Orlistat for Overweight Subjects with Nonalcoholic Steatohepatitis: A Randomized, Prospective Trial

Stephen A. Harrison,[1] Will Fecht,[2] Elizabeth M. Brunt,[3] and Brent A. Neuschwander-Tetri[4]

The aim of this study was to determine if orlistat, an inhibitor of fat absorption, combined with caloric restriction in overweight subjects with nonalcoholic steatohepatitis results in weight loss and improved liver histology. Fifty overweight subjects (body mass index = ≥27) with biopsy proven nonalcoholic steatohepatitis were randomized to receive a 1,400 Kcal/day diet plus vitamin E (800 IU) daily with or without orlistat (120 mg three times a day) for 36 weeks. Liver biopsies were repeated at week 36. Twenty-three subjects in the orlistat/diet/vitamin E group and 18 in the diet/vitamin E group completed the study. The mean age was 47 ± 9.0 (standard deviation) years and mean body mass index was 36.4 ± 6.3 kg/m^2. Four subjects were diabetic. The orlistat group lost a mean of 8.3% body weight compared to 6.0% in the diet plus vitamin E group (not significant). Both groups also had similarly improved serum aminotransferases, hepatic steatosis, necroinflammation, ballooning, and nonalcoholic fatty liver disease activity scores. Stratified according to weight loss instead of treatment group, a loss of ≥5% body weight (n = 24) compared to <5% body weight (n = 17) correlated with improvement in insulin sensitivity ($P = 0.001$) and steatosis ($P = 0.015$). Comparing subjects who lost ≥9% of body weight (n = 16), to those that did not (n = 25), improved insulin sensitivity ($P < 0.001$), adiponectin ($P = 0.03$), steatosis ($P = 0.005$), ballooning ($P = 0.04$), inflammation ($P = 0.045$), and nonalcoholic fatty liver disease activity score ($P = 0.009$) were seen. Increases in adiponectin strongly correlated with improved ballooning and nonalcoholic fatty liver disease activity score ($P = 0.03$). Orlistat did not enhance weight loss or improve liver enzymes, measures of insulin resistance, and histopathology. However, subjects who lost ≥5% of body weight over 9 months improved insulin resistance and steatosis, and those subjects who lost ≥9% also achieved improved hepatic histologic changes. (HEPATOLOGY 2009;49:80-86.)

The optimal treatment for long-term resolution of the complex metabolic and inflammatory disorders that manifest as nonalcoholic steatohepatitis (NASH) remains elusive. The majority of NASH patients is overweight or obese and has insulin resistance that contributes to underlying dysregulated energy metabolism.[1,2] The associated lipotoxicity identified in these patients is responsible for myriad host responses that include altered cytokine profiles, endoplasmic reticulum stress, mitochondrial dysfunction, cellular injury, apoptosis, and ultimately fibrosis. Although novel therapies are being investigated to mitigate or alter these responses, evidence suggests that patients who are able to lose between 5%-10% of their baseline body weight improve not only insulin resistance and serum aminotransferases, but also hepatic histopathologic changes.[3] However, these data are limited in subjects with nonalcoholic fatty liver disease (NAFLD). Furthermore, the majority of patients instructed to lose weight have difficulty achieving and maintaining weight loss. Pharmacologic agents to augment dietary advice have therefore begun to be evaluated as therapy for subjects with NASH. Orlistat, a reversible inhibitor of gastric and pancreatic lipase, blocks absorption of approximately 30% of dietary triglycerides. Preliminary data has shown that orlistat 120 mg three times daily (TID) in association with a low fat diet reduces body mass index (BMI), hemoglobin A1c, and serum amin-

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis.

From the [1]Department of Medicine, Gastroenterology & Hepatology Service, Brooke Army Medical Center, Fort Sam Houston, TX; [2]Indiana Gastroenterology Inc, Indianapolis IN; [3]Department of Pathology and Immunology, Washington University School of Medicine, St. Louis, MO; and [4]Division of Gastroenterology and Hepatology, Saint Louis University, St. Louis, MO.

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Address reprint requests to: Stephen A. Harrison, M.D., Brooke Army Medical Center, Department of Gastroenterology, 3851 Roger Brooke Drive, Fort Sam Houston, TX 78234. E-mail: stephen.harrison@amedd.army.mil; fax: 210-916-5611.

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otransferases. Additionally, histopathologic improvement in hepatic steatosis and fibrosis has been suggested.[4] A more recent 6-month randomized, placebo-controlled trial with orlistat in NASH subjects showed that BMI was reduced significantly, with no difference found between the orlistat and non-orlistat treated groups.[5] In this study, the mean weight loss was 8% in the orlistat group and 6% in the placebo group. Serum alanine aminotransferase (ALT) and steatosis were reduced significantly in both groups with a greater decrease in the orlistat group. Repeat liver biopsies in a subset of subjects showed improvement in steatosis in roughly 50% of subjects in both groups without significant improvement in inflammation or fibrosis. We now report a prospective, parallel, randomized treatment trial of combination orlistat and vitamin E versus diet and vitamin E given for 9 months with liver biopsies obtained before and after treatment.

Patients and Methods

Patient population. Adult patients age 18 and older were eligible for the study if they had a diagnosis of NASH based on clinical evaluation and a liver biopsy that was obtained within 24 months of study enrollment. Subjects were enrolled from two tertiary care academic medical centers (Brooke Army Medical Center, San Antonio, Texas, and Saint Louis University, Saint Louis, Missouri). Subjects were excluded from enrollment if there were causes for liver disease other than NASH, evidence of decompensated liver disease (history of ascites, bleeding varices, or spontaneous encephalopathy), history of alcohol consumption of $>$20 g/day in the past 2 years, prior surgical weight loss procedures including gastroplasty, jejunoileal, or jejunocolic bypass, total parenteral nutrition within the past 6 months, or use of ursodeoxycholic acid, rosiglitazone, pioglitazone, or metformin within a 6-month period before enrollment. Patients were randomized in an open-label fashion to receive either orlistat (120 mg orally TID with meals) plus vitamin E (d-α tocopherol) (800 IU daily), a single multivitamin daily at bedtime, and a standard 1,400-calorie/day diet, or simply the 1,400-calorie/day diet plus vitamin E (800 IU daily), and a multivitamin daily for 36 weeks. Vitamin E was added to both groups to prevent the possibility of orlistat-induced vitamin E deficiency as a confounding variable; moreover, previous data suggested that it may confer a small benefit to patients with NASH. A 36-week duration of treatment was selected due to previous orlistat studies showing clinical and histopathologic benefit in NASH patients when treated for 6-12 months.[4,6]

Dietary guidance was given by both the research nurse and the physician and included a standard 1,400-calorie/day diet for 1 month as outlined by the "Xenicare" program (a web-based nutrition information center designed by Roche Pharmaceuticals). Compliance was assessed by patient recall.

Patients were instructed to increase their level of physical activity from baseline at study start and on intervening clinic visits. No specific exercise recommendations were given.

The primary study endpoint was improvement in steatosis, NAFLD activity score (NAS)[7] and fibrosis score on follow-up liver biopsy obtained at 36 weeks. Secondary endpoints included improvement in biochemical data collected at baseline and at the end of the study. Laboratory data collected included fasting insulin and glucose, liver enzymes, lipid panel, vitamin E, and free fatty acid levels. Assessment of insulin resistance was determined by the quantitative check index (QUICKI) as defined by: log(1/(fasting insulin [mU/L] $\times$ fasting glucose[mg/ml])).[8] Serum was also stored for cytokine analysis at baseline and at the end of the study. Cytokines analyzed included TNFα-α, PAI-1, resistin, and adiponectin. These cytokines were evaluated by using the LINCOplex kit fluorescent immunoassay (Millipore, St. Charles, MO). Liver histology (H&E stain and Masson's trichrome stain) was evaluated in a blinded fashion at study completion by an expert hepatopathologist (E.B.) and all biopsies included in the analysis had $>$5 portal tracts and were deemed adequate for interpretation.

Statistical analysis. In this study, the independent variables are treatment (control, orlistat) and time (baseline, 36 weeks). The dependent variables are weight loss ($<$9%, $\geq$9%), histology, serology, and demographics. The null hypothesis is that there is no difference in weight loss, histology, or serology with respect to treatment or time. The alternative hypothesis is that there is an improvement in weight loss, histology, and serology with time in the orlistat group. Nonparametric tests (Mann-Whitney ranked sum) are used for ordinal and nominal variables.

In preliminary processing, descriptive statistics were obtained to locate out-of-range errors and aliases and data entry errors were corrected. Kolmogorov-Smirnov tests were carried out to identify normally distributed variables and Spearman rank correlation analysis was used to detect significant relationships between dependent and independent variables. A two-factor (treatment, time) analysis of variance (ANOVA) with repeated measures on one factor (time), followed by one-tailed *t* tests corrected for multiple comparisons for normally distributed, interval type variables was carried out on independent variables selected for their significance on correlation analysis. After analyzing for treatment effects, analyses were repeated with weight loss ($<$5%, $\geq$5%) and ($<$9%, $\geq$9%) replac-

Table 1. Baseline Demographics, Clinical, and Laboratory Data

	Overall (n = 41)	Control Group (n = 18)	Orlistat Group (n = 23)	*P* Value
Age	47.0 (9)*	45.8 (10.7)	47.9 (7.5)	0.48
Weight	225 (41)	224 (47)	226 (37)	0.85
BMI	36.4 (6.3)	35.2 (6.5)	37.3 (6.0)	0.31
Gender				1.0
Female	68.3%	66.7%	69.6%	
Ethnicity				0.52
Caucasian	68.3%	78%	61%	
Hispanic	26.8%	22%	30.3%	
AA/Asian	4.8%	0%	8.7%	
ALT (U/L)	97 (80)	83 (57)	108 (95)	0.28
AST (U/L)	63 (39)	61 (38)	64 (39)	0.81
Alkaline phosphate (U/L)	91 (27)	85 (16)	97 (33)	0.17
Fasting glucose (mg/dL)	107 (30)	113 (44)	102 (12)	0.29
Fasting insulin (μIU/mL)	22 (16)	23 (10)	21 (20)	0.76
QUICKI	0.31 (0.04)	0.30 (0.03)	0.31 (0.04)	0.21
Cholesterol (mg/dL)	211 (31)	211 (29)	210 (33)	0.89
LDL cholesterol (mg/dL)	133 (26)	131 (25)	135 (28)	0.65
Hgb A1C (%)	5.7 (0.9)	5.8 (1.2)	5.7 (0.6)	0.68
Vitamin E (mg/L)	15 (7)	15 (8)	14.(6)	0.74
Fatty acids (mmol/L)	0.5 (0.2)	0.5 (0.3)	0.5 (0.2)	0.86
Adiponectin (pg/mL)	9,865 (6,306)	8,721 (3,819)	10,761 (7,689)	0.31
Resistin (pg/mL)	12,262 (9,115)	11,831 (5,734)	12,600 (11,197)	0.78
PAI-1 (pg/mL)	30,851 (12,979)	30,622 (11,509)	31,030 (14,278)	0.92
TNF-α pg/mL	4.6 (4.9)	4.6 (2.7)	4.6 (6.2)	1.0
NAS	4.8 (1.6)	4.4 (1.9)	5.0 (1.4)	0.25

Abbreviations: AA, African American; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; LDL, low-density lipoprotein; NAS, nonalcoholic fatty liver disease; QUICKI, quantitative check index.

*Standard deviation.

ing treatment as an independent variable. This protocol was conducted under a Cooperative Research and Development Agreement with Saint Louis University. The study was approved by the Institutional Review boards of both Brooke Army Medical Center and Saint Louis University. SPSS version 14 was used to analyze the data.

Results

Fifty subjects were enrolled in the study, 25 from each site. Forty-one completed the study and obtained a follow-up liver biopsy. Reasons for not completing the study include: one patient withdrew consent, six patients were lost to follow-up, one patient did not have adequate biopsy tissue for histopathologic examination on follow up, and we were unable to obtain the pretreatment trichrome stain for review for one patient. Overall, the mean age was 47 $\pm$9 (range: 20 to 62 years), mean BMI: 36.4 $\pm$ 6.3, mean weight: 225.3 $\pm$ 40.9 pounds, mean baseline ALT: 97.1 IU/L $\pm$ 80.3, mean aspartate aminotransferase (AST): 62.8 IU/L $\pm$ 38.6, mean QUICKI: 0.30 $\pm$ 0.01 (Table 1). Four subjects were diabetic at baseline. Twenty-three subjects in the orlistat group and 18 subjects in the control group completed the study. No differences in demographic, clinical, laboratory, or histologic characteristics were seen between the two groups at baseline (Tables 1,2).

Orlistat versus control comparison. The orlistat group lost a mean of 8.3% body weight ($P < 0.001$). Serum ALT was reduced from a mean of 108 $\pm$ 95 IU/L to 53 $\pm$41 IU/L, ($P < 0.001$) and the serum AST was

Table 2. Baseline Histopathology

	Control Group (%)	Orlistat Group (%)	*P* Value
Steatosis			0.942
Grade 0	0	0	
Grade 1	38.9	31.8	
Grade 2	38.9	50.0	
Grade 3	22.2	18.2	
Ballooning			0.643
Grade 0	27.8	18.2	
Grade 1	33.3	36.4	
Grade 2	38.9	45.4	
Inflammation			0.589
Grade 1	27.8	4.5	
Grade 2	55.6	95.5	
Grade 3	16.7	0	
NAS			0.254
0	0	0	
1	0	4.3	
2	5.6	0	
3	16.7	8.7	
4	27.8	8.7	
5	11.1	39.1	
6	16.7	30.4	
7	22.1	8.7	

Table 3. Comparison of Pre- and Postclinical and Laboratory Variables Within and Between Groups

	Control Baseline	Control Post	*P* Value	Orlistat Baseline	Orlistat Post	*P* Value	*P* Value Between Groups
Weight	224 (47)	210 (46)	0.01	226 (37)	208 (39)	<0.001	0.86
BMI	35.2 (6.5)	32.6 (6.5)	0.01	37.3 (6.0)	34.5 (6.4)	<0.001	0.36
ALT (U/L)	83 (57)	38 (26)	0.003	108 (97)	53 (41)	<0.001	0.18
AST (U/L)	61 (38)	32 (21)	0.006	64 (39)	36 (17)	<0.001	0.56
Alkaline phosphate (U/L)	85 (16)	77 (24)	0.14	97 (33)	93 (29)	0.32	0.07
Fasting glucose (mg/dL)	113 (44)	101 (12)	0.19	108 (12)	100 (13)	0.55	0.85
Fasting insulin (μIU/mL)	23 (10)	22 (11)	0.88	21 (20)	17 (9)	0.28	0.11
QUICKI	0.30 (0.03)	0.31 (0.03)	0.38	0.31 (0.04)	0.32 (0.03)	0.72	0.17
Cholesterol (mg/dL)	211 (29)	202 (34)	0.27	210 (33)	192 (31)	<0.001	0.34
LDL (mg/dL)	131 (25)	119 (27)	0.29	135 (28)	116 (27)	<0.001	0.73
HgbA1C (%)	5.8 (1.2)	5.5 (0.5)	0.35	5.7 (0.6)	5.5 (0.5)	0.25	0.98
Vitamin E (mg/L)	15.3 (8.5)	30.4 (13.7)	<0.001	14.5 (6.5)	20.6 (8.3)	0.002	0.01
Fatty acid (mmol/L)	0.54 (0.27)	0.46 (0.22)	0.36	0.52 (0.24)	3.5 (13.4)	0.35	0.34
Adiponectin (pg/mL)	8722 (3819)	10,131 (4257)	0.07	10,761 (7689)	12,745 (7761)	0.04	0.21
Resistin (pg/mL)	11,831 (5734)	16,288 (9009)	0.08	12,600 (11,197)	24,170 (18,870)	0.02	0.09
PAI-1 (pg/mL)	30,622 (11,509)	30,704 (10,953)	0.981	31,030 (14278)	33,030 (11,611)	0.53	0.52
TNF-α pg/mL	4.6 (2.7)	7.4 (8.0)	0.177	4.6 (6.2)	55.6 (103.9)	0.03	0.06

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; LDL, low-density lipoprotein; QUICKI, quantitative check index.

significantly decreased from 64 ± 39 IU/L to 36 ± 17 IU/L, $P < 0.001$). Serum cholesterol and low-density lipoprotein (LDL) were also significantly improved. Adiponectin and resistin levels both increased significantly, $P = 0.04$ and $P = 0.02$, respectively. Unexpectedly, in the orlistat group, TNF-α rose from 4.6 ± 6 pg/mL to 56 ± 104 pg/mL, ($P = 0.03$). This increase in the mean value was driven by four outliers, subjects with serum TNF-α levels of 122, 283, 424, and 120 pg/ml. There were no demographic, biochemical, or histologic characteristics of these four subjects that correlated with the markedly increased TNF-α levels. Vitamin E levels rose significantly from 14.5 ± 6.5 mg/dL to 20.6 ± 8.3, $P =$ 0.002 in the orlistat group (Table 3).

Similarly, the non-orlistat group lost a mean of 6.0% body weight ($P = 0.01$). Serum ALT decreased from 83 ± 57 IU/L to 38 ± 26 IU/L ($P = 0.003$), and the serum AST decreased from 61 ± 38 IU/L to 32 ± 21 IU/L ($P = 0.006$). No significant improvement was found in fasting glucose, insulin, QUICKI, total cholesterol, or LDL. Additionally, no significant improvement was found in serum adiponectin, resistin, PAI-1, or TNF-α. Vitamin E levels rose significantly from 15.3 ± 8.5 mg/dL to 30.4 ± 13.7, $P < 0.001$. This was significantly improved when compared to the orlistat group, $P = 0.01$ (Table 3).

Comparing the orlistat group to the non-orlistat group at study completion, no significant differences were identified between the two groups for mean weight loss, serum ALT or AST, insulin resistance, cholesterol, or adipocytokine levels. Furthermore, no significant differences were found between the two groups in biopsy findings for hepatic steatosis, ballooning, inflammation, NAS, or fibrosis at the end of therapy. The only differences identified were that serum vitamin E levels were significantly lower in the orlistat group, although the levels were above baseline in both groups (Table 3). The difference was caused by a smaller increase in the orlistat group compared to the non-orlistat group. This difference could be related to impaired absorption of fat soluble vitamins in orlistat-treated subjects, although this was not specifically examined. Additionally, serum TNF-α levels were significantly higher in the orlistat group.

Comparisons for incremental weight loss. Given that there were no significant differences between the orlistat and non-orlistat groups, the data was reanalyzed to compare subjects who lost ≥5% body weight during the study (n = 24) to those who lost <5%. Additionally, all subjects who lost ≥9% of their body weight during the study (n = 16) were compared with those who lost <9% body weight. Among all subjects who lost ≥5% body weight compared to those that did not, a significant correlation was found between weight loss and glucose ($r = 0.354$, $P = 0.025$), QUICKI ($r = 0.327$, $P = 0.04$), adiponectin ($r = 0.515$, $P = 0.001$), PAI-1 ($r = 0.435$, $P = 0.004$), and hepatic steatosis ($r = 0.388$, $P = 0.01$). Comparing all subjects who lost ≥9% body weight to those that did not, significant correlations between weight loss of ≥9% and improvement in HgbA1c ($r = 0.388$, $P = 0.01$), fasting insulin ($r = 0.389$, $P = 0.01$), QUICKI ($r = 0.385$, $P = 0.01$), adiponectin ($r = 0.461$, $P = 0.002$), resistin ($r = 0.380$, $P = 0.01$), PAI-1 ($r = 0.596$, $P = <0.001$), hepatic steatosis ($r = 0.453$, $P = 0.003$), and NAS ($r = 0.419$, $P = 0.007$) were found.

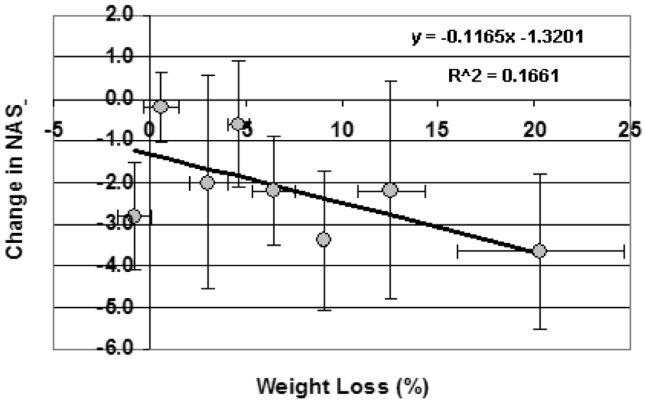

Fig. 1. Change in the NAS as a function of percent weight loss. A greater degree of weight loss was associated with a greater improvement in the NAS. This relationship was driven largely by changes in the steatosis component of the NAS ($R^2 = 0.263$) whereas changes in the ballooning and inflammation components of the NAS were not factors ($R^2 = 0.071$ and 0.054, respectively).

Figure 1 illustrates the correlation between weight loss and change in NAS.

All variables (except histopathology) found to have a significant correlation were then subjected to two factor ANOVA with repeated measures and the following variables were found to be significant for weight loss ≥5%: QUICKI ($P < 0.001$) and hepatic steatosis ($P = 0.015$). For weight loss ≥9%: QUICKI ($P < 0.001$) and adiponectin ($P = 0.03$) were found to be significantly improved. There was a trend toward improvement in fasting insulin (21.2 μIU/mL to 11.9 μIU/mL compared with 22.2 μIU/mL to 24 μIU/mL) but this did not reach statistical significance ($P = 0.07$). In reference to the histopathologic variables, steatosis ($P = 0.005$), ballooning ($P = 0.04$), inflammation ($P = 0.045$), and NAS ($P = 0.009$) were found to be significantly improved using Mann-Whitney rank-sum tests (Fig. 2).

Discussion

As the prevalence of obesity, insulin resistance, and metabolic syndrome continue to increase globally, it is not surprising that NAFLD has become a major epidemic, not only in our society but throughout the world. To date, treatment options remain limited and are founded on weight loss and improvement in insulin resistance. Weight loss has been shown previously to improve liver enzymes and decrease fasting insulin levels. Moreover, weight loss of as little as 6%-8% has been shown to improve insulin sensitivity, and intra-abdominal and hepatic steatosis.[3,9,10] Data specific to NASH subjects is limited to small trials with incomplete histologic follow-up. A pilot trial of 15 subjects treated for 12 months with a 1,400 calorie/day diet consisting of 40%-45% carbohydrate, 35%-40% fat, and 15%-20% protein showed that a weight loss of 7% improved the total NASH score and steatosis.[11] These promising preliminary results were limited by the inclusion of only 9/15 subjects in the subgroup analysis. Additionally, two studies have examined caloric restriction alone in overweight or obese subjects.[12,13] One study by Drenick et al.[12] showed normalization of histology in 9 of 14 patients who were able to lose weight and maintain weight loss over a 17-month period. Additionally, a small, retrospective cohort study of overweight patients showed that a 10% weight reduction achieved on a 600-800 calorie/day diet for a mean of 16 months resulted in improvement in liver enzymes and hepatosplenomegaly; however, no follow-up liver biopsies were carried out.[13]

Given that weight loss is often difficult to obtain with diet and exercise alone, research has focused on medications that may be used to augment weight reduction via diet and exercise. Orlistat has been studied extensively in this regard and it has been shown that roughly one-third of subjects treated with orlistat and a low fat diet lose between 5%-10% body weight with 1 year of therapy.[14] More recent data suggest that orlistat is not associated with a more significant weight loss than lifestyle modifi-

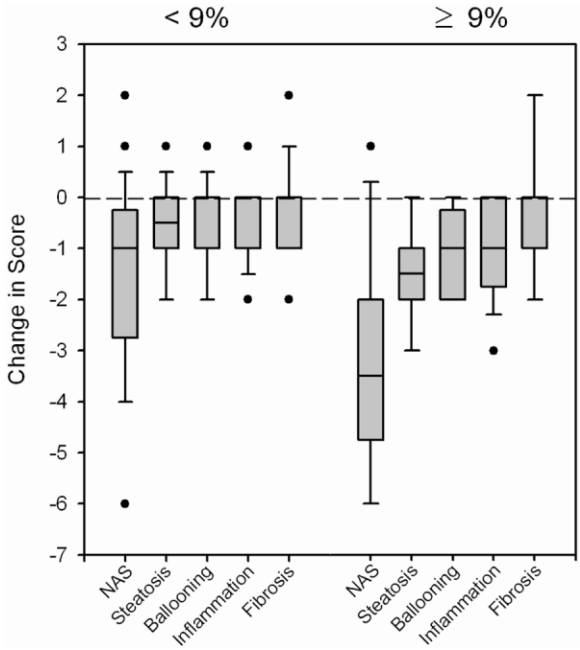

Fig. 2. Histopathologic changes stratified according to whether subjects lost <9% of body weight or ≥9% of body weight. Subjects who lost <9% of body weight did not show improvement in the aggregate NAS, its individual components, or fibrosis. Subjects who lost ≥9% of body weight had significant improvement in their NAS, steatosis, ballooning, and inflammation ($P < 0.05$ for each) but not in fibrosis. Shown are box plots with the ends of each box representing 25th and 75th percentiles, a line at the median, error bars at the 10th and 90th percentiles, and outliers. NAS based on steatosis, ballooning, and inflammation.

cation alone.[5,9] Our data confirm the prior findings, and suggest that 9 months of therapy can result in a loss of about 8% body weight. Inhibition of fat absorption with the use of orlistat can favorably impact serum lipid levels,[15] and in this study, we found improved levels of cholesterol and LDL cholesterol (Table 3).

One subject was diagnosed with NASH by a local pathologist and allowed to enroll in the trial. At the completion of the study, both the pre- and postliver biopsy were reviewed by a separate study hepatopathologist (E.B.) and noted to have a NAS of 1 at baseline without histopathology consistent with NASH. Despite losing 20 pounds (13% of body weight), her repeat liver biopsy was unchanged. This subject remained in the analysis as the data did not change the outcome between groups. Furthermore, the histopathologic significance seen in the group losing $\geq$9% body weight was not affected negatively.

Our study is the first to prospectively define the amount of weight loss necessary to show histopathologic improvement in the active features of steatohepatitis: steatosis, ballooning, and inflammation. Improvement in hepatocellular ballooning is particularly important, as recent data support that ballooning, a feature of cell injury, is associated independently with fibrosis progression.[16] As illustrated in Figure 1, there seems to be a linear relationship with weight loss and improvement in the histologic index of activity, the NAS that continues with even greater amounts of weight loss. The mean NAS improved from 4.8 $\pm$ 1.9 to 1.7 $\pm$ 1.3 in those subjects who lost $\geq$9% of their body weight. Fibrosis, the histologically identifiable deposition of collagen, is a manifestation of longer-term injury and repair processes that may not be as readily reversed by short-term intervention. In this trial, no changes in fibrosis were seen in the biopsies. Previous studies in morbidly obese subjects who underwent bariatric surgery for significantly more restrictive dietary control have shown improvement in fibrosis in repeat liver biopsies 2 years after surgery.[17-19] It can be speculated that with more prolonged follow up, fibrosis might have improved in these patients because improvement in ballooning degeneration was seen after 9 months of treatment.

The roles of the various adipocytokines in NASH pathogenesis are only just beginning to be understood. The adipocytokine with the most data in relation to NASH is probably adiponectin. It is well established that this cytokine is lower in obese subjects and subjects with NASH. In our study, subjects receiving orlistat and all subjects who lost $\geq$5% of their body weight had a significant improvement in adiponectin levels. Improvement in adiponectin in those patients losing $\geq$9% body weight was correlated strongly with improvement in steatosis ($r = 0.63$, $P = 0.009$), ballooning ($r = 0.55$, $P = 0.03$), and change in NAS ($r = 0.56$, $P = 0.03$).

PAI-1 levels have been shown to be elevated in subjects with insulin resistance as well as those with NASH.[20,21] Subsequently, it may be expected that PAI-1 levels would decrease with improvement in insulin resistance and histopathology. However, in our study, although there was a significant correlation with weight loss and PAI-1 levels, we did not find a significant difference with respect to time or weight loss. We found a significant interaction between time and weight loss suggesting a regression to the mean.

Resistin is an adipocyte-derived hormone that is involved in energy regulation and is thought to be a potent pro-inflammatory protein. Although data suggest that resistin levels are elevated in subjects with type 2 diabetes mellitus,[22] previous data in reference to resistin and NAFLD are conflicting. Resistin levels have been shown to be higher in patients with high grade steatosis compared with low grade steatosis[23] and are related to the histopathologic severity of disease.[24] whereas others have shown lower resistin levels in NASH versus simple fatty liver.[25] In our study, resistin levels increased significantly in the subjects receiving orlistat. Additionally, a significant correlation was found between resistin levels and weight loss. However, further analysis (ANOVA) showed that the group losing $\geq$9% had no change in resistin levels whereas the group losing $<$9% weight loss had a significant increase in resistin levels. As some patients in the $<$9% weight loss group actually gained weight, an association between weight gain and rising resistin levels cannot be excluded. Further study is needed to clarify the role of resistin in patients with NASH.

TNF-α levels remained unchanged in subjects who lost $\geq$9% of their body weight. This is a perplexing result as previous investigators have shown elevated levels of TNF-α in NASH patients,[26] and a subsequent decrease in TNF-α with improvement in insulin resistance.[27] In our trial, TNF-α levels in four subjects were elevated significantly at the end of the study compared with baseline despite improvement in adiponectin, serum ALT, and NAS score in all cases. Because there were no demographic, biochemical, or histologic findings that correlated with the markedly elevated TNF-α levels, it is possible that these results were spurious.

Limitations of this study exist and include the lack of a placebo control, although all subjects who lost $\geq$5% and $\geq$9% were compared to those who did not lose this amount of weight. Moreover, because of the gastrointestinal side effects of orlistat, successfully blinding patients to their treatment arm may not be possible. The study number, although relatively large compared to other

NASH trials, still remains small. Additionally, the study duration does not allow for assessment of weight loss maintenance over time. Importantly, the length of follow up does not allow for adequate assessment of potential long-term fibrosis response to the early weight loss.

In conclusion, although this preliminary study does not show a weight loss advantage with the use of orlistat, it does show that moderate weight loss, attainable by approximately 40% of the subjects who completed this trial, is associated with significant improvements in serum aminotransferases, insulin resistance, and specific adipocytokines as well as hepatic steatosis, ballooning, lobular inflammation, and the total NAS.

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