Effects of n-3 polyunsaturated fatty acids from seal oils on nonalcoholic fatty liver disease associated with hyperlipidemia

Feng-Shang Zhu, Su Liu, Xi-Mei Chen, Zhi-Gang Huang, Dong-Wei Zhang

Abstract

AIM: To investigate the efficacy and safety of n-3 polyunsaturated fatty acids (PUFA) from seal oils for patients with nonalcoholic fatty liver disease (NAFLD) associated with hyperlipidemia.

METHODS: One hundred and forty-four patients with NAFLD associated with hyperlipidemia were included in the 24-wk, randomized, controlled trial. The patients were randomized into two groups. Group A (n = 72) received recommended diet and 2 g n-3 PUFA from seal oils, three times a day. Group B (n = 72) received recommended diet and 2 g placebo, three times a day. Primary endpoints were fatty liver assessed by ultrasonography and serum lipid levels after 8, 12, 16, and 24 wk.

RESULTS: A total of 134 patients (66 in group A, 68 in group B) were included in the study except for 10 patients who were excluded from the study. After 24 wk of treatment, no change was observed in body weight, fasting blood glucose (FBG), renal function and blood cells of these patients. Total symptom scores, ALT, serum lipid levels and normalization of ultrasonographic evidence. Further study is needed to confirm these results.

CONCLUSION: Our results indicate that n-3 PUFA from seal oils is safe and efficacious for patients with NAFLD associated with hyperlipidemia and can improve their total symptom scores, ALT, serum lipid levels and normalization of ultrasonographic evidence. Further study is needed to confirm these results.

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Key words: Nonalcoholic fatty liver disease; Polyunsaturated fatty acids; Seal oil; Hyperlipidemia; Therapy

Peer reviewer: Simon D Taylor-Robinson, MD, Department of Medicine A, Imperial College London, Hammersmith Hospital, Du Cane Road, London W12 0HS, United Kingdom


INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a
spectrum of conditions characterized by an excessive accumulation of hepatic fat in the absence of alcohol consumption[1]. In Western countries and some regions of China, the prevalence of nonalcoholic steatohepatitis (NASH) and NAFLD is 1%-5% and 15%-30%, respectively[2]. In most of patients, NAFLD follows a relatively benign course and remains stable for years[1]. However, it was recently reported that many cases of cryptogenic liver cirrhosis may be related to unrecognized NASH[1]. Thus, treatment should be reserved for patients at risk of developing severe liver diseases. Medical therapy for NAFLD and NASH has been disappointing to date[3]. Although a number of treatment modalities are available, they cannot prevent the progression of early liver disease to its advanced stage, and the only recommended therapies are dietary modification and weight loss[4,5]. It has been shown that n-3 polyunsaturated fatty acids (PUFA) is effective on NAFLD[6,7]. In this study, we evaluated the efficacy and safety of n-3 PUFA from seal oils in ameliorating serum lipids and liver enzymes in patients with NAFLD associated with hyperlipidemia.

MATERIALS AND METHODS

Patients
One hundred and forty-four patients with NAFLD associated with mixed dyslipidemia were studied as outpatients in the Tongji Hospital, Tongji University, from September 2006 to June 2008. Written informed consent was obtained from all patients and the study was approved by the Ethics Committee in Tongji Hospital of Tongji University.

The inclusion criteria were as follows: age between 18 and 65 years, lack of excessive alcohol ingestion confirmed by careful questioning by the primary physician and dietitians (consumption of less than 70 g alcohol in female and 140 g in male per week), elevated serum alanine aminotransferase (ALT) and/or aspartate aminotransferase (AST) above the normal limit, but under 5 times the upper limit of the normal range for ALT and 2 times the upper limit of the normal range for AST; grade ≥ 6 mo before the study. Diagnosis of dyslipidemia was based on the presence of one or more of the following findings: fasting serum total cholesterol (TCHO) > 5.7 mmol/L, serum triglyceride (TG) > 1.8 mmol/L and high-density lipoprotein cholesterol (HDL-C) < 0.8 mmol/L, fatty liver diagnosed by abdominal ultrasonography, and ability to give informed consent. Exclusion criteria included overuse of alcohol, viral hepatitis, hemochromatosis, Wilson’s disease, autoimmune hepatitis, α-1 antitrypsin deficiency, primary sclerosing cholangitis or primary biliary cirrhosis; history of any other hepatic, gastrointestinal, renal, cardiovascular, neurological or hematological disorders, psychiatric disorder which might impair the ability of patients to provide written informed consent; as well as pregnancy, breastfeeding, or lack of effective birth control in women at child-bearing age. In addition, patients were excluded if they were on any medications that could influence liver function during the observation period or involved in any clinical trial before the study.

Study design
All the patients meeting the criteria for enrollment agreed to participate in the study. The patients were randomized into two groups to receive a 24-wk treatment. Group A (n = 72) received recommended diet and 2 g n-3 PUFA from seal oils (Shanghai Hengsheng Biology & Medicine CO. Ltd, Shanghai, China), three times a day. Group B (n = 72) received recommended diet and 2 g placebo (Shanghai Hengsheng Biology & Medicine CO. Ltd, Shanghai, China), three times a day. Recommended diet was composed of 50% carbohydrates, 20% protein and 30% fat in accordance with the American Heart Association diet[8]. All obese and overweight patients were advised to lose their weight with a restriction of daily caloric intake to 25-30 kcal/kg per day[9]. All medications the patients received during the 24-wk treatment period were recorded. At the time of enrollment and when the study was completed, body temperature, body mass index (BMI), blood pressure and heart rate were detected and liver ultrasonography was performed. Laboratory tests included serum ALT, AST, γ-glutamyltranspeptidase (GGT), TG, TCHO, HDL-C, LDL-C, FBS, and complete blood cell counts.

During the 24-wk treatment period, total symptom scores, liver enzymes and fasting lipids were monitored at weeks 8, 12, 16, and 24. Hepatic fat infiltration was detected by upper abdominal ultrasonography at weeks 12 and 24. Symptoms included liver discomfort or pain, weakness, abdominal distention, and nausea. The severity of each clinical symptom was scored using a 4-point scale as follows: 0 score = asymptomatic, 1 score = mild, 2 scores = moderate, 3 scores = severe. All patients were investigated after 12 h fasting and underwent ultrasonography for liver steatosis. Ultrasound scans were performed by a trained operator who was blind to the treatment of participants. The severity of steatosis was also scored using a 4-point scale as follows[10]: grade 0 = normal echogenicity, grade 1 = slight, grade 2 = moderate, grade 3 = severe.

Statistical analysis
The data were presented as mean ± SD and analyzed using SPSS11.5 for Windows (SPSS, Chicago, IL, USA). Statistical analysis for baseline characteristics of the study groups was performed using χ² test and t-test. Student’s t-test and Wilcoxon signed rank test were used to evaluate the changes in biochemical parameters before and after treatment. P < 0.05 was considered statistically significant.

RESULTS

Characteristics of the patients
Of the 144 patients enrolled in this study, 134 completed the protocol and were included in the analysis. The baseline clinical and demographic data about the two
groups are shown in Table 1. Patients in the two groups were matched for age, sex, body height and weight, BMI, HR, course of disease, blood pressure, and blood tests including transaminase and lipid concentrations. The ultrasound stages of steatosis were also paired ($P < 0.05$). No patient was classified as grade 0. In group A, 37% of the patients were classified as grade 1, 48% as grade 2, and 15% as grade 3, respectively. In group B, 30% of the patients were classified as grade 1, 56% as grade 2, and 14% as grade 3, respectively.

**Findings in the two groups before and after treatment**

No significant difference was observed in dietary compliance, BMI, blood pressure, HR, HB, RBC, WBC, platelet, serum BUN and creatinine(Cr) between the two groups. At the end of a 24-wk treatment period, a significant improvement in several liver and lipid parameters was observed between the two groups. In particular, total symptom scores, ALT and TG levels decreased more significantly ($P < 0.01$) in Group A (total symptom score = $1.87 \pm 1.18$ to $0.42 \pm 0.72$, ALT = $62.79 \pm 35.92$ U/L to $39.27 \pm 18.94$ U/L, TG = $3.94 \pm 2.69$ mmol/L to $2.08 \pm 1.03$ mmol/L) than in Group B (total symptom score = $1.87 \pm 1.18$ to $0.42 \pm 0.72$, ALT = $79.76 \pm 50.59$ U/L to $42.32 \pm 22.23$ U/L, TG = $3.80 \pm 2.85$ mmol/L to $2.33 \pm 1.42$ mmol/L) (Table 2). As compared to the pretreatment values, total symptom score, ALT and TG levels at weeks 8, 12, and 16 decreased significantly in the two groups after treatment ($P < 0.01$).

As expected, there was a tendency toward improvement in AST, GGT, TCHO, and HDL levels ($P < 0.05$) in the two groups after treatment. However, no significant difference was observed in the two groups.

**Table 1** Baseline characteristics of groups A and B (mean ± SD)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group A ($n = 66$)</th>
<th>Group B ($n = 68$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>45.00 ± 10.91</td>
<td>44.03 ± 11.30</td>
<td>0.74</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>47/19</td>
<td>50/18</td>
<td>0.06</td>
</tr>
<tr>
<td>Body height (cm)</td>
<td>169.07 ± 7.96</td>
<td>169.71 ± 7.89</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>75.71 ± 10.99</td>
<td>75.16 ± 11.33</td>
<td>0.93</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>26.37 ± 3.12</td>
<td>25.96 ± 2.70</td>
<td>0.57</td>
</tr>
<tr>
<td>HR (/min)</td>
<td>75.57 ± 6.24</td>
<td>75.89 ± 6.58</td>
<td>0.32</td>
</tr>
<tr>
<td>Duration of NAFLD (mo)</td>
<td>22.32 ± 38.82</td>
<td>13.65 ± 20.00</td>
<td>0.55</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>126.59 ± 10.97</td>
<td>125.89 ± 9.97</td>
<td>0.58</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>82.26 ± 7.50</td>
<td>81.89 ± 8.02</td>
<td>0.81</td>
</tr>
<tr>
<td>HB (g/L)</td>
<td>143.31 ± 16.49</td>
<td>145.33 ± 15.52</td>
<td>0.58</td>
</tr>
<tr>
<td>RBC (× 10$^9$/L)</td>
<td>4.71 ± 0.57</td>
<td>4.76 ± 0.61</td>
<td>0.84</td>
</tr>
<tr>
<td>WBC (× 10$^9$/L)</td>
<td>6.16 ± 1.58</td>
<td>6.52 ± 1.45</td>
<td>0.18</td>
</tr>
<tr>
<td>Platelet (× 10$^9$/L)</td>
<td>205.79 ± 49.43</td>
<td>195.78 ± 53.33</td>
<td>0.53</td>
</tr>
<tr>
<td>BUN (mmol/L)</td>
<td>5.30 ± 1.67</td>
<td>5.19 ± 1.57</td>
<td>0.63</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>72.56 ± 15.27</td>
<td>76.69 ± 15.35</td>
<td>0.2</td>
</tr>
<tr>
<td>FBC (mmol/L)</td>
<td>5.89 ± 1.20</td>
<td>5.46 ± 1.82</td>
<td>0.43</td>
</tr>
<tr>
<td>Total symptom scores</td>
<td>1.87 ± 1.18</td>
<td>1.79 ± 0.45</td>
<td>0.23</td>
</tr>
<tr>
<td>Steatosis degree 0/1/2/3 (%)</td>
<td>0/30/56/14</td>
<td>0/37/48/15</td>
<td>0.63</td>
</tr>
</tbody>
</table>

BMI: Body mass index; HR: Heart rate; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; HB: Haematoglobin; RBC: Red blood cells; WBC: White blood cells; BUN: Blood urea nitrogen; FBG: Fasting blood glucose.

TCHO: Total cholesterol; TG: Triglycerides; HDL-C: High density lipoprotein cholesterol; LDL-C: Low-density lipoprotein cholesterol. *$P < 0.05$, **$P < 0.01$ vs baseline; ***$P < 0.05$, ****$P < 0.01$ vs group B.

Significant improvements were found in serum LDL-C levels of group A ($P < 0.05$), but not found in group B after treatment.

Ultrasonography showed a normal liver echopattern at the end of treatment in 19.70% (13/66) of the patients, and an overall reduction in 53.03% (35/66) of the patients in group A. In contrast, only five patients (7.35%, 5/68) achieved complete regression ($P = 0.04$), whereas 24 patients (35.29%, 24/68) had a certain reduction ($P = 0.04$) in group B. No change was
observed in the remaining 64.71% of patients.

Gastrointestinal complaints of increased fecal frequency, epigastria, and defecation were occasionally noted in 8 of the 134 patients; but these adverse effects were not significantly different in the two groups. The patients recovered when they completed the treatment. Most of the patients who completed the treatment had no adverse events, indicating that they can tolerate the treatment. No severe adverse event was observed.

**DISCUSSION**

NAFLD is a chronic disease with multiple consequences. The spectrum of this disease ranges from simple steatosis to NASH, which may lead to liver fibrosis and cirrhosis. It has also been well established that NAFLD is intimately related to various clinical and biological markers of the insulin resistance syndrome. The pathogenesis of NASH is multifactorial, including insulin resistance, excessive intracellular fatty acids, oxidant stress, mitochondrial dysfunction and innate immunity. However, the pathogenesis of NAFLD/NASH is yet to be clearly elucidated. Since the most prevailing general theory is the “two-hit” hypothesis proposed by Day and James in 1998, most treatment modalities should be focused on improving the “two-hit” hypothesis or insulin resistance.

Currently, therapeutic options are limited. The present “gold standard” for NAFLD is weight reduction, or more precisely, a reduction in central obesity so as to reverse insulin resistance. Standard practice advocates weight loss and exercise. Such “lifestyle adjustment” or anti-obesity measures (including bariatric surgery when required) can improve insulin sensitivity with only a modest weight loss (2-8 kg), which is difficult for most patients to achieve.

It was reported that dietary supplementation with fatty acids, such as fish and fish oils, can improve NAFLD associated with hyperlipidemia by modifying the function of platelets and leukocytes. Suggested modes of action are through their modulation of eicosanoid synthesis and reduction in plasma TG concentration. The fat composition of seal oils differs significantly from that of fish. In marine mammals, eicosapentaenoic acid (EPA, 20:5 n-3) and docosahexaenoic acid (DHA, 22:6 n-3) are found mainly at the sn-1 and sn-3 positions of TG, whereas in fish, these fatty acids are positioned in sn-2, which may display the better effects on NAFLD associated with hyperlipidemia than fish oils.

Increased fat intake with an excessive amount of n-6 fatty acids can promote NAFLD. However, n-3 PUFA can ameliorate experimental NAFLD. It was reported that n-3 PUFA from different animals is effective against NAFLD. In this study, we evaluated the efficacy and safety of n-3 fatty acids from seal oils in 144 patients with NAFLD associated with hyperlipidemia. The results showed that treatment of NAFLD patients with hyperlipidemia with n-3 PUFA from seal oils significantly reduced their total symptom score, ALT and lipid levels, and normalized ultrasonographic evidence compared to treatment with the recommended diet alone.

As expected, there was a tendency toward improvement in AST, GGT, TC, and HDL levels ($P < 0.05$) in the two groups after treatment. However, no significant difference was seen in the two groups. Spadaro et al. also reported that serum GGT, HDL, ALT, and lipid levels are decreased after treatment. In the present study, the values of LDL were significantly improved in group A ($P < 0.05$), but not in group B at different time points and after a 24-wk treatment period. Ultrasonography showed complete fatty liver regression in 19.70% (13/66) of the patients, and an overall reduction in 53.03% (35/66) of the patients in group A. In contrast, only five patients (7.35%, 5/68) achieved complete regression ($P = 0.04$), whereas 24 patients (35.29%, 24/68) had a certain reduction ($P = 0.04$) in group B. No change was observed in the remaining 64.71% of patients.

Tanaka et al. reported that treatment with EPA, one of the major components of n-3 PUFA, seems to be safe and efficacious for patients with NASH, largely due to its anti-inflammatory and anti-oxidative properties. On the other hand, n-3 PUFA could reduce VLDL production, resulting in decreased serum triglyceride levels. These findings are consistent with our findings, such as improvement in total symptom score, ALT and lipid levels and normalization of ultrasonographic evidence in patients with NAFLD associated with hyperlipidemia. Improvement in serum biochemistry parameters was also observed in group B, indicating that restricted diet and exercise can reverse insulin resistance at a certain extent. In the present study, the two drugs (placebo and seal oils) appeared to be safe and effective in patients with NAFLD associated with hyperlipidemia and no severe side effects were observed during treatment.

The gold standard for diagnosis of NAFLD is liver biopsy, but it is not frequently performed in NAFLD patients due to its low acceptance rate. In our study, ultrasonography was performed to detect and monitor changes in the liver since it is sensitive, cheap, invasive and easy to perform. However, lack of histological findings is a major drawback of this investigation.

In conclusion, treatment of NAFLD associated with hyperlipidemia with PUFA from seal oils seems to be safe and efficacious, and can improve the total symptom score, ALT and lipid levels and normalization of ultrasonographic evidence. Further study is needed to confirm these results.

**COMMENTS**

**Background**

Recent reports suggest that many cases of cryptogenic liver cirrhosis may be related to unrecognized nonalcoholic steatohepatitis (NASH); however, medical therapy for nonalcoholic fatty liver disease (NAFLD) and NASH has been disappointing to date. The only recommended therapies are dietary modification and weight loss. N-3 polyunsaturated fatty acids (PUFA) seems to be efficacious on treating NAFLD from animal and some small samples human studies.
**Research frontiers**

The present “gold standard” for treatment of NAFLD is a reduction in central obesity so as to reverse insulin resistance. Several small samples randomized trials have suggested n-3 PUFA from different animals were effective in the treatment of NAFLD; we explored whether seal oil is efficacious and safe in large samples NAFLD patients.

**Innovations and breakthroughs**

Total symptom scores in NAFLD patients, and large samples were observed besides biochemical indicators and ultrasonography in this study. Seal oils n-3 PUFA can improve liver enzyme, serum lipid levels and normalization of ultrasonographic evidence.

**Applications**

Seal oils PUFA administration seems to be safe and efficacious for patients with NAFLD associated with hyperlipidemia as well as the “lifestyle adjustment” or anti-obesity measures.

**Terminology**

NAFLD refers to the presence of hepatic steatosis not associated with a significant intake of ethanol. Insulin resistance is central to the pathogenesis of NAFLD, thus obesity, diabetes, and the metabolic syndrome are frequently associated with the disease.

**Peer review**

This is an interesting study. Further details need to be given as to the precise ultrasound scoring system that was used to assess the resolution of hepatic steatosis. Was there an objective scoring system used or was this all subjective? The paper is otherwise well written and merits publication.

**REFERENCES**

patients with non-alcoholic fatty liver disease: a pilot study. 


