ORIGINAL ARTICLE

A Placebo-Controlled Trial of Pioglitazone in Subjects with Nonalcoholic Steatohepatitis

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ABSTRACT

BACKGROUND

No pharmacologic therapy has conclusively proved to be effective for the treatment of nonalcoholic steatohepatitis, which is characterized by insulin resistance, steatosis, and necroinflammation with or without centrilobular fibrosis. Pioglitazone is a thiazolidinedione that ameliorates insulin resistance and improves glucose and lipid metabolism in type 2 diabetes mellitus.

METHODS

We randomly assigned 55 patients with impaired glucose tolerance or type 2 diabetes and liver biopsy–confirmed nonalcoholic steatohepatitis to 6 months of treatment with a hypocaloric diet (a reduction of 500 kcal per day in relation to the calculated daily intake required to maintain body weight) plus pioglitazone (45 mg daily) or a hypocaloric diet plus placebo. Before and after treatment, we assessed hepatic histologic features, hepatic fat content by means of magnetic resonance spectroscopy, and glucose turnover during an oral glucose tolerance test ([^{14}C]glucose given with the oral glucose load and [^{3}H]glucose given by intravenous infusion).

RESULTS

Diet plus pioglitazone, as compared with diet plus placebo, improved glycemic control and glucose tolerance (P<0.001), normalized liver aminotransferase levels as it decreased plasma aspartate aminotransferase levels (by 40% vs. 21%, P=0.04), decreased alanine aminotransferase levels (by 58% vs. 34%, P<0.001), decreased hepatic fat content (by 54% vs. 0%, P<0.001), and increased hepatic insulin sensitivity (by 48% vs. 14%, P=0.008). Administration of pioglitazone, as compared with placebo, was associated with improvement in histologic findings with regard to steatosis (P=0.003), ballooning necrosis (P=0.02), and inflammation (P=0.008). Subjects in the pioglitazone group had a greater reduction in necroinflammation (85% vs. 38%, P=0.001), but the reduction in fibrosis did not differ significantly from that in the placebo group (P=0.08). Fatigue and mild lower-extremity edema developed in one subject who received pioglitazone; no other adverse events were observed.

CONCLUSIONS

In this proof-of-concept study, the administration of pioglitazone led to metabolic and histologic improvement in subjects with nonalcoholic steatohepatitis. Larger controlled trials of longer duration are warranted to assess the long-term clinical benefit of pioglitazone. (ClinicalTrials.gov number, NCT00227110.)

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NONALCOHOLIC STEATOHEPATITIS, A chronic liver condition that may progress to cirrhosis, is characterized by insulin resistance, the accumulation of hepatic fat, and predominantly lobular necroinflammation, with or without centrilobular fibrosis. The disorder is thought to be common, since the incidence of its typical features — fatty liver disease, obesity, and type 2 diabetes mellitus — is increasing.[1] Weight loss remains the standard of care because no pharmacologic therapy has conclusively proved to be effective for the treatment of this condition. Multiple pharmacologic interventions have been attempted with variable success; these include pentoxifilline,[2] orlistat,[3] vitamin E,[4-6] ursodeoxycholic acid,[7] and lipid-lowering agents.[8] Trials of glucose-lowering agents such as metformin[6,9] and thiazolidinediones[5,9-12] have yielded promising results, but to our knowledge, no randomized, placebo-controlled studies have provided conclusive support for their use.

Pioglitazone, a thiazolidinedione derivative, is a peroxisome proliferator–activated receptor γ (PPARγ) agonist that ameliorates insulin resistance and improves glucose and lipid metabolism in type 2 diabetes.[13,14] Insulin resistance in nonalcoholic steatohepatitis is frequently associated with chronic hyperinsulinemia, hyperglycemia, and an excessive supply of plasma free fatty acids to the liver; this metabolic milieu promotes hepatic lipogenesis.[15,16] Thiazolidinediones reverse these abnormalities by ameliorating insulin resistance in adipose tissues,[12,17] the liver,[9,10,12,13,17] and muscles.[9,10,12,13] Patients with nonalcoholic steatohepatitis have low plasma adiponectin levels[9,18,19] and adiponectin receptor expression in the liver.[20] Thiazolidinediones increase plasma adiponectin levels,[9,14,19] activate AMP-activated protein kinase,[17,21,22] stimulate fatty acid oxidation, and inhibit hepatic fatty acid synthesis.[15] In patients with nonalcoholic steatohepatitis, there is activation of the intracellular proinflammatory signaling pathways,[23,24] and thiazolidinediones have antiinflammatory effects.[15]

Thiazolidinediones may reverse many of the abnormalities associated with nonalcoholic steatohepatitis. We conducted a randomized, double-blind, placebo-controlled trial to determine whether pioglitazone plus a calorie-restricted diet as compared with placebo plus a calorie-restricted diet may reverse the metabolic and histologic abnormalities in patients with nonalcoholic steatohepatitis.

METHODS

SUBJECTS

We recruited study participants between October 2002 and November 2004 from the University of Texas Health Science Center; the Audie L. Murphy Division, South Texas Veterans Health Care System; and the Brooke Army Medical Center in San Antonio, Texas. After other causes of liver disease had been ruled out, the diagnosis of nonalcoholic steatohepatitis was confirmed in 70 subjects who underwent liver biopsy according to standard clinical indications for liver disease. Initial screening included a medical history, physical examination, routine blood tests, and a 75-g oral glucose tolerance test. Fifty-five subjects with impaired glucose tolerance or type 2 diabetes were enrolled. Twelve subjects were excluded because of normal results on the oral glucose tolerance test and three because of abnormal findings on laboratory tests. Subjects were also excluded if their levels of plasma aspartate aminotransferase and alanine aminotransferase were 2.5 times or more the upper limit of the normal range; if they had a history of heavy alcohol use (>12 to 15 g of alcohol per day, or >12 oz of beer, 5 oz of wine, or 1.5 oz of distilled spirits); if they had a fasting glucose level of 240 mg per deciliter (13.3 mmol per liter) or greater; if they had type 1 diabetes, heart disease, hepatic disease (other than nonalcoholic steatohepatitis), or renal disease; or if they were receiving metformin, thiazolidinediones, or insulin. Ten healthy subjects with normal glucose tolerance and without fatty liver served as controls. Written informed consent was obtained from all subjects before participation in the study, which was approved by the University of Texas Health Science Center at San Antonio.

STUDY DESIGN

During the 4-week run-in period, the subjects were interviewed by the research dietitian at the Frederic C. Bartter General Clinical Research Center and instructed not to change the calorie content of their diet or their level of physical activity. The subjects were given placebo pills, and compliance was assessed by means of a pill count on follow-up visits. Metabolic variables assessed at the general clinical research center at baseline and at the end of the study included hepatic fat content, measured by means of magnetic resonance spectroscopy; whole-body fat by means of dual-energy x-ray absorptiometry (Hologic); fasting plasma glucose,

glycated hemoglobin, lipid, insulin, free fatty acid, cytokine, and adiponectin concentrations; and insulin secretion, endogenous glucose production, and the rate of glucose disappearance after a 75-g oral glucose load by means of a double-tracer oral glucose tolerance test.

ASSESSMENTS AND TREATMENT

After an overnight fast, the subjects were admitted to the general clinical research center at 6 a.m. and [3-³H]glucose was given as a prime (25 μCi × [fasting plasma glucose in milligrams per deciliter ÷ 100]), followed by a continous infusion at a rate of 0.25 μCi per minute. This infusion was continued until the end of the study to measure glucose turnover. After a 3-hour isotopic equilibration period, four blood samples were drawn every 10 minutes for measurements of plasma [³H]glucose radioactivity and glucose, free fatty acid, and insulin concentrations. At 9:30 a.m., subjects received a 75-g oral glucose load labeled with [1-¹⁴C]glucose to quantitate rates of endogenous and exogenous glucose appearance and disappearance. Blood was drawn every 15 to 30 minutes for the next 4 hours to measure levels of plasma [¹⁴C]glucose and [³H]glucose radioactivity and concentrations of glucose, free fatty acids, and insulin.

On a separate day, the subjects reported to the Research Imaging Center at the University of Texas Health Science Center at San Antonio. Localized ¹H nuclear magnetic resonance spectroscopy of the liver was performed with a 1.9-Tesla magnetic resonance imaging scanner (Elscint Prestige), as previously described.[14]

Plasma glucose was measured with a glucose oxidase method (Beckman Instruments), insulin with a radioimmunoassay (Diagnostics Products), and free fatty acids with standard colorimetric methods. Plasma glucose radioactivity was measured from barium hydroxide and zinc sulfate–precipitated plasma extracts. Plasma adiponectin was measured by means of a radioimmunoassay (Linco Research).

Liver biopsy specimens were obtained before and after 6 months of treatment and were evaluated independently by two pathologists who were unaware of the subjects' identity, the timing of the biopsy (before or after treatment), the study group assignment, and all clinical information. Histopathologic changes in specimens before and after the treatment were determined with the use of the criteria of Kleiner et al.[25] The pathologist who served as the primary reader for each biopsy specimen had good-to-excellent intraobserver agreement between readings (weighted kappa coefficient, 0.84 for steatosis, 0.69 for necroinflammation, and 0.82 for fibrosis). The assessment by the second pathologist concurred with that of the primary pathologist for 83 of the 95 liver biopsy specimens. The two pathologists reviewed the 12 discordant assessments together to reach a consensus, and they remained unaware of the subjects' identity, the timing of the biopsy, the study group assignment, and all clinical information.

After baseline metabolic measurements had been performed, the research dietitian instructed the subjects to reduce their caloric intake by 500 kcal per day; this instruction was reinforced by the research dietitian during follow-up visits. The subjects were randomly assigned to receive either placebo or pioglitazone (Actos, Takeda Pharmaceuticals) at a dose of 30 mg per day, which was increased after 2 months to 45 mg per day until the end of the study. Randomization was computer-generated by the research pharmacy, and the investigators were unaware of the treatment assignments. Every 2 weeks, the subjects were seen at the general clinical research center. Vital signs and physical examination, the results of home glucose monitoring, compliance with the study drug (confirmed by pill count), and adverse events were assessed. Blood was drawn for liver aminotransferase levels and metabolic measurements. After 6 months of treatment, the liver biopsy, double-tracer oral glucose tolerance test, and measurements of body fat (by means of dual-energy absorptiometry) and hepatic fat content (by means of magnetic resonance spectroscopy) were repeated.

GLUCOSE TURNOVER CALCULATIONS

In the double-tracer oral glucose tolerance test, the endogenous glucose pool is labeled with [³H]glucose, whereas the oral load is labeled with [¹⁴C]glucose. The relative contribution of exogenous glucose (75 g of glucose given orally) and of endogenous glucose production (primarily by the liver) to the overall glucose excursion (the increase above baseline) during the oral glucose tolerance test was determined with the use of previously validated calculations.[26] In the basal state, where steady-state conditions prevail, the rate of glucose appearance equals the rate of glucose disappearance and represents the basal rate of endogenous glucose production, which is calculated

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by dividing the tracer infusion rate by the basal plasma [³H]glucose-specific activity. After the oral glucose load, when non–steady-state conditions prevailed, the rates of glucose appearance, disappearance, and clearance were calculated with the use of Steele's equation.[26]

Hepatic insulin sensitivity was calculated as the product of the rate of endogenous glucose production and the fasting plasma insulin level, as validated by our group.[27] There is a linear relationship between the rise in the fasting plasma insulin level from 5 to 25 μU per milliliter and the decline in the rate of endogenous glucose production. The higher the rate of endogenous glucose production and the level of fasting plasma insulin, the greater the severity of hepatic insulin resistance. The inverse of this product provides a measure of hepatic insulin sensitivity ([1 ÷ the rate of endogenous glucose production × the level of fasting plasma insulin] ×100), where 100 is a constant to obtain numbers between 0 and 15.

STATISTICAL ANALYSIS

Data were summarized in frequencies (or percentages) for categorical variables and as means ±SD for continuous variables. The chi-square (or Fisher's exact) test and a two-sample t-test were used to compare differences between the groups for categorical and continuous variables, respectively. Changes in continuous measures between basal and glucose tolerance periods were tested by means of a paired t-test, and the differences in these changes between groups were tested by two-way analysis of variance for repeated measures. Nonparametric methods were used for non-normally distributed values. For the histologic scores, weighted kappa coefficients were calculated to examine intraobserver agreement between the two readings by the primary pathologist. For the histologic scores, the Wilcoxon signed-rank test was used to evaluate changes between the baseline and post-treatment scores, and the Wilcoxon rank-sum test was used for the difference between groups. A two-tailed P value of less than 0.05 was considered to indicate statistical significance. Analyses were performed primarily with the use of SAS software for Windows 9.1 (SAS Institute). Takeda Pharmaceuticals North America provided pioglitazone and placebo tablets. This was an investigator-initiated proposal, and Takeda Pharmaceuticals had no direct or indirect involvement in the design of the trial, data collection, or preparation of the manuscript.

RESULTS

SUBJECT COMPLIANCE AND ADVERSE EVENTS

Compliance with the study medication was 95% or greater, with the exception of one subject who received pioglitazone (50% compliance). Seven subjects did not complete the study: one subject in the placebo group had liver aminotransferase levels that were more than 2.5 times the upper limit of the normal range during the run-in period and was withdrawn from the study; two subjects (one in each group) withdrew from the study within 1 week after the start of the study for personal reasons; two subjects (one in each group) withdrew because coronary artery disease was clinically suspected and later confirmed despite a normal electrocardiogram on enrollment; one subject in the placebo group had fatigue and withdrew at 4 weeks; and one subject in the pioglitazone group had fatigue associated with mild lower-extremity edema and was withdrawn at 9 weeks of treatment. Other than for this subject, no clinical or laboratory adverse events were associated with the use of pioglitazone.

BASELINE CLINICAL CHARACTERISTICS

The two groups were similar with respect to baseline clinical characteristics (Table 1) and had severe insulin resistance as suggested by a fasting insulin level that was four times that in the healthy controls (P<0.001). Despite an elevated level of fasting insulin, subjects with nonalcoholic steatohepatitis had a significantly elevated concentration of fasting plasma free fatty acids as compared with the healthy controls (P<0.001), indicating marked adipose-tissue insulin resistance (Table 1).

METABOLIC RESPONSE

The dietary intervention plus placebo had a limited effect on metabolic variables (Table 1). Twelve of 21 subjects lost weight, with an average 4% decrease in mean body fat (−3.2±0.5 kg of body weight). As compared with subjects who received placebo, subjects who received pioglitazone had a modest weight gain (2.5±0.5 kg) and an increase in body fat of 1.5±0.5% (P<0.01 for both comparisons) but also had improvement in all metabolic variables (Table 1). In the placebo group, fasting plasma insulin and free fatty acid levels were unchanged, whereas in the pioglitazone groups, the insulin concentration was reduced by 34% (P<0.001) and free fatty-acid levels by 17% (P=0.04), indicat-

Table 1. Baseline Characteristics of the Subjects and Outcome at 6 Months.*

Variable	Placebo (N=21)			Pioglitazone (N=26)			P Value (Pioglitazone vs. Placebo)†	Healthy Controls (N=10)‡
	Before Treatment	After Treatment	P Value	Before Treatment	After Treatment	P Value		
Age (yr)	51±10			51±7				45±13
Sex (M/F)	7/14			14/12				4/6
Body-mass index§	32.9±4.4	32.7±4.5	0.62	33.5±4.9	34.6±5.7	<0.001	0.005	24.5±3.8
Weight (kg)	90.2±15.4	89.7±14.8	0.53	93.7±18.1	96.2±19.6	<0.001	0.003	65.0±8.4
Body fat (%)	35.7±8.8	34.9±8.8	0.78	33.7±5.6	35.2±6.1	<0.01	0.005	28.2±9
Fasting plasma glucose (mg/dl)	115±28	116±31	0.75	119±35	99±16	0.004	0.011	88±8
Glycated hemoglobin (%)	6.2±1.1	6.1±0.9	0.73	6.2±1.5	5.5±0.8	<0.001	0.008	5.0±0.3
Fasting plasma insulin (μU/ml)	18±13	18±14	0.95	15±9	10±7	<0.001	<0.001	4±2
Fasting plasma free fatty acids (mmol/liter)	720±262	798±314	0.14	716±186	591±294	0.024	0.044	481±154¶
Free fatty acids during oral glucose tolerance test (mmol/liter)	410±166	413±280	0.49	372±165	276±201	0.095	0.042	153±71¶
Cholesterol (mg/dl)								
Total	189±41	191±41	0.62	188±33	193±36	0.48	0.79	211±54
Low-density lipoprotein	117±37	115±36	0.65	118±31	120±35	0.68	0.58	137±52
High-density lipoprotein	37±9	39±9	0.22	40±9	43±9	0.004	0.31	45±11
Triglycerides (mg/dl)	173±142	207±158	0.001	156±87	132±84	0.17	0.003	138±87
Aspartate aminotransferase (U/liter)	42±16	33±10	0.08	47±15	28±7	<0.001	0.04	21±5
Alanine aminotransferase (U/liter)	61±33	40±17	0.033	67±26	28±12	<0.001	<0.001	19±5

* Plus–minus values are means ±SD. To convert the values for glucose to millimoles per liter, multiply by 0.05551. To convert the values for insulin to picomoles per liter, multiply by 7.175. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.0113.
† P values are for the comparison between groups for the effect of treatment (change from baseline).
‡ P<0.01 for all comparisons with both groups before and after administration of the study drug except for total cholesterol and low-density lipoprotein, and except for high-density lipoprotein and triglycerides after pioglitazone treatment.
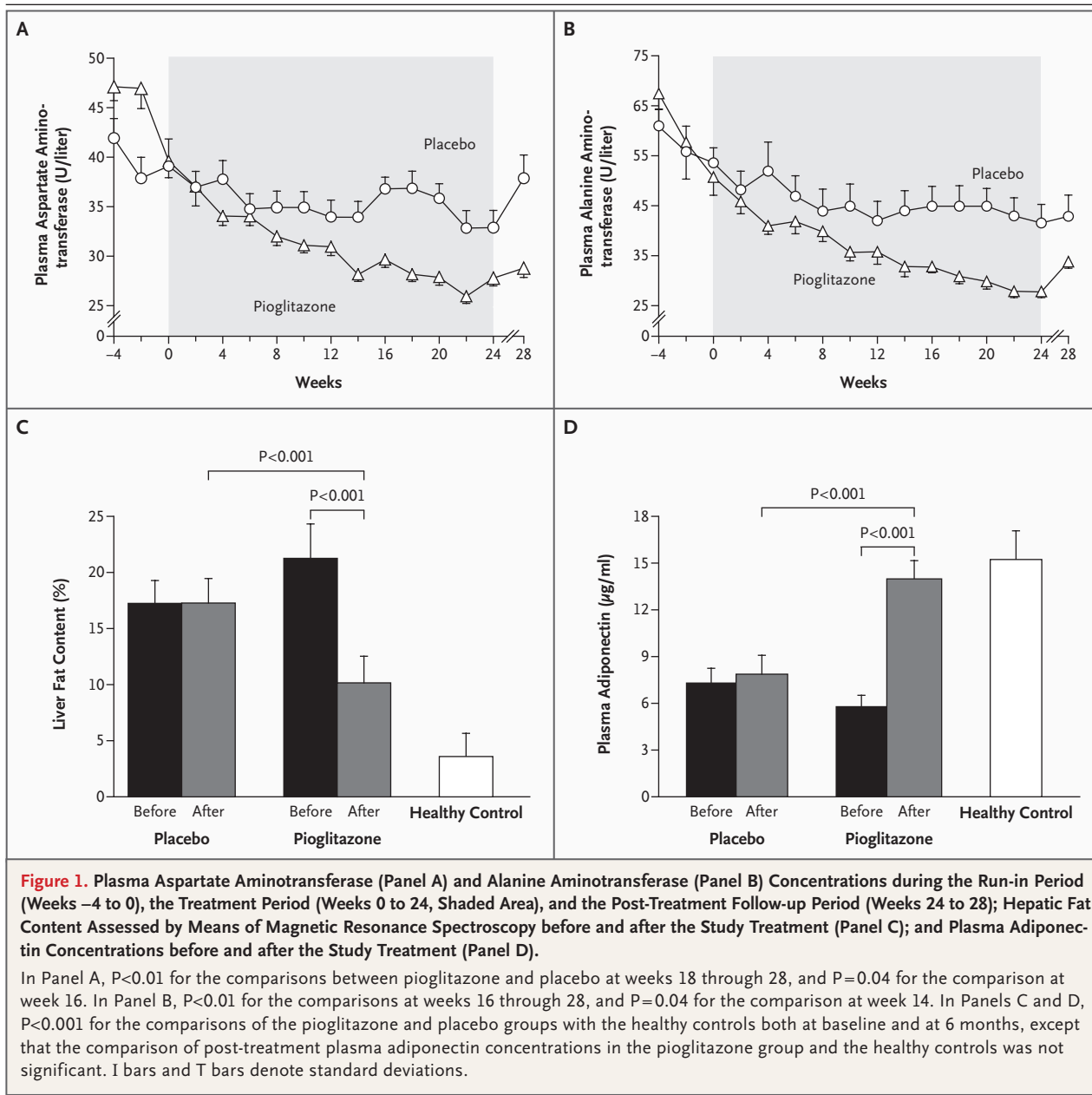§ The body-mass index is the weight in kilograms divided by the square of the height in meters.
¶ P=0.020 vs. placebo and before administration of pioglitazone.

ing improved insulin sensitivity in the peripheral and adipose tissues, respectively (Table 1).

Dietary intervention during the run-in period reduced the levels of aspartate aminotransferase and alanine aminotransferase in both groups (Fig. 1). A modest additional decrease in these levels in the placebo group was explained in part by weight reduction. In contrast, pioglitazone normalized liver aminotransferase levels: plasma aspartate aminotransferase levels decreased by 40% (from 47 to 28 U per liter) in patients who received pioglitazone, as compared with 21% (from 42 to 33 U per liter) in subjects who received placebo (P=0.04), and alanine aminotransferase lev

els were reduced by 58% (from 67 to 28 U per liter) in patients who received pioglitazone, as compared with 34% (from 61 to 40 U per liter) in subjects who received placebo (P<0.001). Normalization of aspartate aminotransferase and alanine aminotransferase levels correlated with improved hepatic insulin sensitivity (for aspartate aminotransferase, r=0.46, P=0.02; for alanine aminotransferase, r=0.40, P=0.05). Levels of tumor necrosis factor α (TNF-α) and transforming growth factor β (TGF-β) were similar at baseline in the two study groups. At 6 months, neither value changed significantly in the placebo group, whereas in the pioglitazone group, the TNF-α level decreased

Figure 1. Plasma Aspartate Aminotransferase (Panel A) and Alanine Aminotransferase (Panel B) Concentrations during the Run-in Period (Weeks –4 to 0), the Treatment Period (Weeks 0 to 24, Shaded Area), and the Post-Treatment Follow-up Period (Weeks 24 to 28); Hepatic Fat Content Assessed by Means of Magnetic Resonance Spectroscopy before and after the Study Treatment (Panel C); and Plasma Adiponectin Concentrations before and after the Study Treatment (Panel D).

In Panel A, P<0.01 for the comparisons between pioglitazone and placebo at weeks 18 through 28, and P=0.04 for the comparison at week 16. In Panel B, P<0.01 for the comparisons at weeks 16 through 28, and P=0.04 for the comparison at week 14. In Panels C and D, P<0.001 for the comparisons of the pioglitazone and placebo groups with the healthy controls both at baseline and at 6 months, except that the comparison of post-treatment plasma adiponectin concentrations in the pioglitazone group and the healthy controls was not significant. I bars and T bars denote standard deviations.

by 11% (P=0.02) and the TGF-β level decreased by 18% (P=0.03).

HEPATIC FAT CONTENT AND PLASMA ADIPONECTIN LEVELS

The mean hepatic fat content, as measured by means of magnetic resonance spectroscopy, was higher in subjects with nonalcoholic steatohepatitis than in healthy controls (19.5±3.1% vs. 3.0±0.7%, P<0.001). In the pioglitazone group, the hepatic fat content was reduced by 54% from baseline to

6 months, whereas it remained unchanged in the placebo group (P<0.001 for the comparison between the two groups) (Fig. 1).

At baseline, the mean plasma adiponectin levels were significantly lower in subjects with nonalcoholic steatohepatitis than in healthy controls (6.7± 1.2 vs. 14.4±1.7 μg per ml, P<0.001). At 6 months, the adiponectin level had increased by a factor of 2.3 in the pioglitazone group, whereas it remained unchanged in the placebo group (P<0.001 for the comparison between the two groups) (Fig. 1).

There was an inverse relationship between the reduction in hepatic fat content and the increase in the plasma adiponectin level (r=−0.60, P<0.001).

BASAL HEPATIC INSULIN SENSITIVITY AND GLUCOSE CLEARANCE

As compared with the dietary intervention plus placebo, pioglitazone decreased plasma glucose and insulin in the fasting state and during the oral glucose tolerance test (Fig. 2) in association with a 26% greater suppression of mean free fatty acids during the test (P=0.04) (Table 1). In subjects with nonalcoholic steatohepatitis, fasting hepatic insulin sensitivity and glucose clearance during the oral glucose tolerance test were 57% and 36% lower than those in controls, respectively (P<0.001 for both comparisons) (Fig. 2). In subjects who received pioglitazone, as compared with those who received placebo, improvement was observed in both hepatic insulin sensitivity (increased by 48% vs. 14%,

P=0.008) and glucose clearance during the oral glucose tolerance test (P<0.001), although the values remained lower than those in the controls with normal glucose tolerance (P<0.001) (Fig. 2).

HEPATIC HISTOLOGIC FINDINGS

In the placebo group, the only histologic improvement from baseline to 6 months was a reduction in inflammation (P=0.03) (Fig. 3). Five of the six subjects in the placebo group in whom inflammation decreased also lost weight (1.8 to 6.5 kg, P=0.18 for the change from baseline), but no such trend was observed for steatosis (P=0.71). In contrast, subjects who received pioglitazone had significant histologic improvements from baseline to 6 months in all variables (Fig. 3 and Table 2). The combined necroinflammation score improved in 85% of subjects who received pioglitazone, as compared with 38% of those who received placebo (P=0.001 for the comparison between the
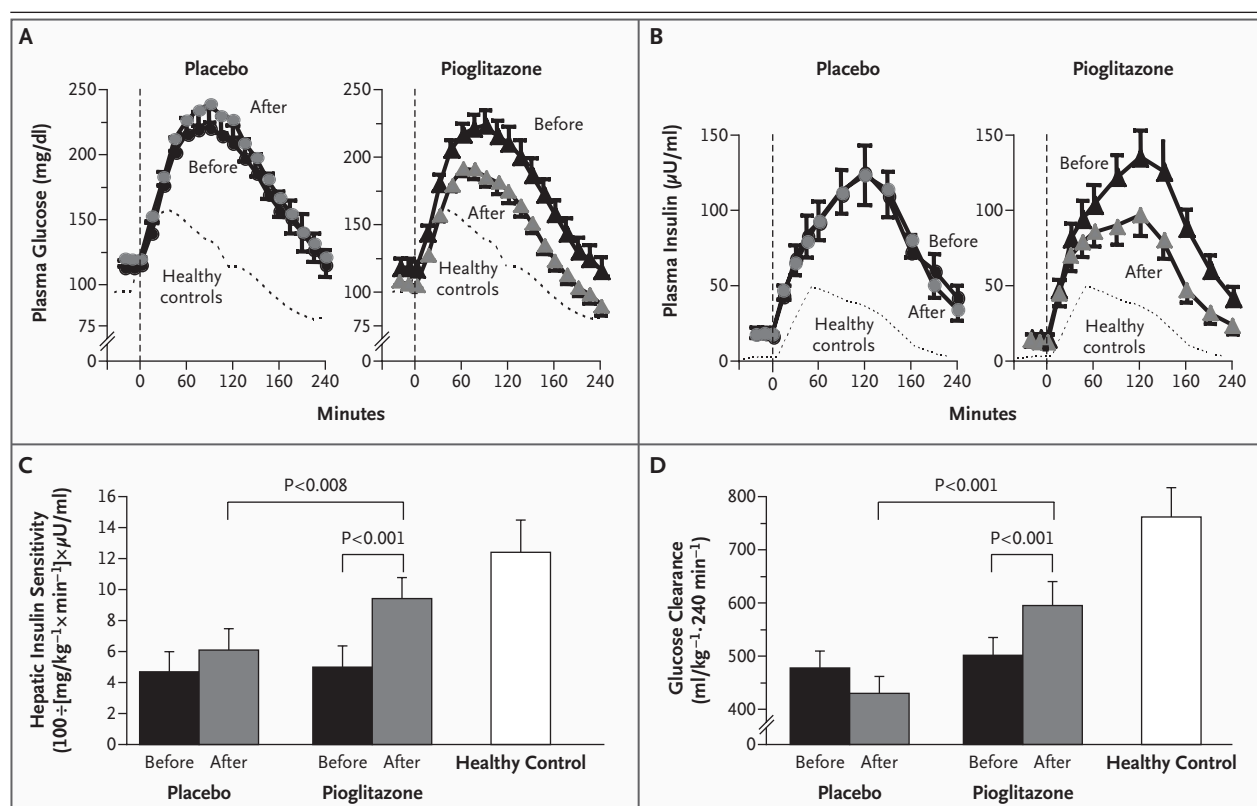

Figure 2. Plasma Glucose (Panel A) and Insulin (Panel B) Concentrations during a 75-g Oral Glucose Tolerance Test, Fasting Hepatic Insulin Sensitivity (Panel C), and Glucose Clearance during a 75-g Oral Glucose Tolerance Test (Panel D).

Fasting hepatic insulin sensitivity was calculated as 100 divided by the product of the rate of endogenous glucose production and the fasting plasma insulin concentration. All plasma glucose and insulin concentrations between 60 and 240 minutes differed significantly before and after treatment with pioglitazone (P<0.01). P<0.001 for the comparisons of the healthy controls with the placebo and pioglitazone groups at baseline and at 6 months. "Before" and "After" denote testing before and at the end of the 6-month treatment period.

groups). The fibrosis scores improved in the pio-glitazone group (P=0.002 for the comparison of scores before and after treatment), but the change from baseline did not differ significantly between the pioglitazone group and the placebo group (P=0.08).

DISCUSSION

Subjects with nonalcoholic steatohepatitis who received pioglitazone for 6 months had improved insulin sensitivity, a reversal of the metabolic milieu permissive of steatosis, and amelioration of cytokine-mediated systemic inflammation (i.e., re-duced plasma TNF-α and TGF-β levels). Increased hepatic insulin sensitivity and glucose clearance led to significant reductions in plasma free fatty acids, glucose, and insulin levels in the fasting state and during an oral glucose tolerance test. The histologic features of steatohepatitis (steato-sis, ballooning necrosis, and centrilobular inflam-mation) were reduced in subjects who received pio-glitazone as compared with those who received placebo, although fibrosis did not differ signifi-cantly between these two groups. Taken togeth-er, these results serve as "proof of concept" that pioglitazone has efficacy in patients with nonal-coholic steatohepatitis.
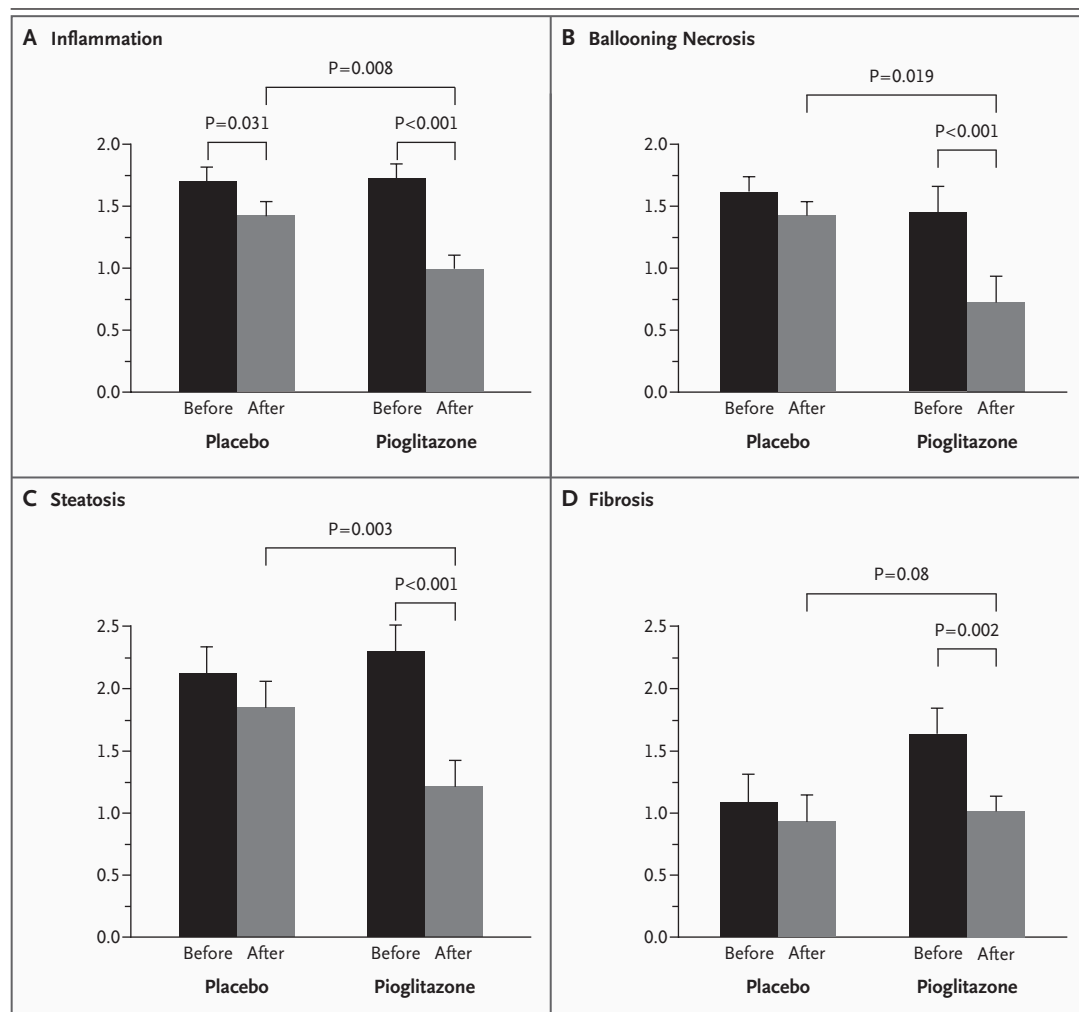

Figure 3. Mean Scores for Inflammation (Panel A), Ballooning Necrosis (Panel B), Steatosis (Panel C), and Fibrosis (Panel D) in Liver Biopsy Specimens.

One subject in the pioglitazone group declined to undergo the end-of-study liver biopsy (for that subject, only metabolic data were included). Between-group differences were compared by means of the Wilcoxon rank-sum test. Within-group differences (before vs. after treatment) were compared by means of the Wilcoxon signed-rank test.

Table 2. Hepatic Histologic Scores.*

Histologic Feature	Placebo			Pioglitazone			P Value (Pioglitazone vs. Placebo)†
	Before Treatment	After Treatment	P Value‡	Before Treatment	After Treatment	P Value‡	
Steatosis							
Score — no. of subjects							
0 (<5%)	0	2		0	6		
1 (5–33%)	7	6		5	12		
2 (>33–66%)	4	6		8	4		
3 (>66%)	10	7		13	4		
Improvement — %		38	0.11		65	<0.001	0.003
Patients with a reduction in score of ≥2 — no./total no. (%)		0/14 (0)			9/21 (43)		0.004§
Ballooning necrosis							
Score — no. of subjects							
0 (None)	0	1		4	13		
1 (Few balloon cells)	8	10		6	7		
2 (Many balloon cells)	13	10		16	6		
Improvement — %		24	0.36		54	<0.001	0.02
Lobular inflammation							
Score — no. of subjects							
0 (0 or 1 focus)	0	0		0	6		
1 (2–4 foci per 200× field)	6	12		5	14		
2 (>4 foci per 200× field)	15	9		21	6		
Improvement — %		29	0.03		65	<0.001	0.008
Combined necroinflammation							
Improvement in score — %		38	0.03		85	<0.001	0.001
Patients with a reduction in score of ≥2 — no./total no. (%)		3/21 (14)			11/24 (46)		0.02§
Fibrosis							
Score — no. of subjects							
0 (None)	6	8		2	5		
1 (Perisinusoidal or periportal)	9	7		12	15		
2 (Perisinusoidal and portal or periportal)	4	3		5	6		
3 (Bridging fibrosis)	2	1		7	0		
4 (Cirrhosis)	0	1		0	0		
Improvement — %		33	0.61		46	0.002	0.08
Patients with a reduction in score of ≥2 — no./total no. (%)		1/6 (17)			5/12 (42)		0.31§

* The scores were calculated according to the criteria of Kleiner et al.[25]
† The between-group comparison for the effect of treatment (change from baseline) was performed with the Wilcoxon rank-sum test.
‡ The P values were calculated with the Wilcoxon signed-rank test.
§ The between-group comparison for the effect of treatment was performed with Fisher's exact test.

The reduction in plasma alanine aminotransferase levels with dietary intervention and placebo (Fig. 1) is consistent with the findings of previous studies,[28,29] although most subjects who received placebo and who had normalized levels of alanine aminotransferase continued to have severe histologic abnormalities. This indicates that plasma alanine aminotransferase per se cannot be used as a surrogate marker to monitor response and highlights the importance of placebo-controlled studies in the assessment of pharmacologic interventions in nonalcoholic steatohepatitis. Progression of fibrosis may occur even in the presence of normal plasma alanine aminotransferase concentrations.[30,31] There were no significant improvements in steatosis, ballooning necrosis, or fibrosis in the subgroup of 12 subjects who lost a mean of 3.2±0.5 kg in body weight, findings that are consistent with the varying results of weight reduction in patients with nonalcoholic steatohepatitis, according to a recent meta-analysis.[28] Although greater weight loss might have decreased hepatic steatosis in our patients,[18] we did not encourage drastic weight reduction because of difficulty with compliance and reports that it may worsen inflammation[32,33] and even fibrosis.[32]

By improving the insulin sensitivity of adipose tissue, thiazolidinediones reduce excessive rates of lipolysis[17] and substrate supply to the liver. Amelioration of hepatic and peripheral insulin resistance[9,10,12,13,17] also mitigates hepatic lipid synthesis, since lower plasma insulin and glucose levels reduce sterol regulatory element-binding protein 1c and carbohydrate response-element–binding protein activity in the liver, respectively.[15] Pioglitazone,[17] as well as rosiglitazone[21] and metformin,[21,34] stimulate AMP-activated protein kinase, a pathway that inhibits hepatic lipogenic enzymes. Adiponectin activates AMP-activated protein kinase[22] and is thought to be an important mediator of the metabolic effects of thiazolidinediones in the liver. When adiponectin is replaced in mice models of nonalcoholic steatohepatitis, there is a marked reduction in steatosis.[35] In patients with nonalcoholic steatohepatitis, plasma adiponectin levels are decreased,[9,18,19] and they increase by a factor of 2 to 3 with thiazolidinedione therapy.[9,14,19] Thus, treatment of patients with both type 2 diabetes and nonalcoholic steatohepatitis may be particularly challenging, because weight loss[18,19] or metformin therapy[9] does not increase adiponectin levels, and administration of insulin may increase steatosis.[36]

Although in our study, treatment with pioglitazone led to clear metabolic and histologic improvements (reductions in steatosis, inflammation, and ballooning necrosis), it did not significantly reduce fibrosis as compared with placebo plus dietary intervention. The issue is complicated not only by the short duration of the study and the small number of subjects, but also by the known variations in assessment of hepatic fibrosis by means of percutaneous biopsy.[37] Earlier, uncontrolled studies in patients with nonalcoholic steatohepatitis showed marginal histologic improvement with troglitazone[11] but promising results with rosiglitazone[10] and pioglitazone.[12] Recently, pioglitazone has been reported to prevent, in a dose-dependent manner, the activation of hepatic stellate cells (mediators of fibrosis) in an animal model of hepatic fibrosis.[38] Because fibrosis may progress in up to one third of patients with nonalcoholic steatohepatitis,[31] with obese patients who have type 2 diabetes at the greatest risk for progression,[30,31] larger clinical trials of longer duration are needed to determine the long-term efficacy and safety of pioglitazone for patients with nonalcoholic steatohepatitis and to gain a better understanding of the mechanisms involved during thiazolidinedione therapy.

REFERENCES

1. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387-95.

2. Adams LA, Zein CO, Angulo P, Lindor KD. A pilot trial of pentoxifylline in nonalcoholic steatohepatitis. Am J Gastroenterol 2004;99:2365-8.

3. Harrison SA, Fincke C, Helinski D, Torgerson S, Hayashi P. A pilot study of orlistat treatment in obese, non-alcoholic steatohepatitis patients. Aliment Pharmacol Ther 2004;20:623-8.

4. Harrison SA, Torgerson S, Hayashi P,

Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2003;98:2485-90.

5. Sanyal AJ, Mofrad PS, Contos MJ, et al. A pilot study of vitamin E versus vitamin E and pioglitazone for the treatment of nonalcoholic steatohepatitis. Clin Gastroenterol Hepatol 2004;2:1107-15.

6. Bugianesi E, Gentilcore E, Manini R, et al. A randomized controlled trial of metformin versus vitamin E or prescriptive diet in nonalcoholic fatty liver disease. Am J Gastroenterol 2005;100:1082-90.

7. Lindor KD, Kowdley KV, Heathcote EJ, et al. Ursodeoxycholic acid for treatment of nonalcoholic steatohepatitis: results of a randomized trial. Hepatology 2004;39:770-8.

8. Merat S, Malekzadeh R, Sohrabi MR, et al. Probucol in the treatment of nonalcoholic steatohepatitis: an open-labeled study. J Clin Gastroenterol 2003;36:266-8.

9. Tiikkainen M, Hakkinen A-M, Korsheninnikova E, Nyman T, Makimattila S, Yki-Jarvinen H. Effects of rosiglitazone and metformin on liver fat content, hepatic insulin resistance, insulin clearance, and gene expression in adipose tissue in patients with type 2 diabetes. Diabetes 2004; 53:2169-76.

10. Neuschwander-Tetri BA, Brunt EM, Wehmeier KR, Oliver B, Bacon BR. Improved nonalcoholic steatohepatitis after 48 weeks of treatment with the PPAR-gamma ligand rosiglitazone. Hepatology 2003;38:1008-17.

11. 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.

12. Promrat K, Lutchman G, Uwaifo GI, et al. A pilot study of pioglitazone treatment for nonalcoholic steatohepatitis. Hepatology 2004;39:188-96.

13. Miyazaki Y, Mahankali A, Matsuda M, et al. Effect of pioglitazone on abdominal fat distribution and insulin sensitivity in type 2 diabetic patients. J Clin Endocrinol Metab 2002;87:2784-91.

14. Bajaj M, Suraamornkul S, Piper P, et al. Decreased plasma adiponectin concentrations are closely related to hepatic fat content and hepatic insulin resistance in pioglitazone-treated type 2 diabetic patients. J Clin Endocrinol Metab 2004;89:200-6.

15. Browning JD, Horton JD. Molecular mediators of hepatic steatosis and liver injury. J Clin Invest 2004;114:147-52.

16. Bugianesi E, Gastaldelli A, Vanni E, et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia 2005; 48:634-42.

17. Saha AK, Avilucea PR, Ye JM, Assifi MM, Kraegen EW, Ruderman NB. Pioglitazone treatment activates AMP-activated protein kinase in rat liver and adipose tissue in vivo. Biochem Biophys Res Commun 2004; 314:580-5.

18. Petersen KF, Dufour S, Befroy D, Lehrke M, Hendler RE, Shulman GI. Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. Diabetes 2005;54:603-8.

19. Abbasi F, Chang S-A, Chu JW, et al. Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. Am J Physiol Regul Integr Comp Physiol 2006;290:R139-R144.

20. Kaser S, Moschen A, Cayon A, et al. Adiponectin and its receptors in non-alcoholic steatohepatitis. Gut 2005;54:117-21.

21. Fryer LGD, Parbu-Patel A, Carling D. The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem 2002;277:25226-32.

22. Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.

23. Samuel VT, Liu Z-X, Qu X, et al. Mechanism of hepatic insulin resistance in nonalcoholic fatty liver disease. J Biol Chem 2004;279:32345-53.

24. Ribeiro PS, Cortez-Pinto H, Sola S, et al. Hepatocyte apoptosis, expression of death receptors, and activation of NF-kappaB in the liver of nonalcoholic and alcoholic steatohepatitis patients. Am J Gastroenterol 2004;99:1708-17.

25. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313-21.

26. Ferrannini E, Simonson DC, Katz LD, et al. The disposal of an oral glucose load in patients with non-insulin-dependent diabetes. Metabolism 1988;37:79-85.

27. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-70.

28. Wang RT, Koretz RL, Yee HF Jr. Is weight reduction an effective therapy for nonalcoholic fatty liver? A systematic review. Am J Med 2003;115:554-9.

29. Hickman IJ, Jonsson JR, Prins JB, et al. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. Gut 2004;53:413-9.

30. Mofrad P, Contos MJ, Haque M, et al. Clinical and histologic spectrum of nonalcoholic fatty liver disease associated with normal ALT values. Hepatology 2003;37: 1286-92.

31. Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132-8.

32. Andersen T, Gluud C, Franzmann MB, Christoffersen P. Hepatic-effects of dietary weight loss in morbidly obese subjects. J Hepatol 1991;12:224-9.

33. Luyckx FH, Desaive C, Thiry A, et al. Liver abnormalities in severely obese subjects: effects of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord 1998;22:222-6.

34. Zhou G, Myers R, Li Y, et al. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest 2001;108:1167-74.

35. Xu A, Wang Y, Keshaw H, Xu L, Lam KS, Cooper GJ. The fat-derived hormone adiponectin alleviates alcoholic and non-alcoholic fatty liver diseases in mice. J Clin Invest 2003;112:91-100.

36. Anderwald C, Bernroider E, Krssak M, et al. Effects of insulin treatment in type 2 diabetic patients on intracellular lipid content in liver and skeletal muscle. Diabetes 2002;51:3025-32.

37. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128:1898-906.

38. Kawaguchi K, Sakaida I, Tsuchiya M, Omori K, Takami T, Okita K. Pioglitazone prevents hepatic steatosis, fibrosis, and enzyme-altered lesions in rat liver cirrhosis induced by a choline-deficient L-amino acid-defined diet. Biochem Biophys Res Commun 2004;315:187-95.

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