Omega-3 supplementation and non-alcoholic fatty liver disease: A systematic review and meta-analysis

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is characterised by increased hepatic fat accumulation in individuals not consuming excessive alcohol and represents a spectrum of disease ranging from 'simple' steatosis to non-alcoholic steatohepatitis [2], which is only distinguishable by histological examination [3,4]. Non-alcoholic steatohepatitis (NASH), the inflammatory component, predisposes to hepatic fibrosis, cirrhosis, and subsequent end-stage liver disease and hepatocellular carcinoma [5–7]. NAFLD is independently associated with coronary heart disease [8], insulin resistance [9,10], and type II diabetes [10,11]. In Western populations, the prevalence of NAFLD may exceed 30% [12], and can be as high as 88% in the obese [13]. Risk factors for the development of NAFLD include central obesity, type II diabetes, dyslipidemia, and hypertension [10,11,13–15]. Given the increasing prevalence and incidence of these conditions [16–21], the global burden of NAFLD is expected to increase.

Currently, the primary treatment for NAFLD is weight loss by lifestyle therapy involving diet and exercise. Weight loss has been shown to improve liver enzymes [22–24], decrease plasma triglycerides [23,25] and improve liver fatness, as measured by magnetic resonance spectroscopy (MRS), ultrasonography or direct histological evaluation [25–27]. Major reductions in weight and consequent improvements in liver pathology can also be achieved by bariatric surgery [23,25], but this is not feasible for the large number of patients presenting with this disease [20,28–31]. Similarly, pharmacotherapy including insulin sensitisers, hypolipidemias and vitamin E [2,32] have been trialled in small cohorts and their effectiveness is limited by poor compliance [33,34], associated weight gain [32], and side-effects [35]. Current evidence from available randomised control trials suggests that only thiazolidinediones actually reduce liver fat [2,32,35].

Although studies investigating the dietary patterns of patients with NAFLD vs. controls have reported conflicting results with respect to the importance of macronutrient composition [36–41], several studies have implicated dietary alteration beyond gross macronutrient change as causal in the development of NAFLD. When compared with controls, individuals with NAFLD have been shown to have lower fish [41] and polyunsaturated fat intake [38] and a higher n−6/n−3 consumption [36]. Accordingly,
Given the well-recognised problems of adherence to lifestyle interventions, achieving sustainable weight loss, and side-effects with pharmacological agents, dietary fish oil supplementation represents a simple and practical alternative therapy. Fish oil provides a convenient source of essential n-3 PUFAs with few side effects [45–48] and may directly reduce hepatic lipogenesis and steatosis [1,49]. A recent systematic review of randomized controlled trials which included subanalysis of the efficacy of n-3 PUFAs for reducing hepatic fat showed a decrease in the relative levels of n-3 PUFAs compared with controls, and an increase in the hepatic n-6/n-3 PUFAs ratio [1,42,43]. These findings have been confirmed in animal studies [44].

Analysis of the composition of hepatic long chain fatty acids has shown a decrease in the relative levels of n-3 PUFAs (polyunsaturated fatty acids) in patients with NAFLD compared with controls, and an increase in the hepatic n-6/n-3 PUFAs ratio [1,42,43]. These concurrent interventions were given to both patients undergoing omega-3 treatment and those who refused treatment were considered as the control group (n = 1; [45]); and uncontrolled design, in which all participants underwent omega-3 treatment (n = 2; [46,47]). There were no reports of adverse effects of omega-3 PUFAs in the studies reviewed.

Data was analysed for 355 individuals who participated in the nine studies; 200 were male (56.3%). The median duration of treatment with omega-3 fatty acids was 6 months (range: 8 weeks to 12 months). The median dose of PUFAs was 4 g/day (range: 0.8–13.7 g/day). Six studies specified the dosage of EPA and DHA (range: 0.375–4.626 g of EPA per day; 0.24–2.24 g of DHA per day). One study used highly purified EPA only [47]. Three studies gave dietary recommendations to all participants [48,53,54] and three studies advised on weight reduction by caloric restriction for participants who were overweight or obese [46,48,54]. These concurrent interventions were given to both the treatment and control groups. In all conditions, subjects were advised to maintain their usual physical activity habits.
Measurement methods used to quantify change in liver fatness included ultrasound (seven studies), magnetic resonance spectroscopy (two studies), and liver biopsy (two studies; with eight patients in total having repeat biopsies).

For the purposes of data pooling and analysis, the ‘high dose’ group was selected as the ‘treatment’ group for analysis in the study by Chen et al. [55]; and it was deemed that there was no appropriate ‘control’ group in the study by Hatzitolios et al. [46] as the two other groups of participants each received lipid-lowering pharmacological agents, not placebo or no treatment.

Effect of fish oil on liver fat and liver tests

The effect of fish oil/omega-3 therapy on liver fatness and function is summarised in Table 1 and Figs. 2–4. Seven studies provided sufficient data to enable calculation of mean differences, effect size and 95% confidence intervals (95% CI) for liver fat, seven studies for ALT, and six studies for AST (Figs. 2–4). For liver fat, six of the seven studies showed an ES favouring PUFA therapy, ranging from −0.48 to −1.72 (p <0.001). Six of these studies showed a statistically significant effect for PUFA supplementation on liver fat. Two of the seven studies showed an ES favouring PUFA therapy on ALT, ranging from −0.90 to −2.35, both of which were statistically significant. One study showed an ES favouring the control condition on ALT which was significant [48]. Of the six studies which measured AST, three studies showed an ES favouring PUFA therapy, ranging from −0.84 to −2.86, all of which were statistically significant. One study showed an ES favouring the control condition on AST which was statistically significant.

Quality analysis

The results of quality analysis are detailed in Supplementary Table 1. External validity, measures of compliance, blinding of subjects and data collectors, and researchers blinding to randomisation until the completion of the study were poorly executed and/or poorly reported. These limitations can be attributed to the pilot nature of some studies where no randomisation or no control group was formed. Four of the nine studies achieved adequate power to detect a change in liver fat; two studies had insufficient data to calculate power for liver fat.

Table 1. Characteristics of included studies.

<table>
<thead>
<tr>
<th>Authors, year [Ref.]</th>
<th>N (total)</th>
<th>Population (method of diagnosis); Gender; Mean BMI category</th>
<th>Dose n-3/day</th>
<th>Duration</th>
<th>Control</th>
<th>Other instructions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Capanni et al., (2006) [45]</td>
<td>56</td>
<td>NAFLD (ultrasound); M/F; Overweight</td>
<td>1 g</td>
<td>12 mo</td>
<td>No treatment</td>
<td>-</td>
</tr>
<tr>
<td>Chen et al., (2008) [55]</td>
<td>46</td>
<td>NAFLD (elevated LFTs and TGs); M/F; Not specified</td>
<td>5 g</td>
<td>24 wk</td>
<td>Placebo</td>
<td>-</td>
</tr>
<tr>
<td>Cussons et al., (2009) [56]</td>
<td>25</td>
<td>Pre-menopausal women with PCOS; Obese</td>
<td>4 g</td>
<td>8 wk</td>
<td>Placebo</td>
<td>Maintain usual dietary and activity habits</td>
</tr>
<tr>
<td>Hatzitolios et al., (2004) [46]</td>
<td>73</td>
<td>Mixed dyslipidemia (&gt;1 of: fasting serum cholesterol &gt;220 mg/dl; serum TG &gt;200 mg/dl; HDL &lt;45 mg/dl); M/F; Overweight</td>
<td>13.7 g</td>
<td>24 wk</td>
<td>Alternative medication (atorvastatin, orlistat)</td>
<td>BMI &gt;25: advised weight reduction</td>
</tr>
<tr>
<td>Sofi et al., (2010) [53]</td>
<td>11</td>
<td>Persistently (&gt;6 mo) elevated serum ALT + ultrasonographic features indicative of fatty liver; M/F; Overweight</td>
<td>0.83 g</td>
<td>12 mo</td>
<td>Placebo</td>
<td>Dietary recommendations (not specified)</td>
</tr>
<tr>
<td>Spadaro et al., (2008) [54]</td>
<td>36</td>
<td>NAFLD (elevated ALT + ultrasound); M/F; Obese</td>
<td>2 g</td>
<td>6 mo</td>
<td>No placebo</td>
<td>Calorie restricted AHA recommended diet</td>
</tr>
<tr>
<td>Tanaka et al., (2008) [47]</td>
<td>23</td>
<td>Biopsy-proven NASH; M/F; Obese</td>
<td>2.7 g</td>
<td>12 mo</td>
<td>-</td>
<td>Maintain usual medications, dietary and activity habits</td>
</tr>
<tr>
<td>Vega et al., (2008) [57]</td>
<td>16</td>
<td>Subset of DHS cohort: elevated HTGC (MRS), + average ALT within reference range; M/F; Obese</td>
<td>9 g</td>
<td>8 wk</td>
<td>Placebo</td>
<td>-</td>
</tr>
<tr>
<td>Zhu et al., (2008) [48]</td>
<td>134</td>
<td>NAFLD associated with mixed dyslipidaemia; M/F; Overweight</td>
<td>2 g</td>
<td>24 wk</td>
<td>Placebo</td>
<td>AHA recommended diet; overweight and obese: advised caloric restriction (25-30 kcal/kg BW/day) for weight loss</td>
</tr>
</tbody>
</table>
Effect of fish oil on liver fat and liver tests (meta-analyses)

Liver fat
There was a significant pooled ES for the efficacy of PUFA therapy on liver fat (ES = -0.84, 95% CI: -0.64 to -1.05; p < 0.001). Significant heterogeneity among studies was observed (I² = 66.12%, p = 0.007). Using a random effect model, there was a significant pooled ES for the efficacy of PUFA therapy on liver fat (ES = -0.96, 95% CI: -0.43 to -1.48; p < 0.001).

ALT
There was a significant pooled ES for the efficacy of PUFA therapy on ALT (ES = -0.25, 95% CI: -0.06 to -0.44, p = 0.01), however, significant heterogeneity was found to exist between studies: I² = 88.32% (p < 0.001). With the random effects model, the pooled ES for ALT showed a trend toward PUFA therapy vs. control on ALT but this did not reach statistical significance (ES = -0.56, 95% CI: -0.03 to -1.16, p = 0.06) (Fig. 3). When only RCT data were analysed, there was no significant pooled ES for the efficacy of PUFA therapy on ALT (ES = -0.18, 95% CI: -0.81 to 0.58; p = 0.74).

AST
There was a significant pooled ES favouring PUFA therapy vs. control on AST (ES = -0.38, 95% CI: -0.16 to -0.60, p < 0.001). Significant heterogeneity was found to exist between studies: I² = 91.62% (p < 0.001). Using random effects model, there was a significant pooled ES favouring PUFA therapy vs. control on AST (ES = -0.72, 95% CI: -2.02 to 0.58; p = 0.28).

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**Fig. 1.** Flowchart showing the process for the inclusion of studies.  
**Fig. 2.** Meta-analysis of effect of omega-3 supplementation on liver fat using a random effects model.
Discussion

The present investigation provides the first meta-analysis of studies investigating the effect of omega-3 PUFA on liver fat in humans. The data show that, despite significant heterogeneity in study design, marine omega-3 fatty acid supplementation in humans is associated with a positive effect on liver fat. Importantly, this effect persisted when only RCTs were examined. Despite a significant benefit of PUFA therapy on AST and a tendency toward a benefit on ALT, these effects were not significant after examination of only RCT data.

Our results build on the findings of a recent review by Musso et al. [32], which provided a meta-analysis of ALT data from three RCTs available at that time. The current review pooled data from a larger number of studies and showed a benefit on liver fatness, but like Musso et al., found no significant benefit on ALT levels. The current data also suggest that AST is unaffected by PUFA supplementation. However, there was a lack of well-controlled, randomised trials, and consequently a lower overall quality rating for the available data. Furthermore, it is well acknowledged that there is high intra-individual variability in liver tests which may reduce the ability to detect significant changes in these parameters with interventions.

Although nine studies were identified that examined the effect of dietary omega-3 PUFA supplementation on liver fat, two studies [46,57] could not be included in liver fat analyses because of insufficient data. Two and three studies had insufficient data for inclusion in ALT analyses [55,57] and AST analyses.
[55–57], respectively. There was significant statistical heterogeneity between studies, and study design varied markedly. Four studies employed non-randomised designs, duration of intervention ranged from 8 weeks to 12 months (median: 6 months), and dose of omega-3 PUFA ranged from 0.8 to 13.7 g per day (median: 4 g/day). When considering the effect of duration of intervention on the study results, the magnitude of change in liver fat and liver enzymes was not a function of duration of dietary supplementation with omega-3 PUFA because studies of long duration (12 months) [45,47,53] yielded similar or even smaller-magnitude changes to those of short [56] or medium [46,48,54,55] duration (Figs. 2–4). Although no studies reported compliance rate, the similar magnitude of effect may be due to lower average compliance over the longer period of intervention. Dose of omega-3 PUFA also did not appear to alter the effect of supplementation: over the same duration of intervention (6 months), omega-3 PUFA dose of 5 g per day gave a reduction in steatosis grade ES: −0.73 (95% CI: −0.02 to −1.44) [55], while 2 g omega-3 PUFA per day gave a similar effect size for a reduction in steatosis grade: −0.63 (−0.97 to −0.28) [48]. Given the limited number of included studies, the variation in design including dose and duration of omega-3 supplementation, it is clear that further research is required before recommendations can be made regarding the optimal dose and duration of therapy for this patient cohort.

**Key Points**

- Omega-3 supplementation decreases liver fat
- The optimal dose required has not been determined, but benefits are seen with ≥0.83 g/day of omega-3 supplementation
- Well-designed randomized controlled trials to quantify the reductions in liver fat and the optimal dose of omega-3 supplementation are urgently required

Given that NAFLD is associated with an increased risk of cardiovascular disease (CVD), potential hepatic benefits of PUFA therapy should be considered in combination with its effects on cardio-metabolic risk factors, including insulin resistance and dyslipidaemia [10,11,13–15]. On the basis of the cohorts included in the present meta-analyses in which these parameters were measured: 8 of 9 studies reported a statistically significant benefit of omega-3 PUFA supplementation on blood triglycerides [45,46,48,53–57]; 2 of 5 showed a significant improvement in low-density lipoprotein cholesterol (LDL-c) [46,48] (3 of 5 reported non-significant changes [53,56,57]) and 1 of 4 studies noted an improvement in fasting glucose concentration [45] (3 of 4 reported non-significant changes [47,53,56]). The sole study that measured blood pressure reported significant improvements in systolic and diastolic blood pressure [56]. However, undertaking meta-analyses on this sub-set of data would not be appropriate as it fails to represent the larger body of evidence available regarding the effect of omega-3 PUFA supplementation on glycaemic control, blood pressure, lipids, and lipoproteins. There is clear evidence from meta-analyses that PUFA supplementation improves blood triglycerides [58–60] and blood pressure [61] but is associated with a small elevation in LDL-c [58–60]. There is some evidence to suggest that PUFA supplementation may elevate fasting blood glucose concentration [59], but the weight of data suggests that this is not significant [58,60]. It should be noted that the study by Zhu et al. [48] used dietary intervention in conjunction with omega-3 PUFA supplementation whereas Chen et al. [55] did not, and the results may be influenced by dietary intake. It is notable that three of the four available RCTs used a combination of omega-3 supplementation with dietary recommendations or diet therapy, and the addition of this dietary component may also have had a marked effect on serum liver enzymes and liver steatosis. A potential limitation of our data is that we pooled data from different measurement techniques for liver fat analysis (MRS, ultrasound). Importantly, we have reported ES which is based on the mean change and variability between intervention and control groups as opposed to quantitative changes, which mitigates this problem. Although pooling data in this way could be confounded by differences in measurement error of the techniques (the reported coefficient of variation for MRS is lower than ultrasound [62]) 6 of the 7 studies in the pooled meta-analysis and all (4 of 4) in the RCT-only meta-analysis used the same measurement technique (ultrasound). We acknowledge that, at present, there is a lack of data from more sensitive, readily-available techniques such as MRS and suggest that this should be a pre-requisite in future studies.

Individuals with NAFLD have been shown to have a lower dietary intake of omega-3 fatty acids than healthy controls [36,63], and biochemical analyses have shown alteration in the hepatic long chain fatty acid composition towards an increase in the n−6/n−3 ratio. Animal data has shown that this is associated with a pro-inflammatory state [64–66] and increased lipogenesis leading to steatosis [42,49,67]. Conversely, omega-3 PUFAs are known to down-regulate sterol regulatory element binding protein 1c (SREBP-1c) and upregulate peroxisome proliferator activated receptor α (PPAR-α) which would favour fatty acid oxidation and reduce steatosis [1]. Given these observations and the fact that omega-3 PUFAs are essential in the human diet and unable to be synthesised de novo, dietary supplementation of n−3 PUFAs has been suggested to be efficacious for the management of NAFLD [67]. Our data support PUFA supplementation as effective for liver fat reduction in human trials, as indicated by a significant and strong effect size. This compares favourably with other pharmaceutical and nutraceutical interventions including metformin, simvastatin, ursodeoxycholic acid, vitamins E and C, and betaine [32]. A reduction in steatosis with omega-3 PUFA supplementation in the absence of weight loss was shown in five of the studies reviewed, which is clinically significant, and supplementation led to the amelioration of liver steatosis as inferred by ultrasonography for 27% of patients who received omega-3 supplementation in these studies [45,47,48,53,54]. However, there is currently little information concerning the magnitude of hepatic benefit as assessed by magnetic resonance imaging (MRI), which is considered to be the gold standard for quantifying liver fat [68,69]. Nine RCTs (clinicaltrials.gov identifier: NCT00819338, NCT00230113, NCT01277237, NCT01285362, NCT00760513, NCT00681408, NCT00845845, NCT01154985) are currently evaluating omega-3 PUFA in NAFLD or NASH and their results should provide additional information.

The present analysis represents the first systematic review suggesting that omega-3 PUFA supplementation can reduce liver
Review

steatosis in humans. From the available data, there is no evidence of a significant effect on liver tests. The limited number of RCTs, variable study designs and potential confounding from differential dietary intake, limit the strength of available data. Additional well-designed and larger randomised controlled trials are required to establish optimal doses, define appropriate patient groups and to quantify the benefit of omega-3 PUFA therapy in patients with NAFLD.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Supplementary data

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References


