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Relationship between Adipose Tissue Insulin Resistance and Liver Histology in NASH: A PIVENS Follow-Up Study

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Abstract

The PIVENS [Pioglitazone versus Vitamin E versus Placebo for the Treatment of Non-diabetic Patients with Nonalcoholic Steatohepatitis (NASH)] trial demonstrated that pioglitazone and vitamin E improved liver histology to varying degrees but mechanisms are unknown. We conducted a study to examine the changes in adipose tissue insulin resistance (Adipo-IR) during the PIVENS trial and its relationship to histological end points. Adipo-IR [fasting non-esterified fatty acids (NEFA) × fasting insulin] was calculated at baseline and after 16 and 96 weeks of therapy. Compared to placebo, the baseline Adipo-IR was not different in either vitamin E group (p=0.34) or pioglitazone group (p=0.29).). Baseline Adipo-IR was significantly associated with fibrosis score (p=0.017) but not with other histological features or NAFLD activity score (NAS). After 16 weeks, compared to placebo, the pioglitazone group had significant reduction in Adipo-IR (−15.7 vs. −1.91, p=0.02) but this effect did not persist at 96 weeks (−3.25 vs. −4.28, p=0.31). Compared to placebo, Adipo-IR in the vitamin E group did not change significantly either after 16 weeks (p=0.70) or after 96 weeks (p=0.85). Change in Adipo-IR at week 16 was not associated with changes in any histological parameters at week 96, but improvement in Adipo-IR at week 96 was significantly associated with improvement in ballooning (p=0.02), fibrosis (p=0.004), and NAFLD activity score (p=0.01).

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Conclusion—Vitamin E improved liver histology independent of changes in Adipo-IR, and pioglitazone treatment acutely improved Adipo-IR but this was not sustained. Changes in Adipo-IR were associated with changes in liver histology, including fibrosis.

Keywords

Nonalcoholic steatohepatitis; vitamin E; pioglitazone; insulin resistance; adipose tissue

Introduction

Despite the increasing incidence of nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH), there are no therapies currently approved for treatment of these common liver disorders. Recently published results from the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) clinical trial showed that pioglitazone and vitamin E improved liver histology to varying degrees (1). Of particular interest is the fact that pioglitazone and vitamin E may target different mechanistic pathways (insulin sensitivity and oxidative stress, respectively) implicated in the pathogenesis of NASH (2–4), but liver histology improved in both treatment groups. Furthermore, although insulin resistance is thought to be directly involved in the development of NASH, liver histology improved in patients taking vitamin E despite no change in general insulin sensitivity as measured by Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) index (1). Delineation of mechanisms involved in the development, progression, and improvement of NASH are clearly needed to better understand natural history and treatment options for this increasingly prevalent disease.

While hepatic and muscle insulin resistance are established hallmark features of NAFLD and NASH (2, 3, 5–9), a report by Gastaldelli *et al.* highlighted an additional component, adipose tissue insulin resistance (Adipo-IR), as an important contributor to the pathogenesis and treatment of NASH (10). In this study, patients with NASH had elevated fasting concentrations of non-esterified fatty acids (NEFA) compared to control subjects, and an index of Adipo-IR, calculated as the product of fasting NEFA×fasting insulin (7, 10), was significantly elevated in NASH patients independent of the degree of obesity. Interestingly, treatment with pioglitazone decreased NEFA concentrations, resulting in a significant reduction in Adipo-IR, and decreases in Adipo-IR were correlated with improvements in hepatic steatosis and necroinflammation. Overall, this study suggests a central role for reduction of adipose tissue insulin resistance in pioglitazone-induced improvement of liver histology in patients with NASH.

The intriguing relationship between changes in adipose tissue insulin resistance and improvements in liver histology (10) led us to investigate Adipo-IR in patients who participated in the PIVENS clinical trial. Specifically, the aims of this study were to: (1) examine the predictors of Adipo-IR at baseline and after 96 weeks, (2) assess changes in Adipo-IR in the placebo, pioglitazone, and vitamin E groups after 16 and 96 weeks of therapy, and (3) determine if early (after 16 weeks) or long term (after 96 weeks) changes in Adipo-IR correlate with changes in liver histology.

Patients and Methods

Study Design

Data included in this analysis were obtained from patients who participated in the PIVENS trial, an adult treatment trial conducted by the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) (1). PIVENS study design has been described previously

(11) and the Clinical Trials.gov identifier is NCT00063622. Briefly, this study investigated the efficacy of pioglitazone or vitamin E for improving liver histology in patients with NASH. Patients enrolled in the PIVENS trial had to be ≥18 years of age and have a histological diagnosis of NASH without cirrhosis (liver biopsy within 6 months of randomization was required). Patients were excluded if they had diabetes, consumed >20 g alcohol/day if female or >30 g alcohol/day if male, had any other form of chronic liver disease besides NASH, took medications known to possibly cause or affect NASH (for example, methotrexate or tamoxifen), used fluctuating doses of lipid-lowering medications, had alanine aminotransferase (ALT) levels >300 U/L or serum creatinine levels ≥2.0 mg/dl, or were females of childbearing age and were pregnant, nursing, or unwilling to use birth control.

The study protocol and informed consent statements were approved by the NASH CRN Steering Committee and by the Institutional Review Boards at each participating site. Patients who met all eligibility criteria gave written informed consent prior to participation in the study, and were randomized to receive pioglitazone (30 mg Actos® once daily), vitamin E (800 IU 100% natural RRR-α-tocopherol once daily) or placebo for 96 weeks. Patients underwent an end-of-treatment liver biopsy after 96 weeks of therapy.

Evaluation of Liver Histology

Baseline and 96-week liver biopsy specimens were formalin-fixed, paraffin-embedded, and unstained slides were cut from tissue blocks and sent to the NASH CRN repository for preparation by a central laboratory and review by the NASH CRN Pathology Committee (10 hepatopathologists blinded to all clinical and group assignment data). Scoring of biopsies was done by consensus according to the NAFLD activity score (NAS) and fibrosis score previously published by the NASH CRN (12).

Laboratory Analyses and Calculation of Indices

Blood was collected into serum separator tubes, allowed to clot for at least 30 min at room temperature, and centrifuged at 1800 × g for 15 min at 4°C. Serum was aliquoted (0.5 ml) and immediately frozen at −70°C. All processing was completed within two hours and samples were free of hemolysis. Routine measurement of fasting insulin and glucose concentrations was carried out at each study site. Fasting serum nonesterified fatty acid (NEFA) concentrations were measured by colorimetric assay (Free Fatty Acids Half Micro Test, Roche Diagnostics Corporation, Indianapolis, IN). Adipo-IR index was calculated as: [fasting NEFA (mM) × fasting insulin (pM)] and HOMA-IR index was calculated as: [fasting glucose (mg/dl) × fasting insulin (μU/ml)] ÷ 405.

Statistical Analyses

Means and standard deviations (SD) were used to show variations across treatment and other subgroups in Adipo-IR, insulin, and NEFA at baseline and follow-up visits. Multiple linear regression models for the outcome measure, log-transformed Adipo-IR index included two indicator variables to represent the separate effects of vitamin E and pioglitazone versus placebo together with other potential confounders: gender, ethnicity (Hispanic vs. non-Hispanic), age at randomization, BMI, waist circumference, total body fat, AST, ALT, glucose, triglycerides, total cholesterol, HDL cholesterol, and histological scores (steatosis, lobular inflammation, hepatocyte ballooning, fibrosis, and the NAFLD Activity Score [NAS]) (12). The log-transform was necessary to correct for the right-skewness in the Adipo-IR index. These regression models were used to assess cross-sectional correlates of the Adipo-IR index at baseline and after 96-weeks of follow-up. Regression models were also used to examine changes in the Adipo-IR index at 16 and 96 weeks in relation to vitamin E and pioglitazone and other potential correlates of change. Two multiple linear

regression models of log-transformed ratios of the Adipo-IR index at 16 weeks vs. baseline and at 96 weeks vs. baseline were used to assess the direction, magnitude, and statistical significance of associations of changes in the Adipo-IR index at each of the two time points with corresponding changes in liver histology at 96 weeks, BMI (16 and 96 weeks), and ALT (16 and 96 weeks), controlling for baseline Adipo-IR, ethnicity, and baseline BMI. Changes in Adipo-IR index from baseline to 16 and 96 weeks were expressed as ratios (rather than absolute change) and analyzed on a log scale to improve normality of the outcome measures used in the linear regression models. Consequently, we reported exponentiated regression coefficients, which are more easily interpretable as ratios of geometric means of the Adipo-IR index per unit change in a given independent variable in the model for otherwise similar patients. All analyses were carried out using SAS 9.2 (SAS Institute, Cary, NC) and Stata 11 (Stata Corp., College Station, TX). Nominal, two-sided *p*-values were used and were considered statistically significant if $p<0.05$.

Results

Study Population

As reported previously, all patients included in this study were previously characterized in the original publication of the PIVENS clinical trial (1). Patients were well-matched across the placebo, pioglitazone, and vitamin E groups at baseline, as there were no significant differences in demographics/anthropometric factors, liver biochemistries, metabolic and lipid profile parameters, or histological features. Effects of pioglitazone and vitamin E therapy on liver histology [as assessed by a composite of standardized scores for histological features (primary outcome) as well as scores for individual components], liver biochemistries, lipid panels, and metabolic factors, compared to placebo, have been previously described in detail (1). Briefly, vitamin E (43% of patients met the primary outcome; $p=0.001$) but not pioglitazone (34% of patients met the primary outcome; $p=0.04$) was superior to placebo (19% of patients met the primary outcome) for treatment of NASH; however, both pioglitazone and vitamin E significantly improved steatosis and lobular inflammation (both $p\leq0.02$) and significantly reduced serum ALT compared to placebo ($p<0.001$). Neither pioglitazone nor vitamin E improved fibrosis scores. Although pioglitazone was beneficial for metabolic endpoints, patients gained significantly more weight ($p<0.001$) compared to the placebo group. As expected, 96 weeks of pioglitazone treatment significantly improved insulin resistance ($p=0.03$; as assessed by HOMA-IR), but improvements in liver histology induced by vitamin E treatment were independent of changes in HOMA-IR.

Changes in Adipo-IR during the PIVENS Clinical Trial

Table 1 shows Adipo-IR index in 3 treatment groups at baseline and at 16 and 96 weeks. Compared to placebo, the baseline Adipo-IR was not different in either vitamin E group ($p=0.34$) or pioglitazone group ($p=0.29$). After 16 weeks, compared to placebo, the pioglitazone group had significant reduction in Adipo-IR (−15.7 vs. −1.91, p=0.02). However, this effect did not persist at 96 weeks as mean Adipo-IR (placebo 61.5 ± 81.5 vs. pioglitazone 48.2 ± 49.0, $p=0.25$) or its mean change from baseline (placebo −4.28 vs. pioglitazone −3.25, p=0.31) (Figure 1; **Panel A**). Compared to placebo, Adipo-IR in the vitamin E group did not change significantly either after 16 weeks or after 96 weeks (Table 1, Figure 1). Baseline NEFA and fasting insulin concentrations as well as their values after 16 and 96 weeks are shown in Table 1.

Cross-sectional Correlates of Adipo-IR

The correlates of baseline Adipo-IR from the multiple linear regression analysis are shown in Table 2. Higher BMI, fibrosis score ≥ 3, and higher serum glucose had significant

positive association with baseline Adipo-IR. The cross-sectional relationship between Adipo-IR at 96 weeks and demographic, anthropometric, laboratory, and histological variables are shown in Table 2. Higher BMI, female gender, and higher cholesterol levels had significant positive association with Adipo-IR at week 96.

Relationship between Changes in Adipo-IR and Changes in Liver Histology

In the multiple linear regression analysis of log-transformed Adipo-IR, after adjusting for baseline BMI, baseline Adipo-IR, treatment, and ethnicity, there were no significant relationships between change in Adipo-IR at 16 weeks and changes in liver histology between baseline and 96 weeks or change in ALT either at 16 or 96 weeks (Table 3). However, Table 3 also shows that, while there was no significant association between change in Adipo-IR after 96 weeks and the primary histological outcome of the PIVENS trial or change in ALT, there were significant associations between improvement in Adipo-IR after 96 weeks and histologic measures: improvement in ballooning (p=0.03), NAS (p=0.01), and fibrosis (p=0.004). There was also a strong association between increase in BMI and increase in Adipo-IR from baseline to week 96.

Discussion

Our study adds significantly to the growing body of literature investigating the significance of Adipo-IR in patients with NAFLD and NASH. Gastaldelli et al. initially described the characteristics of Adipo-IR in 47 subjects with NASH and 20 non-diabetic controls who participated in a randomized controlled trial that demonstrated the efficacy of pioglitazone administered for 6 months in improving liver histology in NASH (10). In this study, there was a strong relationship between Adipo-IR and NASH that was independent of degree of obesity and pioglitazone reduced Adipo-IR significantly in participants with NASH. Interestingly, improvement in Adipo-IR was closely associated with an improvement in steatosis and necroinflammation, suggesting that pioglitazone improves NASH through its effect on dysfunctional adipose tissue. More recently, Lomonaco et al. measured Adipo-IR in 207 individuals with NAFLD and 20 controls without NAFLD (13). This study not only confirmed the strong association between severe Adipo-IR and NAFLD, but showed a strong relationship between Adipo-IR and advanced liver fibrosis. Individuals with Adipo-IR in top two quartiles had more severe liver fibrosis compared to individuals with Adipo-IR in bottom two quartiles. Our study differed from these two studies in that it consisted of a larger sample size which was followed for a much longer treatment period and also examined the characteristics of Adipo-IR in vitamin E treated patients who had robust histological response.

The main observations of our study are (a) there was an early improvement in Adipo-IR with pioglitazone therapy, but it was not maintained throughout the 96 week treatment period, (b) vitamin E treatment had no effect on Adipo-IR despite its benefits on histology, and (c) improvement in Adipo-IR at week 96 correlated with significantly with improvement in ballooning, fibrosis, and NAS, but not with primary histological endpoint of the PIVENS trial. Furthermore, improvement in Adipo-IR at 96 weeks was associated with resolution of NASH at week 96 weeks, but this association was of borderline statistical significance.

In the current study, we observed an initial improvement in Adipo-IR in pioglitazone treated patients but this reduction was not persistent at week 96. The mechanism behind this rebound in Adipo-IR is not entirely clear, but it is possibly related to the weight gained by the pioglitazone treated patients. As shown in Table 3 there was a strong association between increase in Adipo-IR and increase in BMI over the 96-week treatment period (p=0.005) , and 82% of patients in the pioglitazone group experienced an increase in BMI

during the treatment period. Additional measurements of Adipo-IR between weeks 16 and 96 would have helped to further define this rebound in Adipo-IR, but due to sample constraints, we could not perform these interval Adipo-IR measurements.

As Gastaldelli et al. observed a significant relationship between changes in Adipo-IR and changes in steatosis and ballooning in their study, we were interested to explore if early or long term changes in Adipo-IR would predict histological changes observed at week 96. We also observed that change in Adipo-IR at week 96 was significantly associated with changes in many histological parameters in the entire cohort irrespective of the treatment group. But it was surprising that change in Adipo-IR in the pioglitazone treated subjects between baseline and week 96 did not correlate with changes in any histological parameters and we suspect this is because of the rebound in Adipo-IR that we observed between week 16 and week 96.

Of particular interest is the relationship between Adipo-IR and liver fibrosis that we observed in this study. Subjects with F3/F4 had an average 33% higher Adipo-IR than individuals with F1/F2 ($p=0.017$) and improvement in fibrosis at week 96 was associated with significantly decreased Adipo-IR over the treatment period. Considering that Lomonaco et al. also observed a strong relationship between Adipo-IR and fibrosis, we believe that this relationship deserves further investigation. The mechanistic basis for this relationship is unclear. Advanced cirrhosis has been associated with decreased hepatic extraction of insulin which may give rise to higher Adipo-IR (14,15), but none of the patients with PIVENS had advanced cirrhosis. Therefore we do not believe that the relationship observed between fibrosis and Adipo-IR can be explained by abnormal hepatic extraction of insulin. Our observations raise the intriguing possibility that Adipo-IR may serve as a biomarker for predicting changes in liver histology in patients with NASH.

In conclusion, pioglitazone therapy improved Adipo-IR in the short-term but this effect was not sustained, whereas vitamin E treatment had no short- or long-term effects on Adipo-IR. The rebound in Adipo-IR in pioglitazone treated individuals is likely related to weight gain that is common in individuals receiving pioglitazone. The intriguing relationship between Adipo-IR and fibrosis noted in this study as well as the study by Lomonaco et al. requires further investigation.

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Abbreviations

NAFLD	nonalcoholic fatty liver disease
NASH	nonalcoholic steatohepatitis
PIVENS	Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis
NASH CRN	Nonalcoholic Steatohepatitis Clinical Research Network
HOMA-IR	Homeostasis Model Assessment for insulin resistance

Adipo-IR	adipose tissue insulin resistance
NEFA	nonesterified fatty acids
BMI	body mass index
ALT	alanine aminotransferase
NAS	NAFLD activity score
IQR	interquartile range
SD	standard deviation
PPARγ	peroxisome proliferator activated nuclear receptor γ; TGF-β, transforming growth factor-β

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Appendix

Members of the Nonalcoholic Steatohepatitis Clinical Research Network:

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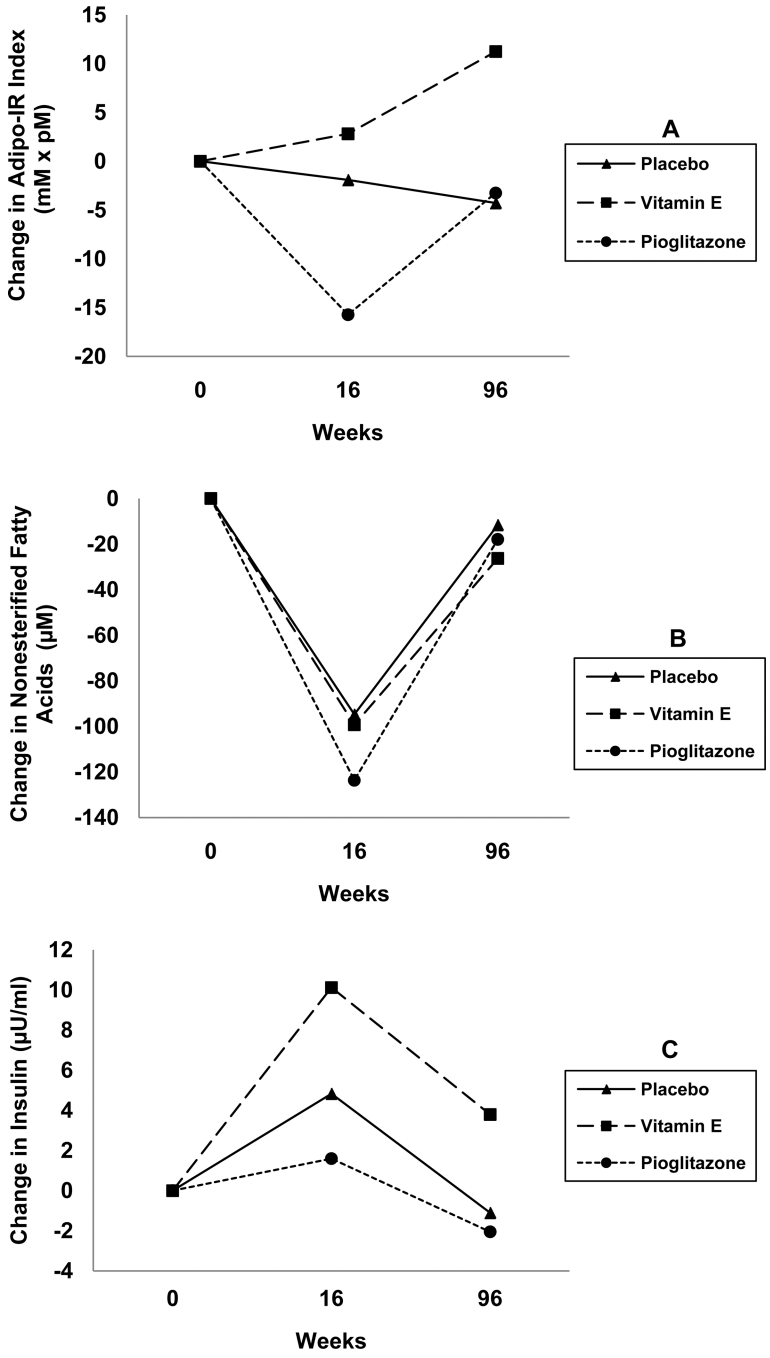

Figure 1. Changes in Adipo-IR throughout the PIVENS clinical trial

Adipo-IR index was calculated [fasting NEFA (mM) × fasting insulin (pM)] at baseline and after 16 and 96 weeks of placebo, pioglitazone, or vitamin E therapy. Throughout the 96 week treatment period, Adipo-IR was unchanged in patients receiving placebo or vitamin E therapy (**Panel A**). In contrast, pioglitazone significantly reduced Adipo-IR after 16 weeks compared to the placebo and vitamin E groups (p=0.005), but this effect was not maintained out to 96 weeks (**Panel A**). Changes in the individual components used to calculate Adipo-IR index, fasting NEFA concentrations (**Panel B**) and fasting insulin levels (**Panel C**), are also shown. Note that conversion of NEFA concentration units (from μM to mM) and insulin concentration units (from μU/ml to pM) was necessary for calculation of Adipo-IR

index. Refer to Table 1 for the standard deviations of the measures and the p values for the comparisons between the treatment group and placebo at each time point presented in the panels.

Table 1

Adipo-IR, Insulin, and NEFA at Baseline, 16 and 96 Weeks of Therapy with Vitamin E, Pioglitazone or Placebo

				p	
	Placebo	**Vit E**	**Pioglitazone**	**Vit E vs. Placebo**	**Pioglitazone vs. Placebo**
Baseline*					
Number	82	84	80		
Adipo-IR (mM × pM)	64.0 ± 85.5	54.1 ± 39.8	52.9 ± 38.2	0.34	0.29
Insulin (μU/ml)§	23.3 ± 21.8	21.5 ± 15.8	21.4 ± 14.9	0.55	0.51
NEFA (μM)	456.6 ± 199.6	452.0 ± 182.9	436.5 ± 193.7	0.88	0.52
After 16 weeks therapy**					
Adipo-IR					
Number	72	73	63		
Mean± s.d.	59.4 ± 62.6	56.0 ± 43.1	37.0 ± 37.3	0.70	0.01
Mean change from baseline± s.d.	−1.91±110.7	2.81±53.5	−15.7±35.6	0.71	0.02
Insulin(μU/ml)					
Number	72	74	64		
Mean± s.d.	27.2 ± 22.2	31.6 ± 28.8	22.7 ± 19.0	0.30	0.21
Mean change from baseline± s.d.	4.83±30.1	10.1±29.8	1.60±17.6	0.30	0.23
NEFA(μM)					
Number	78	80	72		
Mean± s.d.	366.3 ± 190.9	342.2 ± 199.6	303.1 ± 181.6	0.44	0.04
Mean change from baseline± s.d.	−94.7±272.9	−99.3±217.4	−123.7±222.6	0.56	0.08
After 96 weeks therapy					
Adipo-IR					
Number	74	69	66		
Mean± s.d.	61.5 ± 81.5	64.8 ± 119.2	48.2 ± 49.0	0.85	0.25
Mean change from baseline± s.d.	−4.28±120.3	11.2±120.9	−3.25±45.4	0.8	0.31
Insulin(μU/ml)					
Number	74	73	69		

				p	
	Placebo	**Vit E**	**Pioglitazone**	**Vit E vs. Placebo**	**Pioglitazone vs. Placebo**
Mean± s.d.	22.4 ± 16.6	25.8 ± 37.8	19.2 ± 19.1	0.49	0.29
Mean change from baseline± s.d.	−1.11±26.3	3.80±37.7	2.06±19.1	0.37	0.56
NEFA(μM)					
Number	74	74	67		
Mean± s.d	449.7 ± 228.5	409.0 ± 205.8	407.1 ± 218.9	0.26	0.26
Mean change from baseline± s.d.	−11.6±249.8	−26.3±235.2	−17.9±217.8	0.39	0.43

Plus-minus values are mean ± SD. Abbreviations: NEFA, non-esterified fatty acids; Adipo-IR, adipose tissue insulin resistance.

§ Conversion factor used for calculation of Adipo-IR: 1 μU/ml insulin = 6 pM.

* For the means of outcome measures, p values were derived from multiple linear regression models with two indicator variables for the effect of treatment versus placebo.

** For the mean change in scores, p values were calculated with multiple linear regression models with two indicator variables for the effect of treatment versus placebo, adjusting for the baseline value of the outcome.

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Table 2

Cross-sectional Correlates of Adipo-IR at Baseline and at 96 weeks

Correlates at baseline or 96 weeks	Baseline Adipo-IR		Adipo-IR at 96 weeks	
	Estimated ratio of Adipo-IR (95% CI)	P*	Estimated ratio of Adipo-IR (95% CI)	P‡
Demographics				
Female vs. Male	1.13 (0.79, 1.61)	0.50	2.06 (1.40, 3.03)	<0.001
Hispanic vs. other ethnicities	1.16 (0.89, 1.50)	0.27	1.09 (0.80, 1.50)	0.56
Age at Randomization	1.00 (0.99, 1.01)	0.68	0.999 (0.988, 1.010)	0.89
Anthropometrics				
BMI (kg/m^2)	1.06 (1.02, 1.09)	0.001	1.05 (1.01, 1.08)	0.004
Waist circumference (cm)	0.99 (0.98, 1.00)	0.12	1.01 (1.00, 1.02)	0.05
Total body fat (%)	1.02 (0.99, 1.04)	0.16	0.98 (0.96, 1.00)	0.12
Laboratory measures				
AST (U/L)	0.998 (0.993, 1.003)	0.46	1.00 (0.99, 1.01)	0.92
ALT (U/L)	1.001 (0.998, 1.004)	0.39	1.004 (0.999, 1.008)	0.08
Glucose (mg/dl)	1.01 (1.00, 1.02)	0.001	1.01 (1.00, 1.02)	0.001
Triglycerides (mg/dl)	1.00 (1.00, 1.00)	0.62	1.00 (1.00, 1.00)	0.96
Total cholesterol (mg/dl)	1.002 (0.999, 1.005)	0.17	1.004 (1.001, 1.008)	0.03
HDL cholesterol (mg/dl)	0.997 (0.988, 1.006)	0.46	0.995 (0.981, 1.004)	0.29
Histology				
Fibrosis score: 3–4 vs. <3	1.33 (1.05, 1.69)	0.02	1.03 (0.74, 1.41)	0.88
Steatosis Grade 2–3 vs. 0–1	1.21 (0.96, 1.51)	0.10	0.99 (0.75, 1.31)	0.93
Lobular inflammation >2 foci vs. 0–2 foci	1.02 (0.80, 1.28)	0.89	1.08 (0.78, 1.50)	0.63
Ballooning: Many vs. None/Few	1.16 (0.93, 1.45)	0.20	0.99 (0.72, 1.37)	0.97
NAS>=5 vs. <5	0.91 (0.68, 1.21)	0.52	1.00 (0.66, 1.52)	1.00
Resolution of NASH	-	-	0.74 (0.56, 0.98)	0.03

*P values were from multiple linear regression model, regressing the logarithm of Adipo-IR at baseline on the listed correlates at baseline.

‡P values were from multiple linear regression model, regressing the logarithm of Adipo-IR at 96 weeks on the listed correlates at 96 weeks.

Abbreviations: BMI, body mass index; AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDL, low-density lipoprotein; Adipo-IR, adipose tissue insulin resistance; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis.

Table 3

Relationship between change in Adipo-IR and improvements in histologic features at 96 weeks, change in BMI, and change in ALT at 16 or 96 weeks of treatment

Changes in Histologic and Other Features	16 weeks		96 weeks	
	Ratio of Adipo-IR Geometric Means* (95% CI)	p‡	Ratio of Adipo-IR Geometric Means* (95% CI)	p¶
Histologic Improvement at 96 wk‖	0.89 (0.63–1.26)	0.50	1.18 (0.81–1.72)	0.39
Improvement in individual Histologic Features at 96 wk†				
Steatosis	0.89 (0.65–1.22)	0.46	0.88 (0.62–1.24)	0.47
Lobular Inflammation	1.02 (0.76–1.35)	0.91	1.25 (0.90–1.72)	0.18
Hepatocellular Ballooning	1.16 (0.81–1.65)	0.42	0.63 (0.41–0.94)	0.03
Fibrosis	0.91 (0.67–1.25)	0.57	0.59 (0.41–0.84)	0.004
NAS	1.01 (0.67–1.54)	0.95	0.52 (0.33–0.82)	0.01
ΔBMI (kg/m^2) at 16 wk	1.06 (0.97–1.16)	0.20		
ΔBMI (kg/m^2) at 96 wk			1.11 (1.04–1.18)	0.002
ΔALT (U/L) at 16 wk	0.998 (0.998–1.000)	0.35		
ΔALT (U/L) at 96 wk			0.999 (0.999–1.000)	0.73

‖Histologic Improvement required improvement by 1 or more points in the hepatocellular ballooning score; no increase in the fibrosis score; and either a decrease in the activity score for nonalcoholic fatty liver disease to a score of 3 points or less or a decrease in the activity score of at least 2 points, with at least a 1-point decrease in either the lobular inflammation or steatosis score.

†The binary change variables for histology scores are defined as improvement versus no improvement. For each histology score, improvement is: at least a 1-point decrease in the Steatosis score; at least a 1-point decrease in the lobular inflammation score; improvement of 1 or more points in the hepatocellular ballooning score; no increase in the fibrosis score; and at least 2 points decrease in the NAS score, respectively.

*Geometric means estimated as exponentiated regression coefficients from the multiple linear regression model for log-transformed Adipo-IR (see Methods).

‡P values are from multiple linear regression models, regressing the log of the ratio between the outcome variables at 16 weeks and baseline on the five indicator variables for improvement in the histologic features at 96 weeks, the two indicator variables for treatment, the deltas for BMI, and ALT at 16 weeks, adjusting for baseline Adipo-IR, ethnicity, and baseline BMI. N=191; R^2=0.39; RMSE=0.81.

¶P values are from multiple linear regression models, regressing the log of the ratio between the outcome variables at 96 weeks and baseline on the five indicator variables for improvement in the histologic features at 96 weeks, the two indicator variables for treatment, the deltas for BMI, and ALT at 96 weeks, adjusting for baseline Adipo-IR, ethnicity, and baseline BMI. N=205; R^2=0.29; RMSE=0.93.

Abbreviations: Adipo-IR, adipose tissue insulin resistance; NAS, NAFLD activity score; BMI, body mass index; ALT, alanine aminotransferase; NEFA, nonesterified fatty acids; CI, confidence intervals.