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Double blind randomized placebo controlled clinical trial of omega 3 fatty acids for the treatment of diabetic patients with nonalcoholic steatohepatitis

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Abstract

Background—Nonalcoholic steatohepatitis (NASH) is common and severe in patients with diabetes mellitus. Although, there are no effective treatments for NASH in diabetic patients, preliminary reports suggest that polyunsaturated fatty acids (PUFA) may be beneficial in these patients.

Aim—A prospective, randomized, double blind placebo controlled study (NCT 00323414) was performed in NASH patients with diabetes.

Methods—37 patients (50.6±9.8y) with well controlled diabetes (HbA1C<8.5%) were randomized to receive either PUFA containing eicosapentaenoic acid 2160 mg and docosahexaenoic acid 1440 mg daily or an isocaloric, identical placebo containing corn oil for 48 weeks under CONSORT guidelines. Clinical, demographics, biochemical laboratory tests, body composition using DEXA® and liver biopsy were done at randomization and at the end of treatment. Liver biopsy was scored by the NASH CRN criteria. An intention to treat analysis was performed.

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Jaividhya Dasarathy assisted in the analysis of data, drafting and editing the manuscript and the figures.

Carol Ann Hawkins and Ruth Sargent were the research coordinators who assisted with the conduct of the entire study including recruitment of subjects, obtaining necessary regulatory approvals, and collecting and organizing the data.

Amer Khiyami and Lisa Yerian were the pathologists who reviewed all the slides and scored the biopsies.

Trial Registration. Clinicaltrials.gov(NCT 00323414)

No conflict of interest for any of the authors

Results—At inclusion, gender, age, body weight, biochemical tests, glucose control and liver histology were similar in the 2 treatment groups. There was no change in liver enzymes, body weight or body composition during the study in either group. At the end of treatment, hepatic steatosis and the activity score improved ($p < 0.05$) and lobular inflammation worsened ($p < 0.001$) with placebo but was unchanged with PUFA. At the end of treatment, insulin resistance (serum glucose and HOMA) worsened with PUFA but not placebo.

Conclusions—PUFA provided no benefit over placebo in NASH patients with diabetes. The effects of PUFA on histology and insulin resistance were inferior to placebo. These data provide no support for PUFA supplements in NASH.

Keywords

Diabetes mellitus; nonalcoholic steatohepatitis; polyunsaturated fatty acids

Introduction

Both nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes (DM), which affect 30% and 10% of the US adult population respectively(3;13), are common complex metabolic diseases associated with insulin resistance (30). NAFLD is the most common cause of chronic liver disease (56). Nonalcoholic steatohepatitis (NASH) is the most severe form of NAFLD(37). One third of NASH patients have advanced fibrosis and 20% develop cirrhosis (37). Thus, it is estimated that NAFLD has or will cause 6-8 million Americans to develop cirrhosis. Supporting these estimates is the fact that NAFLD is now the third most common indication for liver transplantation with a trajectory to become the most common in 10 years (5). DM, which is present in 30% of NAFLD patients (35), is now recognized as a major risk factor for liver injury in these patients (55;57).

The recognition of the clinical consequences and underlying molecular mechanisms of NASH (51) has led to a number of treatment strategies that have been studied, predominantly in non-diabetic patients (33). To date, only vitamin E (44) and weight loss (40) have been shown to be safe and effective therapies for reversing NASH. There are no established therapies for NASH patients with DM.

N-3 polyunsaturated fatty acids (PUFA) have been shown in nascent human and animal studies to have a beneficial impact in improving hypertension, hyperlipidemia, endothelial dysfunction, cardiovascular disease (25) and improving hepatic steatosis in NAFLD (38). The n-3 PUFAs, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have been shown to regulate a number of transcription factors that control critical components of hepatic fatty acid metabolism (11;22). N-3 PUFAs are potent activators of PPAR α that in turn stimulates fatty acid oxidation (39;60) and PPAR γ that increases insulin sensitivity (29), inhibits hepatic lipogenesis via sterol regulatory binding protein-1 expression (54), down regulates pro-inflammatory genes (1;21;27) and reduces hepatic reactive oxygen species (ROS) (20). Human studies with n-3 EPA supplements resulted in improved lipid profile (15;41). Long term treatment with EPA in humans has reported them to be well tolerated and safe (46). These data provide compelling evidence for a therapeutic role of n-3 PUFA in fatty liver; specifically in patients with DM who have multiple metabolic risk

factors that can potentially be reversed by the administration of n-3 fatty acids, EPA and DHA. Therefore, we performed a randomized double blind controlled trial in NASH patients with DM.

Subjects and Methods

Selection of patients

Patients were recruited from two medical centers, Cleveland Clinic and MetroHealth Medical Center, in Cleveland, Ohio. Patients were considered for the study if they had an established diagnosis of NASH and a NAFLD activity score (NAS) ≥ 4 on liver biopsy performed within 6 months of entry into the study. Other inclusion criteria were (1) adult diabetic patients (age >18) with at least moderate control of blood sugar (HbA1c $<8.5\%$), (2) a stable regimen of anti-diabetic agents (> 4 months) prior to the biopsy and during the time between biopsy and randomization, (3) appropriate exclusion of other liver disease, (4) ability to give informed consent.

The exclusion criteria were (1) cirrhosis defined on liver biopsy or unequivocal clinical evidence of cirrhosis, (2) daily alcohol intake > 30 g for male and > 20 g for females for at least three consecutive months during the previous 5 years assessed by the Skinner lifetime history questionnaire and the self-administered Audit, (3) end stage organ disease associated with diabetes (renal failure defined as a serum creatinine >2 , severe neuropathy, advanced peripheral vascular disease), (4) heart failure (NYHA class 2-4), (5) the use of any amount of fish oil supplements during the 6 months prior to biopsy, (6) the use of medications known to cause steatosis, (7) the use of medications that have shown benefits in previous studies (vitamin E, thiazolidinedione, S-adenosylmethione) (8) other types of liver disease suspected by history, clinical finding or serum biochemistries.

Study Design

This was a pilot and feasibility study of the NASH Clinical Research Network (CRN) designed as a prospective double blinded, randomized, placebo controlled trial. The study was approved by the Institutional Review Boards at both participating centers and conformed to the Helsinki accord on human subjects in research. It was undertaken in accordance with the CONSORT guidelines (Figure 1). An independent Data and Safety Monitoring Board and a regulatory and compliance monitor provided oversight and supervised the conduction of the study. The study was registered in clinicaltrials.gov NCT00323414.

Patients, who met the study criteria, were randomized to one of two study groups and received either an oral dose of purified EPA/DHA or placebo (corn oil). The duration of the study was 48 weeks. At entry and at each study visit, study patients were instructed to perform 30 minutes of aerobic exercises 5 days a week and follow a healthy heart diet.

The EPA/DHA supplementation included 2160 mg of EPA and 1440 mg of DHA in 2 divided doses and contained 72 calories. An identical placebo containing corn oil (72 calories) was administered in the same manner. Patients were randomized by the sealed

envelope technique using a random numbers table. The codes were broken only after primary analysis was completed.

Both formulations were yellow, oblong capsules without markings and with no discernible differences in odor or taste. The PUFA formulation was a marine fish (anchovy and sardines) concentrate (Opti-EPA™) and provided by Douglas Laboratories (Pittsburg PA), which tested the quality and purity of the PUFA. The oil is molecularly distilled fish oil and meets all the specific health limits for dioxins, PCBs and heavy metals as established by the Council for Responsible Nutrition(www.crnusa.org).

Evaluation and Monitoring

Clinical history and physical examination were performed at entry, at 12, 24, 36 and 48 weeks. A follow up liver biopsy was performed at 48 weeks at discontinuation of the study medication. Laboratory studies were performed at entry and at 48 weeks and included blood counts, hepatic function, prothrombin time, renal function, fasting glucose and insulin, HbA1c, and a fasting lipid panel. Patients were followed for 24 weeks after completing the study and clinical and biochemical assessments were repeated. Precise weight and height measurements were obtained. The metabolic syndrome was diagnosed using previously defined criteria (16). Body composition measured using dual energy X-ray absorptiometry (DEXA®) was performed at entry and at the completion of the study. All subjects were evaluated by a dietician for education regarding an American Diabetic Association recommended diet as part of clinical care. However, no specific dietary intervention was planned to avoid additional confounders.

Assessment of Liver Histology

Liver pathologists at each center (A.K at MetroHealth and L.Y. at the Cleveland Clinic) established the histological diagnosis of NASH that was required for entry into the study. Adequacy of the liver biopsy samples was assessed by the study pathologists, who reviewed all entry and end of study biopsies. The histological review used the NASH CRN criteria (23). The grade was based on the individual scores for steatosis, lobular inflammation, ballooning and the composite NAFLD activity score (NAS).

Fibrosis was staged from 0-4 (0 absent, 1a: mild perisinusoidal (seen on Masson Trichrome stain), 1b: moderate perisinusoidal (seen on hematoxylin and eosin stain), 1c: portal/periportal, 2 perisinusoidal and periportal/portal, 3: bridging fibrosis, 4: cirrhosis).

Outcome measures—The primary outcome was defined as an improvement of 2 points in the NAS. Secondary outcome measures included change in serum transaminases, insulin resistance (HOMA score) and measures of diabetes control (fasting blood glucose and HbA1C) before and after therapy.

Statistical Analysis—Qualitative variables were compared using the chi square test. Quantitative and rating variables were compared using the student's 't' test for independent variables and the paired 't' test for serial measurements. For multiple group comparisons, analysis of variance with Bonferroni post hoc analysis was used. The primary outcome

measure was the change in the composite histological score between the two groups. Since this outcome measure is ordinal, a conservative approach to estimating sample size was to convert it to a dichotomous variable (improved histology versus no change or worsening histology). For the placebo group, the percentage with improved histology was estimated to be 15%. This value represents the percentage of patients with NASH secondary to diabetes who have improved histology one year following their initial biopsy (37). For the PUFA group, the percentage of patients with improved histology is estimated to be 60% (improvement of 45%). This difference (about 50% improvement) has been documented in patients treated with PUFA for other components of the metabolic syndrome (i.e. hypertriglyceridemia)(32). Hence, 18 patients per group will allow us to show a rate of improvement of 15% versus 60% with a power of 80% and a type I error of 0.05 (two-tail). This sample size would also allow us to detect an effect size of 0.83 (Difference / Standard Deviation) with a power of 80% and a Type I Error of 0.05(two-tail) for our secondary outcome measures that are interval in nature (i.e., insulin sensitivity, aminotransferases). The difference in the secondary outcome variable: insulin sensitivity and aminotransferases were anticipated to be 50-80% between the treatment and control groups. This number of patients was more than adequate for the secondary outcome measures that are expected to change between the 2 groups by at least 50-100 %.

An intention to treat analysis was performed. The effect of PUFA and placebo were compared for the primary and secondary outcomes on both an absolute difference and delta change during treatment.

Analysis for Efficacy—Even though the primary intent of this study was to obtain pilot data for a larger, multi-centered study, the data was analyzed (as stated above) for efficacy. Patient compliance was monitored and reinforced with protocol telephone calls and study visits with our research personnel. In addition, compliance with the medication was monitored by research personnel using pill counts and patient self-reporting.

Results

Study Patients

Thirty seven subjects met the above criteria and completed the study. The medication was well tolerated by all subjects. Their baseline clinical and demographic features are shown in table 1. Both groups were comparable at entry regarding demographics, laboratory tests, and metabolic parameters. Females were the majority in both study groups. All patients had the metabolic syndrome with at least 3 components and these were similar in the two treatment groups. The mean serum transaminases did not differ between groups. Serum ALT and AST were greater than 40 IU/dl in 8 patients in the PUFA and 12 patients in the placebo group. Measures of diabetes control including fasting blood glucose and HbA1C were similar ($p>0.1$) in the 2 treatment groups. Histological evaluation showed that the NAS and its individual components that included steatosis, inflammation, hepatocyte ballooning, and fibrosis were also similar in the 2 groups.

Body composition

As displayed in table 2, anthropomorphic and body composition measurements including body weight, body mass index (BMI), whole body fat mass, trunk fat, and total lean mass and bone mass were also similar in the 2 study groups. All patients in both groups were overweight with 17 patients in each group being obese (BMI > 30 kg/m²). There was no significant change in body weight or in any of the measures of body composition in either of the groups during the course of the study.

Outcome measures

The primary outcomes are displayed in table 3 and figure 2. End of treatment histological outcome measures showed that steatosis and the NAS significantly improved while lobular inflammation worsened in the placebo group (Table 3). In contrast, there was no significant change in any of the histological measures in the PUFA group (Table 3). However, the mean change from initiation to end of treatment in individual components or the composite NAFLD activity score on liver biopsy in the PUFA and placebo groups were not significantly different (Figure 2). Changes in histological components in individual subjects are shown in table 3. Eight patients in the PUFA group and 9 subjects in the placebo group had at least a 2 point reduction in the NAS. Steatosis was unchanged in 7 patients in the PUFA group and in 8 in the placebo group, worsened in 3 patients on PUFA and 1 in the placebo group. In the remainder, steatosis decreased by at least 1 point on the NAS system. Lobular inflammation was unchanged in 8 and worsened by 1 point in 3 patients in the PUFA group while it was unchanged in 7 and did not worsen in any of those treated with placebo. There was improvement in hepatocyte ballooning by at least 1 point in 6 patients in the PUFA group and in 10 patients in the placebo group. Ballooning score was unchanged in 11 patients in PUFA and 7 in the placebo group while it worsened in 1 in the PUFA and 2 in the placebo group, respectively. In the majority of patients, the fibrosis score did not change (12 in PUFA and 9 in placebo group). These were not significantly different between PUFA and placebo treated subjects.

Displayed in table 4 are the predefined secondary outcome measures. There were no significant changes in hepatic transaminases or total bilirubin in either group. Improvement in serum ALT compared to baseline was observed in 11 subjects in the PUFA and 13 in the placebo. Blood glucose, insulin, HOMA IR, HbA1C, serum triglycerides, HDL and total cholesterol all increased in the PUFA group but only the increases in glucose and HbA1C were significant.

Discussion

This is the first randomized, placebo controlled double blind study comparing omega 3 fatty acid supplementation and isocaloric placebo that used predefined histology as the primary endpoint in diabetic patients with NASH. In these patients with well controlled diabetes, in whom the compliance was excellent and the diagnosis of NASH used well-established histological criteria, PUFA supplementation was not beneficial for either histological or biochemical improvement. In contrast, there was evidence that insulin resistance worsened

with PUFA supplementation. These results differ from previous studies that used different endpoints to evaluate PUFA in NASH.

There have been 10 studies (4;6;9;18;36;47;48;50;52;59) [see table, supplementary digital content] and one meta-analysis (38) that have evaluated the efficacy of PUFA in NAFLD. Five were randomized placebo controlled trials with a total of 286 patients (6;32;36;47;59), one was diet controlled with 40 patients (48) and four were open label with a total of 124 patients (4;18;50;52). Table 5 displays the characteristics of our study and the 5 previously published placebo controlled trials. All of the published trials except one performed in children (36) included adults with nonalcoholic fatty liver seen on ultrasound. The duration of the placebo controlled trials ranged from 8 weeks to 12 months and the dose varied between 830 mg to 6 grams. One study used pure DHA (36), one used pure EPA (50), while all the other trials used a mixture of both EPA and DHA. Only 2 of the published trials (36;50) performed entry liver biopsies and only one (50) obtained post treatment biopsies but only in seven of the 23 (30.4%) enrolled patients. Improvement in the amount of fat on ultrasound was the primary outcome for both the placebo (6;9;36;47;59) and non-placebo (4;18;48;50;52) controlled studies. There was significant heterogeneity in the design, type of PUFA used and the duration of follow-up (supplementary table). Furthermore, most studies did not include diabetic patients.

PUFA supplements significantly decreased the amount of hepatic fat observed on ultrasound in most of these studies (4;6;9;36;47;48;50;59), as was the case in a meta-analysis that included most but not all of the PUFA trials (38). These results support the strategy of PUFA supplements in NAFLD patients, who have been reported to consume less polyunsaturated fat intake (34) and fish (58) and a higher ratio of n-6/n-3 (8) in their diet, as compared to controls.

Although these studies suggest that PUFA are effective in improving liver fat content, the effect of PUFA on other components of NASH, specifically those histological features believed to predict progression, including hepatocyte ballooning and fibrosis, were not assessed. An additional limitation to these trials is the lack of standardized, protocol liver biopsies with predetermined histological end points. This is especially relevant given the limitation of ultrasound to precisely quantify hepatic fat content, and inability to identify histological measures of hepatocellular injury (10). To our knowledge, the present study is the first of its kind to examine the role of both EPA and DHA in a double blind, randomized study in well characterized NASH patients with diabetes. In the current study, both DHA and EPA were used rather than purified DHA or EPA because each has a different effect on hepatic fatty acid metabolism (14;53). Although the optimal dose of PUFA supplements remains unclear (28), we chose the dose of 3.6 g because similar doses have shown to be beneficial for cardiovascular disease (45) hypertriglyceridemia (32) and NAFLD.

Our data showed that over 48 weeks of treatment, there were no significant beneficial effects of PUFA in this population. The two treatment groups were similar in terms of demographic characteristics and diabetic control as determined by HbA1C. Importantly, there was no change in body weight or body composition over the course of study in either study group. Of note, patients treated with PUFA did not show a statistically significant improvement in

histology while patients on placebo had significant improvement in steatosis and in the NAFLD activity score. In addition, markers of glycemic control worsened in the PUFA treated patients while lipid control did not change. These data are in contrast to previous data on the analysis of 23 trials using omega 3 PUFA in type 2 diabetes mellitus (17). Plasma triglyceride levels decreased while glycemic control and HDL did not change. The dose of PUFA in the present study was similar to those previously reported with beneficial effects.

Our observations in the present study differ from the randomized and open labeled studies previously reported. Even though the number of patients who showed improvement in overall histology was similar between the PUFA and placebo groups, it is interesting that the mean NAS improved in the placebo group but not in the PUFA. Improvement of patients in the placebo arm in this study is consistent with previous reports in randomized controlled studies on non diabetic patients with NASH that spontaneous improvement in liver histology occurs in 15-26% (7;19). These observations also suggest that PUFA might adversely affect NASH patients with DM. This could be due to worsening of glucose control as seen in the current study. Another possible explanation could be the use of other supplements including vitamin E but none of these patients had reported ingestion of vitamin E or other food supplements for the 6 months prior to or during the study period. Use of n-3 PUFA could have increased hepatic mitochondrial fatty acid oxidation by activation of PPAR α with increased oxidative stress (26;49) that could also have resulted in failure of beneficial effects. In addition, recent studies have shown a lack of clinical efficacy of PUFA in cardiovascular trials (24;25;42;43) and even potential harm in a cancer trial (2).

One of the limitations of the present study was that neither tissue nor plasma EPA and DHA were quantified and this could have contributed to the negative results. However, compliance was carefully assessed by clinical criteria (pill count technique and patient recall). Variable absorption and metabolism may also contribute to differential effects of the medications. Another limitation is the relatively small sample size that might have allowed for a type 2 error and potentially missing a positive effect of PUFA. However, this is unlikely given the relative positive effect in the placebo group compared to PUFA. Finally, patients were not rigorously monitored for dietary compliance and this may have contributed to worsening glycemic control and failure to respond to PUFA.

We therefore believe that it is principally our study design of using change in liver histology as an outcome measure and the study population of diabetic patients that explains our essentially negative observations. In fact, as mentioned earlier, PUFA may be worse than placebo in this patient group. Our data suggests that patients with type 2 diabetes may demonstrate metabolic heterogeneity determined by the presence or absence of NASH. The potential mechanisms by which omega 3 PUFAs lower plasma triglycerides include suppression of SREBP-1 with resultant reduced lipogenesis, decreased VLDL secretion and enhanced hepatic clearance of lipoproteins requiring a hepatic response (12). It is possible that patients with NASH do not respond appropriately to PUFA. This is the first study of its kind to specifically evaluate the response of an intervention that depends on its hepatic effects in diabetic patients with NASH. These data therefore provide a paradigm shift in

developing therapeutic interventions for diabetic patients and suggest that response to therapy may be determined by the severity of underlying hepatic dysfunction.

We conclude that despite strong rationale for the use of PUFA in NAFLD (31) the present study shows that in a well characterized population of NASH patients with diabetes, supplementation of n-PUFA provided no beneficial effects, and may potentially be inferior to placebo in terms of histological progression of the disease. Results from long term studies that are ongoing on the use of n-3 PUFA in NASH will need to be evaluated before PUFA can be recommended as a therapeutic intervention in this population at high risk of progression to cirrhosis. (Clinicaltrials.gov NCT 00323414).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Statement of Interests

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List of abbreviations

NASH	Non alcoholic steatohepatitis
PUFA	polyunsaturated fatty acids
HOMA	homeostatic model of assessment
DEXA	dual energy Xray absorptiometry
NAFLD	non alcoholic fatty liver disease
DM	diabetes mellitus
EPA	eicosapentaenoic acid
DHA	docosahexaenoic acid
ROS	reactive oxygen species

NAS	NAFLD activity score
CRN	clinical research network
BMI	body mass index
SD	standard deviation
SEM	standard error of mean

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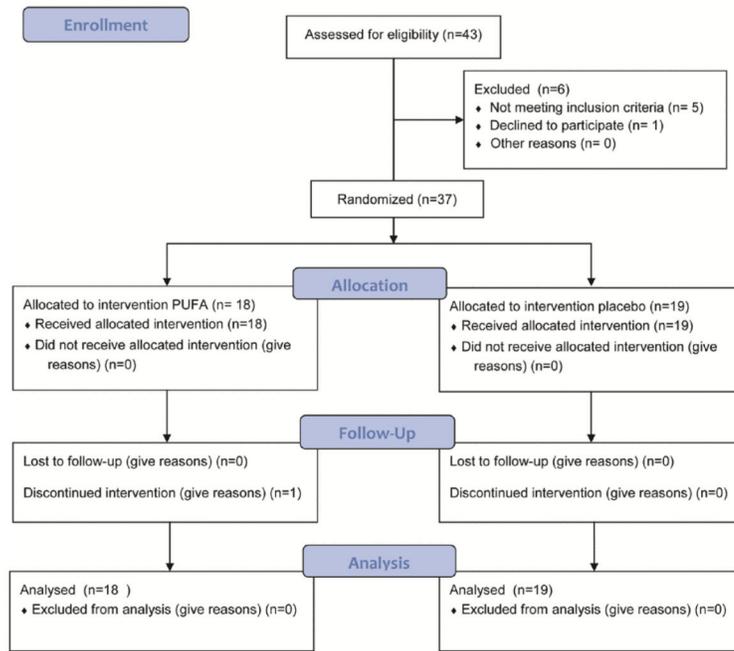


Figure 1.
CONSORT statement of study design.

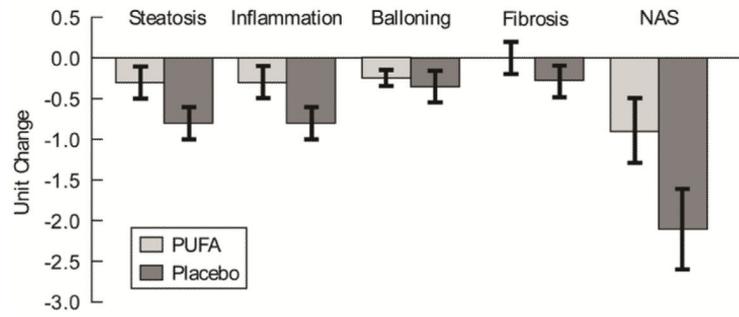


Figure 2. Histograms showing mean (\pm SEM) of difference in histological scores on liver biopsy in the PUFA and placebo groups.

Table 1

Clinical and laboratory characteristics

Characteristic	PUFA	Placebo
Number	18	19
Gender (M:F)	6:12	2:17
Age (y)	51.5±6.9	49.8±12.1
Ethnicity		
Caucasian	17 (94.4%)	17 (89.5%)
Hispanic	0	2 (10.5%)
Black	1 (5.6%)	0
Hypertension	17 (94.4%)	13 (68.4%)
Hyperlipidemia	9 (50%)	11 (47.9%)
<i>Laboratory results</i>		
AST (units/l)	47.7±22.8	49.1±22.1
ALT (units/l)	60.1±27.6	66.0±43.7
Total bilirubin (mg/dl)	1.1±0.3	1.0±0.1
Fasting glucose (mg/dl)	129.9±36.5	120.5±37.6
HOMA IR	12.01±6.8	15.5±4.9
HbA1C (g/dl)	6.7±0.9	6.7±0.7
Total cholesterol (mg/dl)	177.9±40.2	189.6±47.4
HDL (mg/dl)	40.3±7.0	41.1±9.7
Triglycerides (mg/dl)	190.2±108.9	233.0±169.6
<i>Liver biopsy findings</i>		
Steatosis grade		
1	3 (16.7%)	4 (21.1%)
2	7 (38.9%)	9 (47.4%)
3	8 (44.4%)	6 (31.6%)
Lobular inflammation grade		
1	2 (11.1%)	4 (21.0%)
2	12 (66.6%)	9 (47.4%)
3	4 (22.2%)	6 (31.6%)
Ballooning grade		

Characteristic	PUFA	Placebo
1	6 (33.3%)	7 (36.8%)
2	12 (66.7%)	12 (63.2%)
Fibrosis grade		
1	6 (33.3%)	7 (36.8%)
2	6 (33.3%)	7 (36.8%)
3	6 (33.3%)	5 (26.3%)

All values mean±SD

Table 2

Body composition in diabetic patients with NASH.

	PUFA		Placebo	
Weight	98.2±14.8	96.4±14.3	97.9±27.4	98.6±25.1
BMI (kg/m ²)	34.8±4.6	34.1±4.6	35.7±7.0	35.9±6.2
Waist circumference (cm)	113.1±10.2	113.0±11.7	110.8±15.7	112.1±14.6
Trunk fat	21.6±5.6	20.8±5.5	20.9±6.7	18.7±5.0
Total body fat	35.2±8.8	33.1±8.9	39.5±15.3	35.1±8.3
Total body lean mass	57.6±6.2	58.6±6.3	52.3±7.3	52.7±6.1
Total body bone mass	24.6±3.6	24.8±3.8	23.9±4.3	24.6±5.4

All values mean±SD

All measurements in kg.

Table 3

Primary histological outcomes

Characteristic	PUFA			Placebo		
	Entry	18	End of trt	Entry	19	End of trt.
<u>NAFLD activity score</u>	6.12±0.99		5.41±1.20	5.83±1.25		4.11±1.45 ^a
Decrease by 2 points		8 (44.4%)			9 (47.4%)	
Unchanged		7 (38.9%)			8 (42.1%)	
Worsened by 2 points		3 (16.7%)			2 (10.5%)	
<u>Steatosis</u>	2.29±0.77		2.06±0.75	2.06±0.75		1.47±0.72 ^b
Improved		8 (44.4%)			10 (52.6%)	
Unchanged		7 (38.9%)			8 (42.1%)	
Worsened		3 (16.7%)			1 (5.3%)	
<u>Lobular inflammation</u>	1.88±0.60		2.12±0.60	1.50±0.51		2.17±0.51 ^a
Improved		7 (38.9%)			10 (52.6%)	
Unchanged		8 (44.4%)			8 (42.1%)	
Worsened		3 (16.7%)			1 (5.3%)	
<u>Ballooning</u>	1 (5.3%)		1.47±0.51	1.69±0.48		1.38±0.50
Improved		6 (33.3%)			10 (52.6%)	
Unchanged		11 (61.1%)			7 (36.8%)	
Worsened		1 (5.6%)			1 (5.6%)	
<u>Fibrosis</u>	2.13±1.02		2.06±0.85	1.94±0.77		2.00±0.82
Improved		3 (16.7%)			3 (16.7%)	
Unchanged		12 (66.7%)			9 (47.4%)	
Worsened		3 (16.6%)			4 (21.1%)	

^a p<0.001 compared to Entry;

^b p<0.05 compared to entry.

Table 4

Secondary outcome measures

Group	PUFA		Placebo	
	Baseline	48 w	Baseline	48 w
Aspartate amino transferase (IU/dl)	47.7±22.8	41.7±17.4	47.8±22.1	47.9±38.1
Alanine amino transferase (IU/dl)	60.1±27.6	56.9±30.9	66.7±44.9	59.6±43.8
Serum bilirubin (mg/dl.)	1.1±0.3	1.1±0.3	1.0±0.1	1.0±0.0
Blood glucose (mg/dl)	129.9±36.5	150.4±43.7 ^a	121.5±38.4	123.5±22.9
Insulin (mIU/L)	37.7±17.7	42.3±20.7	55.4±50.6	43.6±21.6
HOMA	12.0±6.8	16.1±10.3 ^a	15.8±15.2	13.1±7.3
HbA1C (g/dl)	6.7±0.9	7.5±2.2 ^b	6.7±0.7	6.9±1.1
Serum triglycerides (mg/dl)	175.2±91.6	211.5±160.8	232.1±174.5	177.4±66.0
Serum HDL (mg/dl.)	40.6±7.1	43.2±8.9	40.8±9.4	41.6±9.3
Total cholesterol	177.9±40.2	182.5±55.5	189.6±47.4	187.2±32.1

All values mean±SD

^a p<0.05 compared to baseline;

^b p= 0.059 compared to baseline

Table 5

Placebo Controlled Trials in NAFLD

Author (Year)	n	Method of Diagnosis	Dose	Duration	Outcome ¹
Zhu [34]	144	Ultrasound	6,000	24 weeks	FOU, ALT, Lipids
Chen [35]	46	Ultrasound	5,000	24 weeks	FOU
Cussons [36] ²	25	Ultrasound	4,000	8 weeks	FOU
Sofi [33]	11	Ultrasound	830	12 months	FOU
Nohili [32] ³	60	Ultrasound/ Liver Biopsy	250/500	6 months	FOU
Dasarathy (present study)	37	NASH on Biopsy	3,600	48 weeks	Liver Histology

¹FOU = Fat on ultrasound

²This was a crossover study

³Only DHA used at two different doses: 250 and 500 mg