their consent for similar reasons (46).

Overall adherence to study medication during the first 18 months was 95.3%. There were no severe adverse events associated with pioglitazone requiring study discontinuation. One patient discontinued placebo use because of an increase in liver enzyme levels to more than 2.5 times the ULN. Hypoglycemia in both groups was usually associated with the use of sulfonylureas, insulin, or both. No patient developed bladder cancer, osteoporosis, or osteoporotic bone fractures. **Appendix Table 2** (available at www.annals.org) provides a detailed description of adverse events.

Liver Histologic Outcomes

Primary Outcome

Both groups had similar severity of liver disease at baseline (**Table 1**). **Appendix Table 1** summarizes the observed histologic scores at baseline and month 18. Results for the primary histologic outcome (≥2-point reduction in NAS without worsening of fibrosis) are provided in **Table 2**. In the multiple-imputation analysis, more patients in the pioglitazone group (58%) achieved the primary outcome than in the placebo group (17%) (treatment difference, 41 percentage points [95% CI, 23 to 59 percentage points]; $P < 0.001$). When the same analysis was limited to patients with definite NASH at baseline, 67% achieved the primary outcome with pioglitazone versus 17% with placebo (treatment difference, 50 percentage points [CI, 30 to 69 percentage points]; $P < 0.001$). When patients who did not have a second liver biopsy were labeled as treatment failures, more patients in the pioglitazone group achieved the primary outcome than in the placebo group (52% vs. 16%; treatment difference, 36 percentage points [CI, 19 to 53 percentage points]; $P < 0.001$). **Appendix Figure 1** (available at www.annals.org) gives information on patients with paired biopsies.

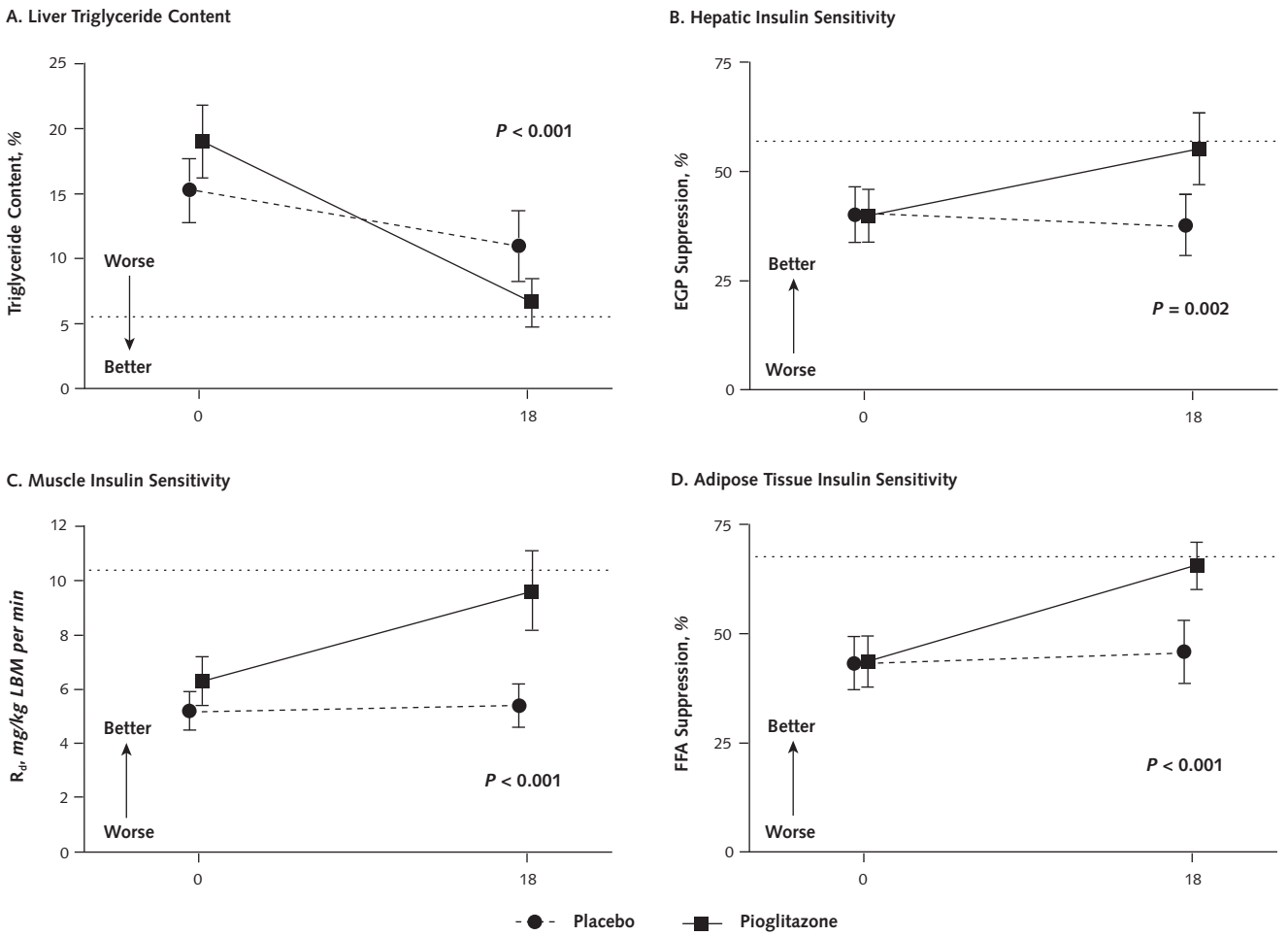
Secondary Outcomes

Resolution of NASH occurred in 51% of pioglitazone-treated patients versus 19% of those receiving placebo (treatment difference, 32 percentage points [CI, 13 to 51 percentage points]; $P < 0.001$) (**Table 2**). Similar results were obtained when patients who did not reach month 18 were considered to be treatment failures (46% vs. 18%; treatment difference, 28 percentage points [CI, 11 to 46 percentage points]; $P = 0.002$). More patients in the pioglitazone group had improvements in steatosis ($P < 0.001$), inflammation ($P = 0.004$), and ballooning necrosis ($P = 0.004$), with the overall NAS improving in 66% versus 21% of those in the placebo group ($P < 0.001$). The mean histologic scores (**Appendix Figure 2**, available at www.annals.org; $P \leq 0.001$ for all) and the fibrosis score ($P = 0.039$) also improved significantly with pioglitazone (**Table 2**). Progression of any fibrosis over 18 months occurred in only 12% of pioglitazone-treated patients compared with 28% of those receiving placebo (treatment difference, −16 percentage points [CI, −34 to 0 percentage points]; $P = 0.039$).

Liver Fat and Insulin Sensitivity

Pioglitazone markedly reduced hepatic triglyceride content from 19% to 7% versus from 15% to 11% in the placebo group (treatment difference, −7 percentage points [CI, −10 to −4 percentage points]; $P < 0.001$) (**Figure 2**, *A*). Fasting and OGTT levels of plasma glucose and insulin decreased with pioglitazone (**Table 3**).

Figure 2. Liver fat and insulin sensitivity before and after 18 mo of pioglitazone or placebo in patients with nonalcoholic steatohepatitis and prediabetes or type 2 diabetes mellitus.



Data include 101 observations at baseline (51 in the placebo group and 50 in the pioglitazone group) and 83 at month 18 (42 and 41, respectively). Bars represent 95% CIs. EGP = endogenous glucose production; FFA = free fatty acid; LBM = lean body mass; R_d = rate of glucose disappearance. **A.** Liver triglyceride content, measured by magnetic resonance and proton spectroscopy. The dotted line represents the threshold for a diagnosis of nonalcoholic fatty liver disease. **B.** Hepatic insulin sensitivity, representing suppression of EGP during the low-dose insulin infusion in a euglycemic insulin clamp. **C.** Muscle insulin sensitivity, representing whole-body insulin-stimulated R_d during the high-dose insulin infusion in a euglycemic insulin clamp. **D.** Adipose tissue insulin sensitivity, representing suppression of plasma FFA concentration during the low-dose insulin infusion in a euglycemic insulin clamp. The dotted lines in panels *B*, *C*, and *D* represent values based on previous studies (18, 19) for obese control participants without type 2 diabetes mellitus or nonalcoholic fatty liver disease.

Pioglitazone improved hepatic, muscle, and adipose tissue insulin action, measured as improvement in fasting hepatic insulin resistance index (−58% vs. 7% in the placebo group), insulin-induced suppression of EGP (**Figure 2**, *B*), R_d (**Figure 2**, *C*), fasting adipose tissue insulin resistance index (**Figure 3**, *D*), low-dose insulin-induced suppression of FFA (**Figure 2**, *D*) ($P \leq 0.001$ for all vs. placebo), and FFA suppression during the OGTT (approximately 11% [$P = 0.016$]).

Effects on Weight, Plasma Aminotransferase Levels, and Other Biomarkers

Compared with placebo, pioglitazone treatment was associated with significant weight gain (2.5 kg [CI, 0.4 to 4.5 kg]; $P = 0.020$) and a significant decrease in hemoglobin A_{1c} level in patients with T2DM (**Table 3**). Mean aminotransferase levels normalized with pioglitazone versus placebo by month 3 (35 vs. 56 IU/L; $P = 0.005$), reaching a plateau by month 5 and remaining normal thereafter (**Figure 3**, *A* and *B*). In contrast, patients receiving placebo had a modest decrease in aminotransferase levels. After patients switched from placebo to pioglitazone at month 18, AST and ALT levels normalized within 2 months.

Patients were insulin-resistant at the level of liver, muscle, and adipose tissue. Compared with placebo, pioglitazone significantly improved the HOMA-IR score (predominantly an indicator of hepatic insulin resistance) (**Figure 3**, *C*) and adipose tissue insulin resistance (**Figure 3**, *D*) at 18 months, an effect that persisted at 36 months. Mean plasma adiponectin levels

Table 3. Metabolic and Hepatic Characteristics After 18 mo of Pioglitazone Treatment

Characteristic	Mean Value After 18 mo (SD)		Treatment Difference (95% CI)*	*P* Value*
	Placebo	Pioglitazone		
Weight, *kg*	99.5 (16.7)	99.4 (16.6)	2.5 (0.4 to 4.5)	0.020
Body mass index, *kg/m²*	34.6 (5.0)	34.6 (4.8)	0.9 (0.1 to 1.6)	0.019
Total body fat by DXA, %	36 (8)	36 (7)	2 (1 to 3)	<0.001
Fasting plasma glucose level				0.020
mmol/L	6.6 (1.3)	6.1 (0.8)	−0.6 (−1.1 to −0.1)	
mg/dL	119 (24)	110 (14)	−11 (−19 to −2)	
2-h plasma glucose level				<0.001
mmol/L	12.0 (3.8)	9.6 (3.9)	−2.7 (−3.8 to −1.6)	
mg/dL	216 (69)	173 (70)	−48 (−69 to −28)	
Hemoglobin A_{1c} level, %				
Patients without T2DM	5.8 (0.3)	5.6 (0.3)	−0.1 (−0.3 to 0.0)	0.124
Patients with T2DM	6.5 (0.7)	6.2 (0.7)	−0.6 (−1.1 to −0.2)	0.009
Fasting plasma insulin level				0.041
pmol/L	102 (96)	48 (90)	−36 (−72 to 0)	
μU/mL	17 (16)	8 (15)	−6 (−12 to 0)	
Free fatty acid level, *mmol/L*	0.46 (0.17)	0.36 (0.16)	−0.04 (−0.14 to 0.05)	0.38
Liver fat content, %	11 (7)	7 (5)	−7 (−10 to −4)	<0.001
Aspartate aminotransferase level, *U/L*	38 (31)	29 (10)	−14 (−22 to −6)	0.001
Alanine aminotransferase level, *U/L*	44 (33)	27 (12)	−24 (−35 to −12)	<0.001
Triglyceride level				0.018
mmol/L	1.7 (0.8)	1.4 (0.7)	−0.6 (−1.0 to −0.1)	
mg/dL	149 (72)	127 (63)	−50 (−92 to −9)	
Total cholesterol level				0.92
mmol/L	3.9 (0.9)	4.0 (0.9)	0.0 (−0.5 to 0.5)	
mg/dL	149 (36)	153 (34)	1 (−18 to 19)	
LDL cholesterol level				0.59
mmol/L	2.0 (0.7)	2.2 (0.7)	0.1 (−0.3 to 0.6)	
mg/dL	79 (28)	84 (28)	5 (−13 to 22)	
HDL cholesterol level				<0.001
mmol/L	1.0 (0.2)	1.1 (0.3)	0.1 (0.1 to 0.2)	
mg/dL	40 (9)	44 (10)	5 (3 to 8)	

DXA = dual-energy x-ray absorptiometry; HDL = high-density lipoprotein; LDL = low-density lipoprotein; T2DM = type 2 diabetes mellitus.
* All randomly assigned patients were included in analyses (n = 101). Data were analyzed using mixed-effects linear regression, assuming that data were missing at random.

increased 2.6-fold with pioglitazone (from 8.7 to 22.8 μg/mL; $P < 0.001$), consistent with the improvement in adipose tissue function (**Figure 3**, *E*). Plasma cytokeratin-18 levels were elevated in patients with NASH and decreased significantly with pioglitazone treatment (**Figure 3**, *F*).

Long-Term Liver Histologic and Metabolic Effects

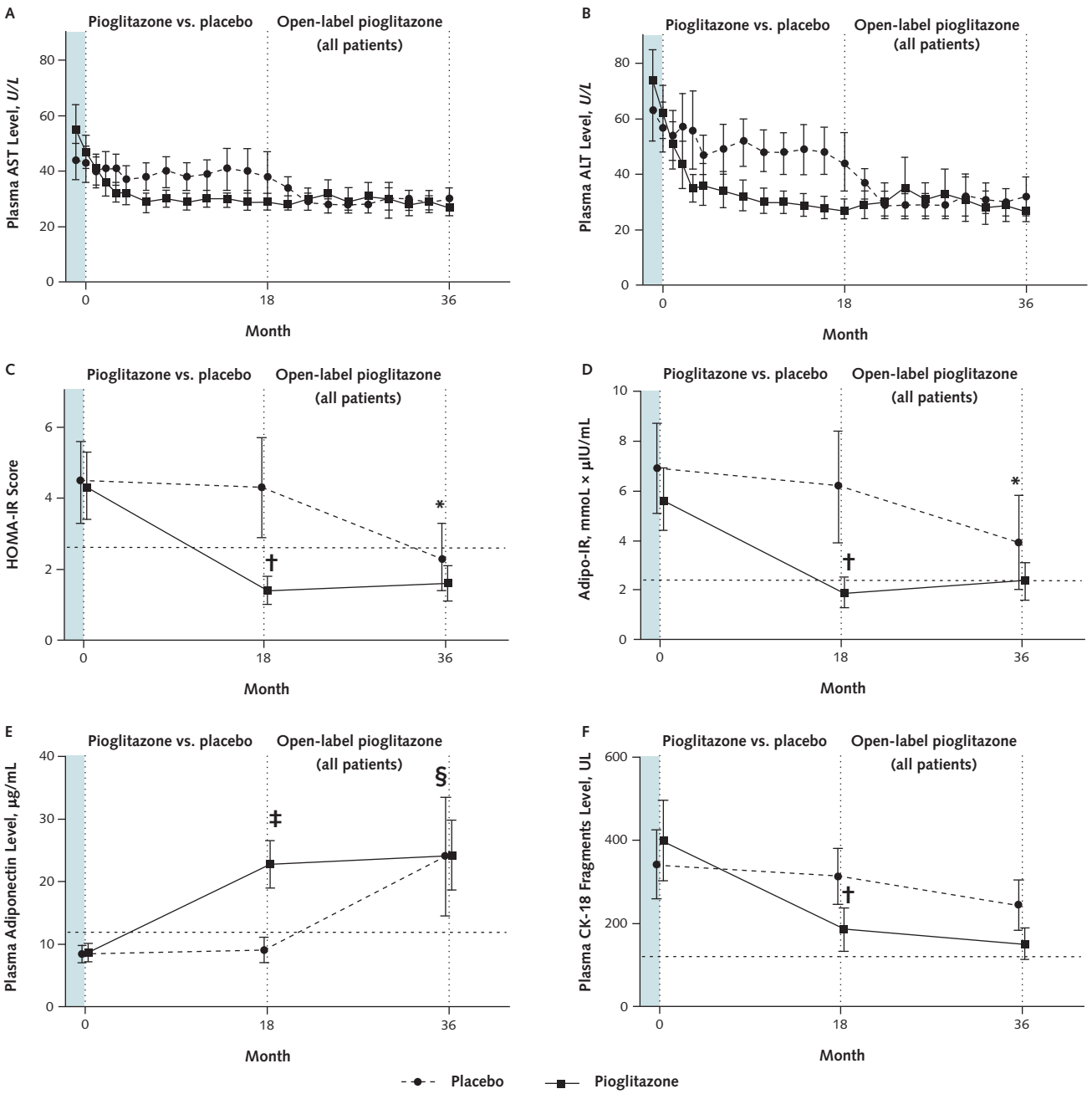
The histologic benefit of pioglitazone treatment, as measured by mean individual histologic scores (**Appendix Figure 1**) or expressed as a reduction of at least 2 points in the NAS without worsening of fibrosis (69%) or resolution of NASH (59%) (**Appendix Table 3**, available at www.annals.org), was maintained after 36 months of therapy. The same was true for normalization of plasma concentrations of AST and ALT (**Figure 3**, *A* and *B*), glucose, lipid profile, adiponectin (**Figure 3**, *E*), and cytokeratin-18 (**Figure 3**, *F*). These results were similar when all patients who completed 18 months of thiazolidinedione treatment were analyzed together (that is, those who received pioglitazone during months 0 to 18 plus those who received placebo from months 0 to 18 and switched to pioglitazone after 18 months) (n = 70) (**Appendix Table 4**, available at www.annals.org).

Discussion

Nonalcoholic steatohepatitis is a frequently overlooked and undertreated condition among patients with T2DM. Recent work from our laboratory (14, 21) and others (8–12) indicates that most obese patients with T2DM have NAFLD on imaging. Moreover, in studies involving liver biopsy, about 30% to 50% of patients have steatohepatitis even in the presence of normal plasma aminotransferase levels (9, 14, 21, 48). Given this background, by using gold standard metabolic and imaging techniques and serial liver biopsies, our study offered a unique opportunity to evaluate the effect of prolonged thiazolidinedione therapy in this population. Because the intervention proved to be safe and effective, these results may encourage early diagnosis and treatment of patients with prediabetes or T2DM and NASH.

Treatment led to marked improvements in steatosis, inflammation, and ballooning, with 58% of patients in the pioglitazone group achieving the primary outcome after 18 months (**Table 2**). The benefit was even more evident in patients with definite NASH at baseline, with 67% achieving the primary outcome ($P <$ 0.001 vs. placebo for each). This histologic benefit, combined with improvement in the mean fibrosis

Figure 3. Plasma aminotransferase levels and other biomarkers at baseline, after 18 mo of pioglitazone or placebo, and after 18 or 36 mo of pioglitazone.



Data include 101 observations at baseline (51 in the placebo group and 50 in the pioglitazone group), 83 at month 18 (42 and 41, respectively), and 63 at month 36 (29 and 34, respectively). Bars represent 95% CIs. Adipo-IR = adipose tissue insulin resistance; ALT = alanine aminotransferase; AST = aspartate aminotransferase; CK-18 = cytokeratin-18; HOMA-IR = homeostatic model assessment of insulin resistance. **A.** Plasma AST level during run-in (month −1 to 0 [*shaded area*]) and treatment (month 0 to 36) periods. **B.** Plasma ALT concentration during run-in [*shaded area*] and treatment periods. All plasma AST and ALT concentrations normalized with pioglitazone treatment by month 3, differed significantly compared with placebo ($P < 0.001$ to 0.010), and remained within the normal range thereafter during treatment. In contrast, levels decreased only modestly among patients receiving placebo but normalized within 2 mo after patients switched from placebo to pioglitazone after month 18. **C.** HOMA-IR score. **D.** Adipose tissue insulin resistance. **E.** Plasma adiponectin level. **F.** CK-18 fragment concentration. Dotted lines in panels *C* through *F* represent values based on previous studies (18, 19, 47) for obese control participants without type 2 diabetes mellitus or nonalcoholic fatty liver disease.
* $P \leq 0.042$ for change in placebo group after starting open-label pioglitazone therapy.
† $P \leq 0.026$ for effect of pioglitazone vs. placebo.
‡ $P < 0.001$ for effect of pioglitazone vs. placebo.
§ $P < 0.001$ for change in placebo group after starting open-label pioglitazone therapy.

score, suggests that pioglitazone may alter the natural history of the disease. Evidence of this was the reduc-

tion in fibrosis progression over 18 months in patients treated with pioglitazone compared with those receiving placebo (12% vs. 28%; treatment difference, −16 percentage points [CI, −34 to 0 percentage points]; $P = 0.039$). Of note, the relatively high rate of fibrosis progression without pharmacologic intervention in the placebo group confirms recent observational studies of fibrosis progression within relatively short periods in diabetes (49, 50) and adds significance to our study. In contrast, treatment discontinuation was associated with a progressive return of plasma aminotransferase levels to the elevated baseline levels over the following 12 months (data not shown), which suggests recurrence of steatohepatitis (51).

This study also has implications for patients with prediabetes and NASH (about half of our participants) because hepatic steatosis is a risk factor for T2DM, even in nonobese patients (52). Pioglitazone halts the progression from prediabetes to diabetes (37). Future studies may test whether reversal of hepatic steatosis or NASH with pioglitazone in patients with prediabetes may be a predictor of success in halting the development of T2DM. This is important to address at a time when 37.2% of U.S. adults (86 million) have prediabetes (36). The single-center nature of this study is a limitation that calls for additional work from a larger, longer-term (>3 years), multicenter trial. Future work should be done to compare the effects of pioglitazone in patients with prediabetes versus those with T2DM and to examine its effects in patients with more advanced liver fibrosis.

Although the role of lipotoxicity in the development of NASH is well-established (7, 25–27), the molecular mechanisms by which thiazolidinediones may improve insulin sensitivity or liver histologic outcomes remain elusive (24, 39, 53). From a clinical perspective, we aimed to define the profile of treatment "responders." However, no single clinical or metabolic parameter at baseline unequivocally predicted histologic response, such as overall adiposity; AST, ALT, or cytokeratin-18 level; severity of hepatic or muscle insulin resistance; or degree of steatosis on ^{1}H-MRS. Although treatment enhanced insulin sensitivity across hepatic, muscle, and adipose tissue to levels similar to those in well-matched control participants without NAFLD (**Figure 2**), the correlation between metabolic change and histologic response was modest overall. This suggests that intrinsic cellular mechanisms trigger steatohepatitis beyond the permissive role of systemic insulin resistance. Consistent with the role of dysfunctional adipose tissue in NASH, an increase in plasma adiponectin level was the best metabolic predictor of histologic response. As shown in previous studies by our group, adiponectin levels increase within 1 to 3 months (17, 35) and remain elevated during pioglitazone treatment in patients with NASH (**Figure 3**, *E*, and **Appendix Table 3**). Patients receiving placebo had minimal, if any, increases in plasma adiponectin level, and nonresponders had a blunted response compared with responders who had at least a 2.5-fold increase in adiponectin level (54). We envision that better identification of potential thiazolidinedione responders will be possible with the combination of genetic polymorphisms, existence of certain high-risk clinical profiles (elevated NAS or fibrosis at baseline), and improved imaging techniques or plasma biomarkers. This will allow better tailoring of treatment to limit long-term therapy to patients who are more likely to benefit.

Pioglitazone was well-tolerated, and there were no major drug-related adverse events. Recent prospective data suggest that pioglitazone does not increase the risk for bladder cancer (55, 56) and are encouraging in terms of the long-term safety of the drug. Close monitoring is necessary to identify patients with undiagnosed diastolic dysfunction who are at risk for congestive heart failure with pioglitazone treatment and to assess long-term effects on bone metabolism, particularly in women (38, 39, 55, 56). Pioglitazone treatment induced only modest weight gain (2.5 kg over 18 months) versus placebo (3.1 kg at 36 months vs. baseline). To our knowledge, this is the only study in patients with biopsy-proven NASH in which active dietary advice extended beyond 12 months. The limited effect of a prescribed diet with a deficit of 500 kcal/d was consistent with prior lifestyle studies and highlights the need for trials to determine more efficacious long-term dietary interventions (22).

In summary, 3 years of pioglitazone treatment was associated with long-term metabolic and histologic improvement in patients with prediabetes or T2DM and NASH. These results suggest that NASH progression may be halted and the natural history of the disease may be modified with the use of pioglitazone in patients with prediabetes or T2DM.

From University of Florida and Malcom Randall Veterans Affairs Medical Center, Gainesville, Florida, and University of Texas Health Science Center at San Antonio and Audie L. Murphy Veterans Affairs Medical Center, San Antonio, Texas.

Note: Dr. Cusi, as principal investigator of the study, 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.

Acknowledgment: The authors thank the volunteers and the nursing, nutrition, and laboratory staff at the Clinical Translational Science Institute for their skilled work; Jonathan J. Shuster, PhD, Professor and Co-Director of Biostatistics, Epidemiology, and Research Design at the Clinical Translational Science Institute at the University of Florida for his support and advice during manuscript preparation; and Takeda Pharmaceuticals for providing the pioglitazone and placebo tablets.

Grant Support: By the Burroughs Wellcome Fund (1006762.01 [Dr. Cusi]) and the American Diabetes Association (1-08-CR-08 [Dr. Cusi]). Additional support was provided by the National Center for Research Resources (MO1-RR-01346), the UTHSCSA Clinical Research Center and Research Imaging Center, and the Veterans Affairs Medical Research Fund.

Disclosures: Dr. Cusi reports nonfinancial support from Takeda Pharmaceuticals (provision of study medication and placebo); grants from Novartis and Janssen Research & Development; and consultancies for Eli Lilly and Company, Tobira Therapeutics, and Pfizer outside the submitted work. Authors not named here have disclosed no conflicts of interest. Disclosures can also be viewed at www.acponline.org/authors/icmje/ConflictOfInterestForms.do?msNum=M15-1774.

Reproducible Research Statement: *Study protocol:* The authors chose not to provide readers access to the study protocol with this article. *Statistical code:* Available from Dr. Cusi (e-mail, kenneth.cusi@medicine.ufl.edu). *Data set*: Not available.

Requests for Single Reprints: Kenneth Cusi, MD, Professor of Medicine, Chief, Division of Endocrinology, Diabetes and Metabolism, University of Florida, 1600 SW Archer Road, Room H-2, Gainesville, FL 32610; e-mail, Kenneth.Cusi@medicine.ufl.edu.

Current author addresses and author contributions are available at www.annals.org.

References

1. **Loomba R, Sanyal AJ.** The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013;10:686-90. [PMID: 24042449] doi:10.1038/nrgastro.2013.171
2. **Caldwell S, Argo C.** The natural history of non-alcoholic fatty liver disease. Dig Dis. 2010;28:162-8. [PMID: 20460906] doi:10.1159/000282081
3. **Caldwell SH, Crespo DM.** The spectrum expanded: cryptogenic cirrhosis and the natural history of non-alcoholic fatty liver disease. J Hepatol. 2004;40:578-84. [PMID: 15030972]
4. **Bugianesi E, Vanni E, Marchesini G.** NASH and the risk of cirrhosis and hepatocellular carcinoma in type 2 diabetes. Curr Diab Rep. 2007;7:175-80. [PMID: 17547834]
5. **Targher G, Chonchol M, Bertolini L, Rodella S, Zenari L, Lippi G, et al.** Increased risk of CKD among type 2 diabetics with nonalcoholic fatty liver disease. J Am Soc Nephrol. 2008;19:1564-70. [PMID: 18385424] doi:10.1681/ASN.2007101155
6. **Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al.** Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care. 2007;30:1212-8. [PMID: 17277038]
7. **Cusi K.** Role of obesity and lipotoxicity in the development of nonalcoholic steatohepatitis: pathophysiology and clinical implications. Gastroenterology. 2012;142:711-725. [PMID: 22326434] doi:10.1053/j.gastro.2012.02.003
8. **Younossi ZM, Gramlich T, Matteoni CA, Boparai N, McCullough AJ.** Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol. 2004;2:262-5. [PMID: 15017611]
9. **Fracanzani AL, Valenti L, Bugianesi E, Andreoletti M, Colli A, Vanni E, et al.** Risk of severe liver disease in nonalcoholic fatty liver disease with normal aminotransferase levels: a role for insulin resistance and diabetes. Hepatology. 2008;48:792-8. [PMID: 18752331] doi:10.1002/hep.22429
10. **Kotronen A, Juurinen L, Hakkarainen A, Westerbacka J, Cornér A, Bergholm R, et al.** Liver fat is increased in type 2 diabetic patients and underestimated by serum alanine aminotransferase compared with equally obese nondiabetic subjects. Diabetes Care. 2008;31:165-9. [PMID: 17934148]
11. **Leite NC, Salles GF, Araujo AL, Villela-Nogueira CA, Cardoso CR.** Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int. 2009;29:113-9. [PMID: 18384521] doi:10.1111/j.1478-3231.2008.01718.x
12. **Williamson RM, Price JF, Glancy S, Perry E, Nee LD, Hayes PC, et al; Edinburgh Type 2 Diabetes Study Investigators.** Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: the Edinburgh Type 2 Diabetes Study. Diabetes Care. 2011;34:1139-44. [PMID: 21478462] doi:10.2337/dc10-2229
13. **Mofrad P, Contos MJ, Haque M, Sargeant C, Fisher RA, Luketic VA, et al.** Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology. 2003;37:1286-92. [PMID: 12774006]
14. **Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al.** High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. J Clin Endocrinol Metab. 2015;100:2231-8. [PMID: 25885947] doi:10.1210/jc.2015-1966
15. **Portillo P, Yavuz S, Bril F, Cusi K.** Role of insulin resistance and diabetes in the pathogenesis and treatment of nonalcoholic fatty liver disease. Curr Hepatol Rep. 2014;13:159-70.
16. **Ortiz-Lopez C, Lomonaco R, Orsak B, Finch J, Chang Z, Kochunov VG, et al.** Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). Diabetes Care. 2012;35:873-8. [PMID: 22374640] doi:10.2337/dc11-1849
17. **Gastaldelli A, Harrison S, Belfort-Aguiar R, Hardies J, Balas B, Schenker S, et al.** Pioglitazone in the treatment of NASH: the role of adiponectin. Aliment Pharmacol Ther. 2010;32:769-75. [PMID: 20662773] doi:10.1111/j.1365-2036.2010.04405.x
18. **Lomonaco R, Bril F, Portillo-Sanchez P, Ortiz-Lopez C, Orsak B, Biernacki D, et al.** Metabolic impact of nonalcoholic steatohepatitis in obese patients with type 2 diabetes. Diabetes Care. 2016. [PMID: 26861926]
19. **Bril F, Sninsky JJ, Baca AM, Superko HR, Portillo Sanchez P, Biernacki D, et al.** Hepatic steatosis and insulin resistance, but not steatohepatitis, promote atherogenic dyslipidemia in NAFLD. J Clin Endocrinol Metab. 2016;101:644-52. [PMID: 26672634] doi:10.1210/jc.2015-3111
20. **Fabbrini E, Mohammed BS, Magkos F, Korenblat KM, Patterson BW, Klein S.** Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology. 2008;134:424-31. [PMID: 18242210] doi:10.1053/j.gastro.2007.11.038
21. **Maximos M, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, et al.** The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. Hepatology. 2015;61:153-60. [PMID: 25145475] doi:10.1002/hep.27395
22. **Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al.** The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology. 2012;55:2005-23. [PMID: 22488764] doi:10.1002/hep.25762
23. **Neuschwander-Tetri BA.** NASH: the tribulations of conducting NASH trials. Nat Rev Gastroenterol Hepatol. 2014;11:274-6. [PMID: 24709815] doi:10.1038/nrgastro.2014.51
24. **Utzschneider KM, Kahn SE.** Review: the role of insulin resistance in nonalcoholic fatty liver disease. J Clin Endocrinol Metab. 2006;91:4753-61. [PMID: 16968800]
25. **Neuschwander-Tetri BA.** Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role of nontriglyceride fatty acid metabolites. Hepatology. 2010;52:774-88. [PMID: 20683968] doi:10.1002/hep.23719
26. **Armstrong MJ, Adams LA, Canbay A, Syn WK.** Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology. 2014;59:1174-97. [PMID: 24002776] doi:10.1002/hep.26717
27. **Birkenfeld AL, Shulman GI.** Nonalcoholic fatty liver disease, hepatic insulin resistance, and type 2 diabetes. Hepatology. 2014;59:713-23. [PMID: 23929732] doi:10.1002/hep.26672

28. **Ratziu V, Caldwell S, Neuschwander-Tetri BA.** Therapeutic trials in nonalcoholic steatohepatitis: insulin sensitizers and related methodological issues. Hepatology. 2010;52:2206-15. [PMID: 21105109] doi:10.1002/hep.24042
29. **Caldwell SH, Hespenheide EE, Redick JA, Iezzoni JC, Battle EH, Sheppard BL.** A pilot study of a thiazolidinedione, troglitazone, in nonalcoholic steatohepatitis. Am J Gastroenterol. 2001;96:519-25. [PMID: 11232700]
30. **Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver D, Bacon BR.** Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology. 2003;38:1008-17. [PMID: 14512888]
31. **Promrat K, Lutchman G, Uwaifo GI, Freedman RJ, Soza A, Heller T, et al.** A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology. 2004;39:188-96. [PMID: 14752837]
32. **Aithal GP, Thomas JA, Kaye PV, Lawson A, Ryder SD, Spendlove I, et al.** Randomized, placebo-controlled trial of pioglitazone in nondiabetic subjects with nonalcoholic steatohepatitis. Gastroenterology. 2008;135:1176-84. [PMID: 18718471] doi:10.1053/j.gastro.2008.06.047
33. **Ratziu V, Giral P, Jacqueminet S, Charlotte F, Hartemann-Heurtier A, Serfaty L, et al; LIDO Study Group.** Rosiglitazone for nonalcoholic steatohepatitis: one-year results of the randomized placebo-controlled Fatty Liver Improvement with Rosiglitazone Therapy (FLIRT) Trial. Gastroenterology. 2008;135:100-10. [PMID: 18503774] doi:10.1053/j.gastro.2008.03.078
34. **Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al; NASH CRN.** Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med. 2010;362:1675-85. [PMID: 20427778] doi:10.1056/NEJMoa0907929
35. **Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al.** A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med. 2006;355:2297-307. [PMID: 17135584]
36. **Dall TM, Yang W, Halder P, Pang B, Massoudi M, Wintfeld N, et al.** The economic burden of elevated blood glucose levels in 2012: diagnosed and undiagnosed diabetes, gestational diabetes mellitus, and prediabetes. Diabetes Care. 2014;37:3172-9. [PMID: 25414388] doi:10.2337/dc14-1036
37. **DeFronzo RA, Tripathy D, Schwenke DC, Banerji M, Bray GA, Buchanan TA, et al; ACT NOW Study.** Pioglitazone for diabetes prevention in impaired glucose tolerance. N Engl J Med. 2011;364:1104-15. [PMID: 21428766] doi:10.1056/NEJMoa1010949
38. **Yau H, Rivera K, Lomonaco R, Cusi K.** The future of thiazolidinedione therapy in the management of type 2 diabetes mellitus. Curr Diab Rep. 2013;13:329-41. [PMID: 23625197] doi:10.1007/s11892-013-0378-8
39. **Soccio RE, Chen ER, Lazar MA.** Thiazolidinediones and the promise of insulin sensitization in type 2 diabetes. Cell Metab. 2014;20:573-91. [PMID: 25242225] doi:10.1016/j.cmet.2014.08.005
40. **Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al; Nonalcoholic Steatohepatitis Clinical Research Network.** Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313-21. [PMID: 15915461]
41. **Zein CO, Yerian LM, Gogate P, Lopez R, Kirwan JP, Feldstein AE, et al.** Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. Hepatology. 2011;54:1610-9. [PMID: 21748765] doi:10.1002/hep.24544
42. **Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al; NASH Clinical Research Network.** Farnesoid X nuclear receptor ligand obeticholic acid for noncirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet. 2015;385:956-65. [PMID: 25468160] doi:10.1016/S0140-6736(14)61933-4
43. **Ratziu V, Harrison SA, Francque S, Bedossa P, Lehert P, Serfaty L, et al; GOLDEN-505 Investigator Study Group.** Elafibranor, an agonist of the peroxisome proliferator-activated receptor-α and -δ, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology. 2016;150:1147-1159. [PMID: 26874076] doi:10.1053/j.gastro.2016.01.038
44. **Sanyal AJ, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, et al.** Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology. 2011;54:344-53. [PMID: 21520200] doi:10.1002/hep.24376
45. **Bril F, Ortiz-Lopez C, Lomonaco R, Orsak B, Freckleton M, Chintapalli K, et al.** Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. Liver Int. 2015;35:2139-46. [PMID: 25847730] doi:10.1111/liv.12840
46. **Lewis JD, Ferrara A, Peng T, Hedderson M, Bilker WB, Quesenberry CP Jr, et al.** Risk of bladder cancer among diabetic patients treated with pioglitazone: interim report of a longitudinal cohort study. Diabetes Care. 2011;34:916-22. [PMID: 21447663] doi:10.2337/dc10-1068
47. **Cusi K, Chang Z, Harrison S, Lomonaco R, Bril F, Orsak B, et al.** Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol. 2014;60:167-74. [PMID: 23973932] doi:10.1016/j.jhep.2013.07.042
48. **Leite NC, Villela-Nogueira CA, Pannain VL, Bottino AC, Rezende GF, Cardoso CR, et al.** Histopathological stages of nonalcoholic fatty liver disease in type 2 diabetes: prevalences and correlated factors. Liver Int. 2011;31:700-6. [PMID: 21457442] doi:10.1111/j.1478-3231.2011.02482.x
49. **Stepanova M, Rafiq N, Makhlouf H, Agrawal R, Kaur I, Younoszai Z, et al.** Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD). Dig Dis Sci. 2013;58:3017-23. [PMID: 23775317] doi:10.1007/s10620-013-2743-5
50. **Pais R, Charlotte F, Fedchuk L, Bedossa P, Lebray P, Poynard T, et al; LIDO Study Group.** A systematic review of follow-up biopsies reveals disease progression in patients with non-alcoholic fatty liver. J Hepatol. 2013;59:550-6. [PMID: 23665288] doi:10.1016/j.jhep.2013.04.027
51. **Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al.** The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. Hepatology. 2007;46:424-9. [PMID: 17559148]
52. **Fan JG, Li F, Cai XB, Peng YD, Ao QH, Gao Y.** Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. J Gastroenterol Hepatol. 2007;22:1086-91. [PMID: 17608855]
53. **Koliaki C, Roden M.** Hepatic energy metabolism in human diabetes mellitus, obesity and non-alcoholic fatty liver disease. Mol Cell Endocrinol. 2013;379:35-42. [PMID: 23770462] doi:10.1016/j.mce.2013.06.002
54. **Bril F, Portillo Sanchez P, Maximos M, Lomonaco R, Orsak B, Hecht J, et al.** Metabolic predictors of response to pioglitazone treatment in patients with prediabetes or type 2 diabetes mellitus (T2DM) and nonalcoholic steatohepatitis (NASH). Diabetes. 2015;64:A337.
55. **Levin D, Bell S, Sund R, Hartikainen SA, Tuomilehto J, Pukkala E, et al; Scottish Diabetes Research Network Epidemiology Group.** Pioglitazone and bladder cancer risk: a multipopulation pooled, cumulative exposure analysis. Diabetologia. 2015;58:493-504. [PMID: 25481707] doi:10.1007/s00125-014-3456-9
56. **Lewis JD, Habel LA, Quesenberry CP, Strom BL, Peng T, Hedderson MM, et al.** Pioglitazone use and risk of bladder cancer and other common cancers in persons with diabetes. JAMA. 2015;314:265-77. [PMID: 26197187] doi:10.1001/jama.2015.7996

Current Author Addresses: Drs. Cusi, Bril, Lomonaco, and Portillo-Sanchez: University of Florida, 1600 SW Archer Road, Room H-2, Gainesville, FL 32610.
Ms. Orsak; Ms. Hecht; Drs. Ortiz-Lopez, Tio, Hardies, Musi, and Webb; and Ms. Darland: University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, DTL Room 3.380S, San Antonio, TX 78229.

Author Contributions: Conception and design: K. Cusi, A. Webb.
Analysis and interpretation of the data: K. Cusi, F. Bril, C. Ortiz-Lopez, F. Tio, J. Hardies, N. Musi, A. Webb, P. Portillo-Sanchez.
Drafting of the article: K. Cusi, F. Bril.
Critical revision of the article for important intellectual content: K. Cusi, F. Bril, P. Portillo-Sanchez.
Final approval of the article: K. Cusi, B. Orsak, F. Bril, R. Lomonaco, J. Hecht, C. Ortiz-Lopez, F. Tio, J. Hardies, C. Darland, N. Musi, A. Webb, P. Portillo-Sanchez.
Provision of study materials or patients: K. Cusi, B. Orsak, R. Lomonaco, C. Darland, A. Webb.
Statistical expertise: K. Cusi, F. Bril.
Obtaining of funding: K. Cusi.
Administrative, technical, or logistic support: K. Cusi, J. Hecht, N. Musi.
Collection and assembly of data: K. Cusi, B. Orsak, F. Bril, R. Lomonaco, C. Ortiz-Lopez, P. Portillo-Sanchez.

Appendix: Methods

Inclusion and Exclusion Criteria

A total of 176 patients were recruited, and 101 were randomly assigned after initial screening. Inclusion and exclusion criteria were evaluated by the investigators.

Patients had to meet the following inclusion criteria:

1. Able to communicate meaningfully with the investigator and legally competent to provide written informed consent.

2. Aged 18 to 70 years.

3. Diagnosis of prediabetes or T2DM based on results from a fasting plasma glucose, hemoglobin A_{1c}, or oral glucose tolerance test, according to American Diabetes Association guidelines.

4. Diagnosis of NASH based on results from a liver biopsy.

5. Female patients were eligible if they were postmenopausal for at least 1 year, were using adequate mechanical contraceptive precautions (for example, intrauterine device, diaphragm with spermicide, or condom with spermicide), had a history of surgical sterilization (bilateral tubal ligation or bilateral oophorectomy), or had undergone a hysterectomy.

6. Female patients who had not undergone a hysterectomy or a bilateral oophorectomy were eligible if they had negative pregnancy test results throughout the study period.

7. Hemoglobin level of at least 120 g/L (men) or at least 110 g/L (women), leukocyte count of at least 3.0×10^9 cells/L, neutrophil count of at least 1.5×10^9 cells/L, platelet count of at least 100×10^9 cells/L, albumin level of at least 30 g/L, serum creatinine level of 159.1 μmol/L (1.8 mg/dL) or less, and AST and ALT levels no more than 3 times the ULN (patients were not definitively excluded if either level [but not both] was >3 times the ULN, but plasma aminotransferase measurement was repeated within 1 to 8 weeks to confirm that both levels were ≤3 times the ULN).

Exclusion criteria were as follows:

1. Past or current history of alcohol abuse (>20 g of ethanol consumed per day). Alcohol abuse was ruled out on the basis of physicians' judgment, self-reported alcohol use, and family members' report of the patient's alcohol use. In addition, the Alcohol Use Disorders Identification Test was used to assess alcohol use.

2. Receipt of long-term therapy with medications known to have adverse effects on glucose tolerance, unless the patient had been receiving a stable dose of such agents for 4 weeks before study entry.

3. Use of medications that could induce steatosis, such as estrogen or other hormonal replacement therapy, tamoxifen, raloxifene, oral glucocorticoids, or chloroquine.

4. Any cause of chronic liver disease other than NASH (including but not restricted to alcohol or drug abuse, medication, chronic hepatitis B or C virus infection, autoimmune liver disease, hemochromatosis, Wilson disease, or α1-antitrypsin deficiency). The following tests were done to rule out these differential diagnoses:

- Hepatitis B virus infection: positive result on a hepatitis B surface antigen test.
- Hepatitis C virus infection: positive result on a hepatitis C antibody test.
- Autoimmune liver disease: positive result on an antinuclear antibody, anti–smooth-muscle antibody, antimitochondrial antibody, or anti–liver-kidney microsomal antibody test or previous histologic features consistent with autoimmune hepatitis.
- Wilson disease: ceruloplasmin levels below the limits of normal.
- α1-Antitrypsin deficiency: α1-antitrypsin level below normal.
- Hemochromatosis or history of iron overload: presence of 3+ or 4+ stainable iron on liver biopsy or history of iron overload.
- Drug-induced liver disease: history of exposure.
- History of primary or metastatic liver cancer.

5. Presence of other medical conditions known to cause fatty liver disease.

6. Any clinical evidence of hepatic decompensation, such as history of ascites, esophageal bleeding varices, or spontaneous encephalopathy.

7. Prior or scheduled surgical procedures, including gastroplasty or jejunoileal or jejunocolic bypass.

8. Prior exposure to organic solvents, such as carbon tetrachloride.

9. Total parenteral nutrition within the past 6 months.

10. Presence of type 1 diabetes mellitus.

11. History of clinically significant heart disease (New York Heart Association Classification greater than grade II), peripheral vascular disease (history of claudication), or diagnosed pulmonary disease (dyspnea on exertion of ≤1 flight; abnormal breath sounds on auscultation).

12. Presence of severe osteoporosis (T-score of −3.0 at the level of the spine and hip).

13. Pregnancy or lactation in women.

Measurement of Liver, Muscle, and Adipose Tissue Insulin Sensitivity During the Euglycemic Insulin Clamp

After an overnight fast, hepatic, muscle, and adipose tissue insulin sensitivity were measured at the Clinical Research Center as previously reported by our group (21, 57–60). In brief, a primed (25 μCi/min × fasting plasma glucose/100), continuous (0.25 μCi/min) [3-^{3}H] glucose infusion was started and continued until the end of the study to measure glucose turnover. After a 3-hour isotopic equilibration, insulin was administered as a primed continuous infusion at 10 mU/m^2 per minute for 120 minutes to assess suppression of EGP and lipolysis (plasma FFA levels), followed by another primed continuous 120-minute insulin infusion at 80 mU/m^2 per minute to assess whole-body insulin-stimulated R_d. A variable 20% glucose infusion maintained plasma glucose at approximately 5.0 to 5.6 mmol/L (90 to 100 mg/dL) (coefficient of variation <5%). Blood was drawn every 5 to 10 minutes at baseline and for the next 4 hours to measure plasma [3-^{3}H] glucose radioactivity and plasma glucose, insulin, and FFA concentrations.

Calculations

During the insulin clamp, a 2-hour low-dose insulin infusion was used to assess adipose tissue insulin sensitivity (represented as insulin-induced suppression of plasma FFA concentration) and hepatic insulin sensitivity (represented as insulin-induced suppression of EGP). During the 2-hour high-dose insulin infusion, skeletal muscle insulin sensitivity was measured as insulin-stimulated whole-body R_d per kilogram of lean body mass. Both EGP and R_d were calculated as previously reported (57). Indexes of fasting insulin resistance in hepatic tissue (fasting plasma insulin × EGP) and adipose tissue (fasting plasma insulin × FFA) were calculated as previously reported (21, 35, 57–60).

Analytic Determinations

Plasma glucose level was measured in the Clinical Research Center by the glucose oxidase method (Analox Glucose Analyzer [Analox Instruments]). Other samples were placed on ice at the bedside, processed within 15 to 20 minutes, and frozen at −80 °C until final analysis. Plasma insulin level was determined by radioimmunoassay, FFA concentration by standard colorimetric methods, hemoglobin A_{1c} level by high-performance liquid chromatography (Tosoh G7), adiponectin level by magnetic bead MILLIPLEX technology (Luminex xMAP), and cytokeratin-18 concentration by enzyme-linked immunosorbent assay (M30 Apoptosense [Diapharma]). Tritiated plasma glucose–specific activity was measured from barium hydroxide/zinc sulfate–deproteinized plasma extracts (14, 16, 35).

Statistical Methods

For the primary analyses, missing values were considered to be missing at random because most discontinuations occurred after patients were warned about the possibility of bladder cancer with pioglitazone; the number of patients discontinuing for this reason was similar between treatment groups. We used multiple imputation to predict the histologic outcomes of patients not having a second liver biopsy. Treatment group, age, sex, presence of diabetes, and baseline histologic parameters were used to impute missing histologic parameters at month 18; 40 data sets were created. Calculated proportions for the different histologic outcomes in each data set were combined according to Rubin's rules.

Sensitivity analyses were done for the analyses of the primary outcome and resolution of NASH to examine the effect of assumptions about the missing data (**Appendix Table 5**). First, as prespecified in the protocol and based on previous approaches in the field (34, 40–42), for the analysis of histologic outcomes, patients not reaching month 18 were considered to be treatment failures (lack of improvement). Second, an analysis of only those completing 18 months of therapy was performed, followed by an analysis of only completers who had a baseline diagnosis of definite NASH based on the final biopsy specimen. Finally, worst- and best-case scenarios were calculated. For the worst-case scenario, patients not reaching month 18 were considered to have failed to achieve the primary outcome if they were randomly assigned to pioglitazone and to have achieved this outcome if they were randomly assigned to placebo. The opposite assumption was made for the best-case scenario. Analyses based on the outcome used in the PIVENS (Pioglitazone vs. Vitamin E vs. Placebo for Treatment of Non-Diabetic Patients With Non-alcoholic Steatohepatitis) study were also performed (**Appendix Figure 3**). This outcome was defined as improvement in ballooning, with a reduction of at least 2 points in the NAS or an absolute NAS of 3 or lower

(with improvement in steatosis or inflammation) without worsening of fibrosis.

Web-Only References

57. **Kashyap S, Belfort R, Gastaldelli A, Pratipanawatr T, Berria R, Pratipanawatr W, et al.** A sustained increase in plasma free fatty acids impairs insulin secretion in nondiabetic subjects genetically predisposed to develop type 2 diabetes. Diabetes. 2003;52:2461-74. [PMID: 14514628]

58. **Belfort R, Mandarino L, Kashyap S, Wirfel K, Pratipanawatr T, Berria R, et al.** Dose-response effect of elevated plasma free fatty acid on insulin signaling. Diabetes. 2005;54:1640-8. [PMID: 15919784]

59. **Lomonaco R, Ortiz-Lopez C, Orsak B, Finch J, Webb A, Bril F, et al.** Role of ethnicity in overweight and obese patients with nonalcoholic steatohepatitis. Hepatology. 2011;54:837-45. [PMID: 21674556] doi:10.1002/hep.24483

60. **Bril F, Lomonaco R, Orsak B, Ortiz-Lopez C, Webb A, Tio F, et al.** Relationship between disease severity, hyperinsulinemia, and impaired insulin clearance in patients with nonalcoholic steatohepatitis. Hepatology. 2014;59:2178-87. [PMID: 24777953] doi:10.1002/hep.26988

Appendix Table 1. Liver Histologic Variables at Baseline and After 18 mo, Based on Observed Data*

Variable	Placebo		Pioglitazone	
	Baseline (*n* = 51)	**18 mo (*n* = 42)**	**Baseline (*n* = 50)**	**18 mo (*n* = 40)**
Steatosis, *n (%)*				
Patients with grade 0 (<5%)	0 (0)	2 (5)	2 (4)	13 (32)
Patients with grade 1 (5%–33%)	17 (33)	18 (43)	11 (22)	19 (48)
Patients with grade 2 (>33%–66%)	20 (39)	10 (24)	21 (42)	6 (15)
Patients with grade 3 (>66%)	14 (28)	12 (29)	16 (32)	2 (5)
Inflammation, *n (%)*				
Patients with grade 0 (no foci)	0 (0)	1 (2)	1 (2)	8 (20)
Patients with grade 1 (<2 foci per 200 × field)	16 (31)	15 (36)	16 (32)	24 (60)
Patients with grade 2 (2–4 foci per 200 × field)	34 (67)	25 (60)	32 (64)	8 (20)
Patients with grade 3 (>4 foci per 200 × field)	1 (2)	1 (2)	1 (2)	0 (0)
Ballooning, *n (%)*				
Patients with grade 0 (none)	8 (16)	14 (33)	9 (18)	30 (75)
Patients with grade 1 (few balloon cells)	42 (82)	27 (64)	40 (80)	10 (25)
Patients with grade 2 (many balloon cells)	1 (2)	1 (2)	1 (2)	0 (0)
Fibrosis, *n (%)*				
Patients with stage 0 (none)	20 (39)	18 (43)	15 (30)	22 (55)
Patients with stage 1 (perisinusoidal or periportal)	22 (43)	16 (38)	22 (44)	13 (32)
Patients with stage 2 (perisinusoidal and portal or periportal)	4 (8)	3 (7)	6 (12)	2 (5)
Patients with stages 3–4 (bridging fibrosis or cirrhosis)	5 (10)	5 (12)	7 (14)	3 (8)

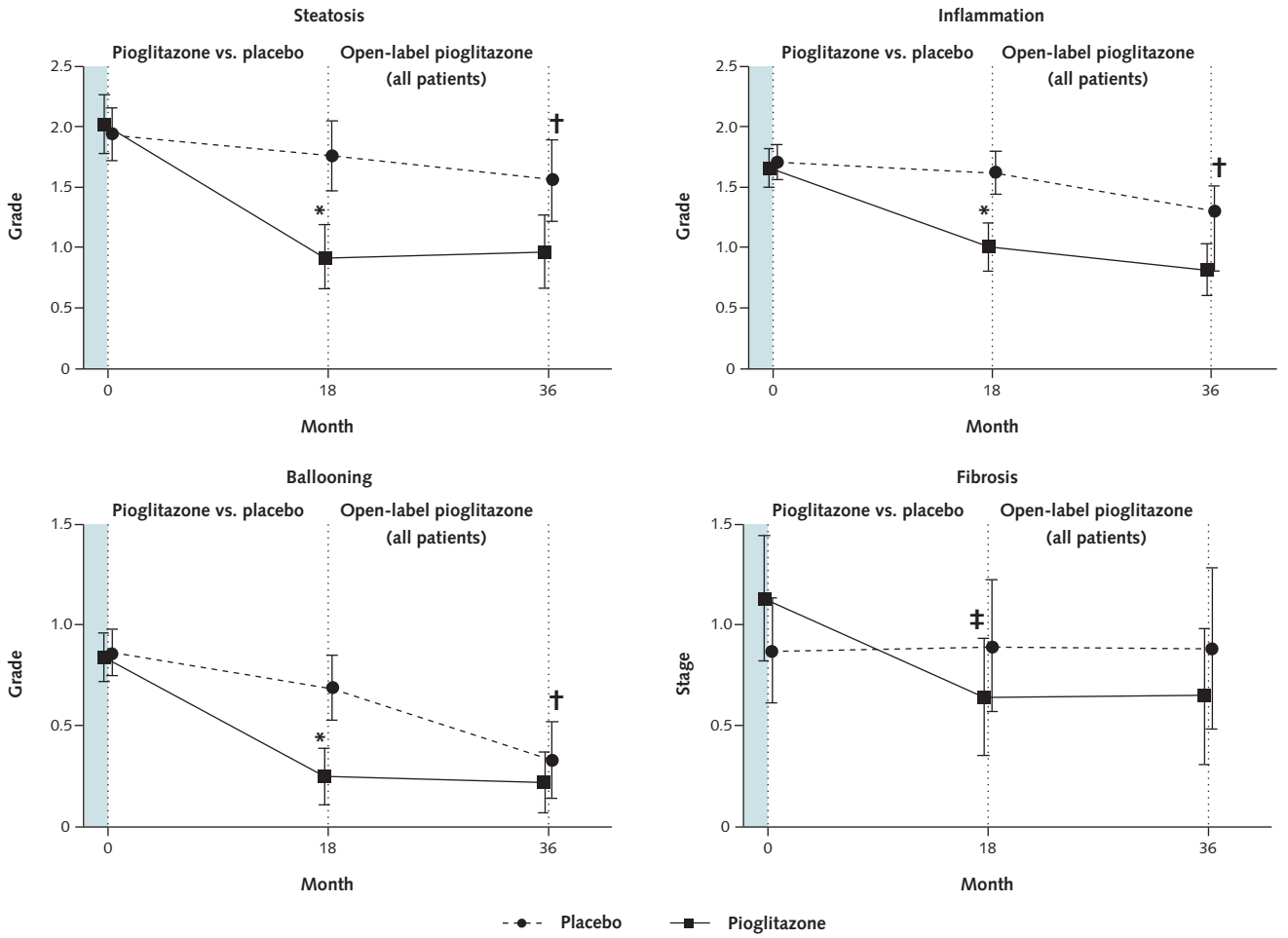
* Percentages may not sum to 100 due to rounding.

Appendix Table 2. Adverse Events

Adverse Events	First 18 mo		Open-Label Phase	
	Placebo (*n* = 51)	Pioglitazone (*n* = 50)	Patients Starting Pioglitazone Therapy (*n* = 36)	Pioglitazone (months 18–36) (*n* = 40)
Mild adverse events, *n*				
Cardiovascular	6	2	10	6
Respiratory/otolaryngologic	12	14	15	14
Gastrointestinal	17	13	12	14
Endocrinologic	0	0	0	1
Neurologic	6	6	8	5
Gynecologic	2	1	0	0
Urologic	3	6	6	7
Hematologic	3	7	7	5
Dermatologic	6	6	3	7
Musculoskeletal	21	23	22	26
Asthenia	8	5	0	3
Other	11	8	4	7
Moderate to severe adverse events, *n*				
Cardiovascular				
Atypical chest pain	1	1	0	2
Pulmonary thromboembolism	0	0	1	0
Palpitations/arrhythmia	1	0	1	0
Hypertension/hypotension	0	0	1	2
Chronic lower limb edema	3	11	5	0
Gastrointestinal				
Pancreatitis	0	1	0	0
Cholelithiasis	0	0	1	2
Diverticulitis	0	0	2	0
Gastritis	1	0	1	2
Alanine/aspartate aminotransferase level elevations	1	0	0	1
Endocrinologic				
Hypoglycemic episodes	8*	4	16†	10
Osteoporotic fractures	0	0	0	0
≥0.5-point reduction in T-score in femoral neck	2	1	2	3
Diagnosis of adrenal carcinoma	0	0	1	0
Neurologic				
Dissociative amnesia	0	1	0	0
Newly diagnosed peripheric neuropathy	1	1	2	1
Cephalea/migraine	2	0	1	0
Dizziness	1	0	0	0
Insomnia	0	1	0	0
Gynecologic				
Ovarian cyst rupture	0	0	0	1
Uterine bleeding	0	1	0	0
Vaginal yeast infection	0	0	1	0
Urologic				
Diagnosis of bladder cancer	0	0	0	0
Diagnosis of prostate cancer	0	0	0	1
Urinary tract infection	1	1	1	1
Urine retention	0	0	2	0
Kidney stones	0	0	0	2
Hematologic				
Anemia	0	2	2	0
Thrombocytopenia	1	0	0	0
Other				
Biopsy-related	3	1	1	1
Motor vehicle accident	0	1	0	0
Perforation secondary to diverticulosis	0	0	1	0
Concussion	0	0	1	0

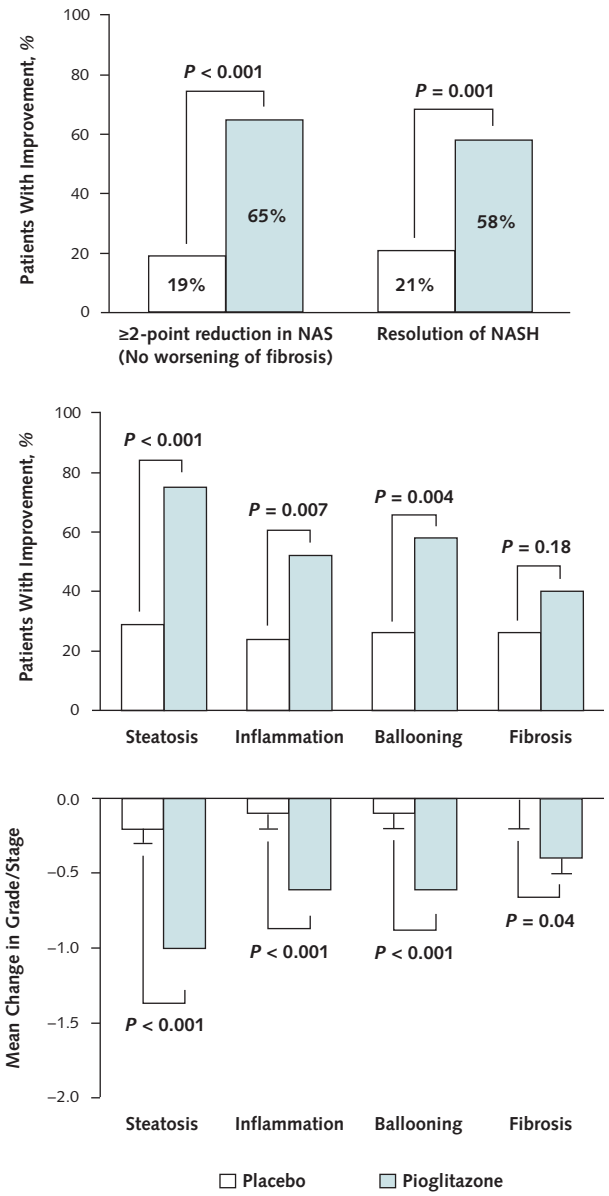
* 2 patients (both receiving glipizide and 1 also receiving insulin) had 3 episodes each.
† 1 patient (also receiving glipizide) had 7 episodes, whereas another (also receiving glyburide and insulin) had 4.

Appendix Figure 1. Histologic changes after 18 and 36 mo of pioglitazone treatment.



Data include 101 observations at baseline (51 in the placebo group and 50 in the pioglitazone group), 82 at month 18 (42 and 40, respectively), and 63 at month 36 (29 and 34, respectively). Bars represent 95% CIs.
* $P < 0.001$ for effect of pioglitazone vs. placebo.
† $P \leq 0.007$ for change in placebo group after starting open-label pioglitazone.
‡ $P < 0.05$ for effect of pioglitazone vs. placebo.

Appendix Figure 2. Histologic changes after 18 mo of pioglitazone (n = 40) or placebo (n = 42).



NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis.

Appendix Table 3. Response to Pioglitazone in Patients Who Completed 36 mo of Treatment*

Outcome	Patients Who Completed 36 mo of Pioglitazone (*n* = 34)						
	Before Therapy	After 18 mo of Therapy	Pioglitazone Effect: 0 vs. 18 mo (95% CI)	*P* Value (0 vs. 18 mo)	After 36 mo of Therapy	Pioglitazone Effect: 18 vs. 36 mo (95% CI)	*P* Value (18 vs. 36 mo)
Histologic							
Primary outcome, *n (%)*	–	23 (68)	68 (49 to 83)	–	22 (69)	−1 (−24 to 21)	0.92
Resolution of NASH, *n (%)*	–	20 (59)	59 (41 to 75)	–	19 (59)	0 (−24 to 23)	0.96
Mean NAS (SD)	4.5 (1.5)	1.9 (1.4)	−2.6 (−3.2 to −2.0)	<0.001	2.0 (1.5)	0.1 (−0.4 to 0.6)	0.70
Mean steatosis grade (SD)	2.0 (0.9)	0.8 (0.7)	−1.2 (−1.5 to −0.9)	<0.001	1.0 (0.8)	0.2 (−0.1 to 0.5)	0.184
Mean inflammation grade (SD)	1.7 (0.5)	0.9 (0.6)	−0.8 (−1.0 to −0.5)	<0.001	0.8 (0.6)	−0.1 (−0.3 to 0.2)	0.45
Mean ballooning grade (SD)	0.8 (0.4)	0.2 (0.4)	−0.6 (−0.8 to −0.4)	<0.001	0.2 (0.4)	0 (−0.2 to 0.2)	0.99
Mean fibrosis stage (SD)	1.0 (1.1)	0.6 (0.8)	−0.4 (−0.7 to −0.1)	0.007	0.7 (0.9)	0 (−0.2 to 0.3)	0.80
Metabolic							
Mean weight (SD), *kg*	99.0 (15.6)	101.1 (16.2)	2.2 (0.2 to 4.1)	0.029	102.1 (16.6)	0.9 (−1.2 to 3.1)	0.38
Mean body mass index (SD), kg/m^2	34.1 (4.4)	34.9 (4.8)	0.8 (0.1 to 1.5)	0.024	35.2 (4.8)	0.3 (−0.4 to 1.0)	0.40
Mean fasting plasma glucose level (SD)				<0.001			0.72
mmol/L	7.0 (1.7)	6.2 (0.8)	−0.8 (−1.3 to −0.4)		6.1 (0.8)	−0.1 (−0.4 to 0.3)	
mg/dL	126 (30)	111 (14)	−15 (−23 to −8)		110 (14)	−1 (−7 to 5)	
Mean hemoglobin A_{1c} level (SD), %	6.5 (1.0)	6.0 (0.6)	−0.5 (−0.8 to −0.2)	0.002	6.0 (0.7)	0 (−0.1 to 0.2)	0.63
Mean fasting plasma insulin level (SD)				<0.001			0.71
pmol/L	84 (66)	30 (24)	−54 (−78 to −30)		36 (30)	6 (−6 to 18)	
μU/mL	14 (11)	5 (4)	−9 (−13 to −5)		6 (5)	1 (−1 to 3)	
Mean fasting free fatty acid level (SD), *mmol/L*	0.50 (0.19)	0.39 (0.10)	−0.11 (−0.18 to −0.04)	0.005	0.45 (0.16)	0.06 (−0.01 to 0.13)	0.066
Mean adiponectin level (SD), *μg/mL*	6.7 (2.8)	23.0 (10.6)	16.3 (12.3 to 20.3)	<0.001	22.0 (11.3)	−1.0 (−5.3 to 3.3)	0.64
Mean triglyceride level (SD)				<0.001			0.068
mmol/L	2.2 (1.6)	1.4 (0.7)	−0.8 (−1.2 to −0.4)		1.2 (0.5)	−0.2 (−0.3 to 0)	
mg/dL	194 (144)	121 (64)	−73 (−109 to −36)		105 (43)	−15 (−31 to 1)	
Mean total cholesterol level (SD)				<0.001			0.30
mmol/L	4.6 (1.1)	3.9 (0.9)	−0.7 (−1.1 to −0.4)		3.7 (0.7)	−0.2 (−0.4 to 0.1)	
mg/dL	178 (42)	149 (34)	−29 (−41 to −17)		143 (29)	−6 (−17 to 5)	
Mean LDL cholesterol level (SD)				0.001			0.57
mmol/L	2.7 (1.0)	2.1 (0.7)	−0.6 (−0.9 to −0.3)		2.0 (0.7)	−0.1 (−0.3 to 0.2)	
mg/dL	104 (38)	81 (28)	−22 (−35 to −10)		78 (26)	−3 (−12 to 7)	
Mean HDL cholesterol level (SD)				<0.001			0.83
mmol/L	0.9 (0.2)	1.1 (0.3)	0.2 (0.2 to 0.3)		1.1 (0.3)	0 (−0.1 to 0.1)	
mg/dL	36 (9)	44 (11)	8 (6 to 10)		44 (10)	0 (−2 to 2)	
Mean aspartate aminotransferase level (SD), *U/L*	52 (29)	28 (10)	−24 (−35 to −14)	<0.001	27 (8)	−1 (−5 to 3)	0.63
Mean alanine aminotransferase level (SD), *U/L*	72 (42)	27 (12)	−45 (−58 to −31)	<0.001	27 (13)	0 (−5 to 4)	0.97
Mean cytokeratin-18 fragment level (SD), *U/L*	417 (375)	166 (155)	−251 (−401 to −102)	0.002	163 (108)	−3 (−73 to 67)	0.93

HDL = high-density lipoprotein; LDL = low-density lipoprotein; NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis.
* Patients originally randomly assigned to pioglitazone who continued its use during the open-label phase for a total of 36 mo of therapy.

Appendix Table 4. Response to Pioglitazone in Patients Who Completed 18 mo of Treatment*

Outcome	Patients Who Completed 18 mo of Pioglitazone (n = 70)			
	Before Therapy	After 18 mo of Therapy	Pioglitazone Effect (95% CI)	*P* Value
Histologic				
Primary outcome, *n (%)*	–	40 (60)	60 (47 to 72)	–
Resolution of NASH, *n (%)*	–	37 (55)	55 (43 to 67)	–
Mean NAS (SD)	4.5 (1.4)	2.6 (1.6)	−1.9 (−2.3 to −1.5)	<0.001
Mean steatosis grade (SD)	2.0 (0.9)	1.2 (0.9)	−0.8 (−1.0 to −0.6)	<0.001
Mean inflammation grade (SD)	1.7 (0.5)	1.1 (0.6)	−0.6 (−0.7 to −0.4)	<0.001
Mean ballooning grade (SD)	0.8 (0.4)	0.3 (0.5)	−0.6 (−0.7 to −0.4)	<0.001
Mean fibrosis stage (SD)	1.1 (1.1)	0.7 (0.9)	−0.4 (−0.6 to −0.2)	0.001
Metabolic				
Mean weight (SD), *kg*	99.3 (16.8)	102.8 (17.2)	3.4 (2.1 to 4.7)	<0.001
Mean body mass index (SD), kg/m^2	34.2 (4.8)	35.4 (5.3)	1.2 (0.8 to 1.7)	<0.001
Mean fasting plasma glucose level (SD)				<0.001
mmol/L	6.8 (1.5)	6.1 (0.7)	−0.8 (−1.1 to −0.4)	
mg/dL	123 (27)	109 (13)	−14 (−19 to −8)	
Mean plasma hemoglobin A_{1c} level (SD), *%*	6.3 (0.9)	5.9 (0.5)	−0.4 (−0.5 to −0.2)	<0.001
Mean fasting plasma insulin level (SD)				0.004
pmol/L	102 (84)	60 (108)	−42 (−66 to −12)	
μU/mL	17 (14)	10 (18)	−7 (−11 to −2)	
Mean fasting free fatty acid level (SD), *mmol/L*	0.46 (0.17)	0.41 (0.12)	0.05 (0 to 0.10)	0.048
Mean plasma adiponectin level (SD), *μg/mL*	7.5 (4.4)	21.8 (13.0)	14.4 (11.2 to 17.5)	<0.001
Mean triglyceride level (SD)				0.003
mmol/L	2.0 (1.3)	1.6 (0.9)	−0.4 (−0.7 to −0.1)	
mg/dL	178 (118)	140 (77)	−39 (−64 to −14)	
Mean total cholesterol level (SD)				0.011
mmol/L	4.3 (1.1)	3.9 (0.7)	−0.3 (−0.6 to −0.1)	
mg/dL	165 (43)	151 (29)	−13 (−23 to −3)	
Mean LDL cholesterol level (SD)				0.004
mmol/L	2.4 (1.0)	2.1 (0.6)	−0.3 (−0.6 to −0.1)	
mg/dL	93 (38)	80 (25)	−13 (−22 to −4)	
Mean HDL cholesterol level (SD)				<0.001
mmol/L	1.0 (0.2)	1.1 (0.3)	0.2 (0.1 to 0.2)	
mg/dL	37 (9)	44 (10)	6 (5 to 8)	
Mean plasma aspartate aminotransferase level (SD), *U/L*	48 (32)	29 (10)	−19 (−26 to −11)	<0.001
Mean plasma alanine aminotransferase level (SD), *U/L*	62 (39)	29 (14)	−32 (−41 to −23)	<0.001
Mean cytokeratin-18 fragment level (SD), *U/L*	342 (288)	206 (153)	−136 (−201 to −70)	<0.001

HDL = high-density lipoprotein; LDL = low-density lipoprotein; NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis.

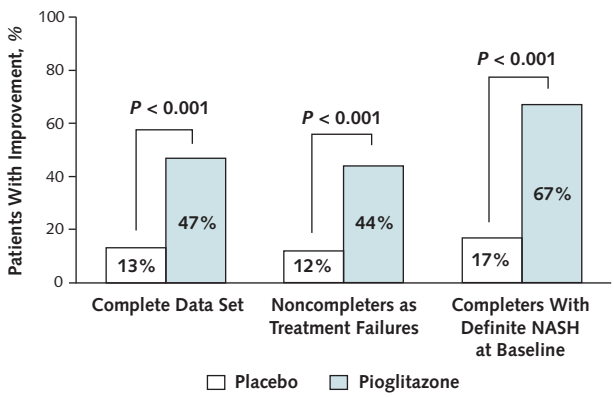
* Patients treated with pioglitazone when first randomly assigned (from 0–18 mo; n = 41) or after being initially assigned to placebo and later switched to pioglitazone during the open-label 18-to-36–mo period (n = 29).

Appendix Table 5. Sensitivity Analysis for the Effect of 18 mo of Pioglitazone Versus Placebo on the Primary Histologic Outcome, Using Various Scenarios

Variable	Placebo, *n/N (%)*	Pioglitazone, *n/N (%)*	Treatment Difference (95% CI), *percentage points*	*P* Value
Primary outcome				
Multiple imputation for missing data*	9/51 (17)	29/50 (58)	41 (23 to 59)	<0.001
Considering dropouts as treatment failures†	8/51 (16)	26/50 (52)	36 (19 to 53)	<0.001
Including only patients with complete data‡	8/42 (19)	26/40 (65)	46 (27 to 65)	<0.001
Only completers with definite NASH at baseline	7/36 (19)	25/33 (76)	56 (37 to 76)	<0.001
Worst-case scenario§	17/51 (33)	26/50 (52)	19 (0 to 38)	0.058
Best-case scenario‖	8/51 (16)	36/50 (72)	56 (40 to 72)	<0.001
Resolution of NASH				
Multiple imputation for missing data*	10/51 (19)	26/50 (51)	32 (13 to 51)	<0.001
Considering dropouts as treatment failures†	9/51 (18)	23/50 (46)	28 (11 to 46)	0.002
Including only patients with complete data‡	9/42 (21)	23/40 (58)	36 (16 to 56)	<0.001
Only completers with definite NASH at baseline	9/36 (25)	23/33 (70)	45 (24 to 66)	<0.001
Worst-case scenario§	18/51 (35)	23/50 (46)	11 (−8 to 30)	0.27
Best-case scenario‖	9/51 (18)	33/50 (66)	48 (32 to 65)	<0.001

NASH = nonalcoholic steatohepatitis.
* Primary analysis, with missing data on histologic outcomes imputed using a multiple imputation model (described in detail in the Appendix), was included for comparisons.
† Patients who did not complete 18 mo were imputed as "no improvement".
‡ Only completers were included (with biopsy performed before and after treatment).
§ Missing data were imputed as "no improvement" for patients randomly assigned to pioglitazone and as "improvement" for the placebo group.
‖ Missing data were imputed as "improvement" for patients randomly assigned to pioglitazone and as "no improvement" for the placebo group.

Appendix Figure 3. Response to pioglitazone or placebo at 18 mo, as defined in a prior trial of pioglitazone in nondiabetic patients (34).



"Response" was defined as improvement of ≥1 point in ballooning score, reduction of ≥2 points in NAS (with ≥1-point reduction in either steatosis or inflammation) or absolute NAS ≤3, and no worsening of fibrosis. Data include 101 observations for the complete data set and with noncompleters labeled as treatment failures (51 in the placebo group and 50 in the pioglitazone group) and 69 observations for completers with definite NASH at baseline (36 and 33, respectively). NAS = nonalcoholic fatty liver disease activity score; NASH = nonalcoholic steatohepatitis.