

Systematic review and meta-analysis of controlled intervention studies on the effectiveness of long-chain omega-3 fatty acids in patients with nonalcoholic fatty liver disease

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Context: Treatment options for nonalcoholic fatty liver disease (NAFLD) are needed. **Objective:** The aim of this review was to systematically assess the effects of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), particularly eicosapentaenoic acid and docosahexaenoic acid, on liver-related and metabolic outcomes in adult and pediatric patients with NAFLD. **Data Sources:** The online information service ProQuest Dialog was used to search 8 literature databases. **Study Selection:** Controlled intervention studies in which the independent effects of n-3 LC-PUFAs could be isolated were eligible for inclusion. **Data Extraction:** The 18 unique studies that met the criteria for inclusion were divided into 2 sets, and data transcriptions and study quality assessments were conducted in duplicate. Each effect size was expressed as the weighted mean difference and 95%CI, using a random-effects model and the inverse of the variance as a weighting factor. **Results:** Based on the meta-analyses, supplementation with n-3 LC-PUFAs resulted in statistically significant improvements in 6 of 13 metabolic risk factors, in levels of 2 of 3 liver enzymes, in liver fat content (assessed via magnetic resonance imaging/spectroscopy), and in steatosis score (assessed via ultrasonography). Histological measures of disease [which were assessed only in patients with nonalcoholic steatohepatitis (NASH)] were unaffected by n-3 LC-PUFA supplementation. **Conclusions:** Omega-3 LC-PUFAs are useful in the dietary management of patients with NAFLD. Additional trials are needed to better understand the effects of n-3 LC-PUFAs on histological outcomes in patients with NASH. **Systematic Review Registration:** PROSPERO CRD42017055951.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is present when the following 2 conditions are met: (1) there is evidence (via imaging or histology) of hepatic steatosis

and (2) there are no causes for secondary hepatic fat accumulation (eg, inborn errors of metabolism, Wilson's disease, excessive alcohol intake, hepatitis, iron toxicity, or hepatotoxic drugs or toxins).¹ Histologically, NAFLD is present when $\geq 5\%$ of hepatocytes contain

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macrovesicular steatosis (reviewed by Petäjä and Yki-Järvinen²). NAFLD encompasses a range of conditions with increasing severity, including nonalcoholic fatty liver (NAFL), nonalcoholic steatohepatitis (NASH) with or without fibrosis, and cirrhosis. With NAFL (also called simple steatosis), there is hepatic steatosis without hepatocellular injury (ie, there is no hepatocyte ballooning or fibrosis). In contrast, with NASH, hepatic steatosis is accompanied by inflammation and hepatocyte injury (ballooning), with or without fibrosis.¹ In some individuals, NASH can progress to hepatocellular carcinoma.^{1,3} Factors that increase the risk of NAFLD include obesity, type 2 diabetes mellitus, dyslipidemia, and metabolic syndrome.¹

As reviewed by Neuschwander-Tetri,³ 20% to 30% of adults living in Westernized countries have NAFLD, 2% to 5% have NASH, and 1% to 2% have cirrhosis of the liver. The prevalence of NAFLD is reported to be even greater in certain population subgroups. For example, in adults with diabetes, the prevalence of NAFLD was reported to be 46.2% in the United Kingdom and 69.5% in Italy (reviewed by Bellentani⁴). In children and adolescents in Europe, the prevalence of NAFLD was reported to range from 2% to 12.5%; however, the prevalence among obese children was much higher: 36% in Germany and 44% in Italy.⁴ In the United States, cirrhosis and hepatocellular carcinoma related to NAFLD have emerged as the second leading cause of liver transplant, and projections show that NAFLD will become the leading indication for liver transplantation in the next 10 years.^{3,4} Reductions in body weight—achieved via reduced caloric intake and increased physical activity—appear to be effective in the treatment of NAFLD; however, most patients cannot achieve the required degree of weight loss or have trouble maintaining weight loss over the long term.⁵ Currently, there are no drugs approved for the management of NAFLD.

The efficacy of omega-3 long-chain polyunsaturated fatty acids (n-3 LC-PUFAs), particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in the dietary management of patients with NAFLD is actively being investigated. Both EPA and DHA are potent modulators of hepatic gene expression, promoting genes involved in hepatic fatty acid β -oxidation and export from the liver and inhibiting genes involved in hepatic fatty acid synthesis and storage.⁶ Specifically, n-3 LC-PUFAs suppress sterol regulatory element-binding protein 1 (SREBP-1), a transcription factor that regulates both the rate of triglyceride synthesis and its storage in the liver.^{6–8} Of note, SREBP-1 is stimulated by insulin; thus, the inhibition of SREBP-1 by n-3 LC-PUFAs is of particular importance in patients with NAFLD, as they are often also affected by

hyperinsulinemia and insulin resistance and are thus predisposed to insulin-induced stimulation of SREBP-1 and, consequently, the synthesis and accumulation of liver fatty acids. The n-3 LC-PUFAs further act to stimulate both hepatic peroxisome proliferator-activated receptor- α (PPAR- α), thereby increasing fatty acid β -oxidation, and peroxisome proliferator-activated receptor- γ (PPAR- γ), which increases insulin sensitivity.⁹ Collectively, the effects of n-3 LC-PUFAs on SREBP-1, PPAR- α , and PPAR- γ result in a net reduction in hepatic fat accumulation and a net increase in hepatic fatty acid β -oxidation. Thus, mechanistically, insufficient hepatic levels of EPA and DHA could tip the balance toward hepatic fatty acid lipogenesis, rather than toward hepatic fatty acid β -oxidation. Therefore, it seems biologically plausible that patients with NAFLD may benefit from an increased intake of EPA and DHA.

The clinical efficacy of n-3 LC-PUFAs, primarily EPA and DHA, in the management of adult and pediatric patients with NAFLD has been investigated in several clinical studies. The results of these studies have been critically appraised in 3 systematic reviews and meta-analyses, the first of which was published in 2012.¹⁰ Since that publication, many additional clinical studies of the effects of n-3 LC-PUFAs in patients with NAFLD have been published. The remaining systematic reviews did not include studies conducted in children and also did not include studies if the data were not reported in a manner that permitted the inclusion of the study in the meta-analyses.^{11,12} In the systematic review and meta-analyses presented here, the effects of n-3 LC-PUFA supplementation in adult and pediatric patients with NAFLD on liver-related outcomes and metabolic risk factors are presented. Of importance, if a study did not report data for liver outcomes in a manner that permitted inclusion of the study in the meta-analyses, the corresponding author of the study was contacted a minimum of 3 times to request the data, in order to reduce publication bias and have as robust and inclusionary a data set as possible.

METHODS

The objective of the current review was to systematically assess the effects of n-3 LC-PUFA supplementation on liver-related and metabolic outcomes in adult and pediatric patients with NAFLD. The research question was defined by the PICOS (Population, Intervention, Comparator, Outcomes, Study design) criteria presented in [Table 1](#). The systematic review and meta-analyses were conducted in compliance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines.¹³ This systematic review is registered with PROSPERO (an

Table 1 PICOS criteria for inclusion of studies

Data domain	Categories used for data extraction
Participants	Adults with NAFLD (either NAFL or NASH) Children with NAFLD (either NAFL or NASH)
Interventions	Supplementation with n-3 LC-PUFAs, predominantly EPA and/or DHA
Comparators	Placebo No intervention
Outcomes	Liver fat content or steatosis score, as measured by liver imaging Liver fibrosis score, hepatocellular ballooning score, steatosis score, lobular inflammation score, or NAFLD activity score, as measured by liver biopsy Liver enzymes (ALT, AST, GGT) Metabolic risk factors: blood lipid levels (TC, LDL-C, HDL-C, TGs), measures of glycemic control (fasting blood glucose, fasting insulin, HbA1c, HOMA-IR, adiponectin), body weight/composition (BMI, body weight, waist circumference), other (systolic BP, diastolic BP)
Study design	Randomized controlled trials (including parallel or crossover studies) Nonrandomized controlled trials (including parallel or crossover studies)

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BP, blood pressure; BMI, body mass index; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GGT, γ -glutamyl transferase; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment of insulin resistance; n-3 LC-PUFA, omega-3 long-chain polyunsaturated fatty acid; LDL-C, low-density lipoprotein cholesterol; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; TC, total cholesterol; TG, triglyceride.

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Literature search

To retrieve relevant literature on the effects of n-3 LC-PUFAs in patients with NAFLD, the electronic search tool ProQuest Dialog was used to search the following 8 literature databases: Allied & Complementary Medicine Database (AMED); BIOSIS Previews; CAB Abstracts; Embase; Foodline: Science; FSTA—Food Science and Technology Abstracts; MEDLINE; and, National Technical Information Service (NTIS). Two literature searches were conducted, one on September 26, 2016 (for studies conducted in adults), and the other on October 19, 2016 (for studies conducted in children and adolescents). At least one search term for the exposure [“docosahexaenoic,” “eicosapentaenoic,” “DHA,” “EPA,” “fish oil,” “cod oil,” “krill oil,” “algal oil,” “marine lipid,” “omacor,” “lovaza,” or (“long chain,” “long-chain,” “omega-3,” “n-3,” or “omega 3”) within two words of (“fatty” or “PUFA”)], the study population (“men,” “women,” “man,” “woman,” “human,”

“subject,” “participant,” “volunteer*,” “patient,” “elder*,” “senior,” “geriatric,” “older,” “adult,” “people,” “person,” “individual,” “breastfe*,” “breast-fe*,” “breast fe*,” “pediatric,” “paediatric,” “teen*,” “adolescen*,” “child*,” “boy,” “girl,” “toddler*,” “baby,” “babies,” “infant,” or “neonat*”), and the outcome [(“liver” or “hepat*”) AND (“*steat*,” “fibrosis,” “cirrhosis,” “fatty liver,” “non-alcoholic,” “nonalcoholic,” “injury,” “NAFLD,” “NAFL,” “inflammation,” or “NASH”)] had to appear in either the title or the abstract of the article. Furthermore, to limit the search to studies conducted in humans, animal terms (“rat,” “mice,” “mouse,” “dog,” “pig,” “piglet,” “rabbit,” “hamster,” “monkey,” “rodent*,” “chick,” “broiler,” “cow,” “cattle,” “sheep,” “ewe,” “porcine,” “horse,” “equine,” “in vitro,” or “ex vivo”) were required not to appear in the title of the article. The use of an asterisk in some of the above search terms allowed for flexibility in the word ending (eg, “adolescen*” would have resulted in the identification of “adolescents” or “adolescence”). No limitations were placed on the literature search with respect to language; however, the year of publication was restricted to 1960 and onward.

Inclusion and exclusion criteria

Once the search strategy was implemented and the publication titles were retrieved, the eligibility of the publications for inclusion was determined at 3 stages using the titles, abstracts, and, subsequently, the full text of each publication. A study was included if it met all of the following inclusion criteria: (1) it was a full-length article published in a peer-reviewed journal; (2) it was a controlled intervention study conducted in patients (adults or children) with NAFLD (either NAFL or NASH); and (3) the investigational product was composed of n-3 LC-PUFAs (predominantly EPA and/or DHA). A study was excluded if it met one or more of the following exclusion criteria: (1) it was a full-length article published in a non-peer-reviewed source (eg, website, magazine); (2) it was published in abstract form only or as a short communication (eg, letter to the editor, commentary, etc); (3) it was an animal or in vitro study; (4) it was an uncontrolled human intervention study; (5) the investigational product was not composed of n-3 LC-PUFAs or was composed of additional bioactive agents, the independent effects of which could not be isolated; (6) the route of administration was not oral; (7) the study population consisted of individuals with serious diseases, other than NAFLD or diet-related diseases; (8) it was a secondary research paper (eg, narrative review, systematic review, meta-analysis, etc); or (9) the study was a duplicate publication. Although secondary research papers were

Table 2 Liver imaging and biopsy scoring

Measure	Scoring algorithm	Studies in adults	Studies in children
Steatosis score (via ultrasonography)	Scored on a 4-grade scale as 0 (absent steatosis), 1 (mild steatosis), 2 (moderate steatosis), or 3 (severe/advanced steatosis) ^a	Capanni et al (2006) ¹⁴ ; Sofi et al (2010) ¹⁵ ; Spadaro et al (2008) ¹⁶ ; Zhu et al (2008) ¹⁷	Boyratz et al (2015) ¹⁸ ; Janczyk et al (2015) ¹⁹ ; Nobili et al (2011, 2013) ^{20–22}
Liver biopsy measures (NASH-CRN criteria) ^{b,c}			
Fibrosis score	Scored on a 5-grade scale as 0 (none), 1 (perisinusoidal or periportal), 2 (perisinusoidal and portal/periportal), 3 (bridging fibrosis), or 4 (cirrhosis)	Argo et al (2015) ²⁵ ; Dasarathy et al (2015) ²⁶ ; Li et al (2015) ²⁴ ; Nogueira et al (2016) ²⁷	None
Hepatocellular ballooning score	Scored on a 3-grade scale as 0 (none), 1 (few), or 2 (many)	Argo et al (2015) ²⁵ ; Dasarathy et al (2015) ²⁶ ; Li et al (2015) ²⁴ ; Nogueira et al (2016) ²⁷	None
Steatosis score	Scored on a 4-grade scale as 0 (< 5%), 1 (5%–33%), 2 (34%–66%), or 3 (> 66%)	Argo et al (2015) ²⁵ ; Dasarathy et al (2015) ²⁶ ; Li et al (2015) ²⁴ ; Nogueira et al (2016) ²⁷	None
Lobular inflammation score	Scored on a 4-grade scale as 0 (none), 1 (< 2), 2 (2–4), or 3 (> 4)	Argo et al (2015) ²⁵ ; Dasarathy et al (2015) ²⁶ ; Nogueira et al (2016) ²⁷	None
NAFLD activity score	Scored by summing the scores for hepatocellular ballooning (0–2), steatosis (0–3), and lobular inflammation (0–3), resulting in a possible NAFLD activity score of 0 (best) to 8 (worse)	Argo et al (2015) ²⁵ ; Dasarathy et al (2015) ²⁶ ; Nogueira et al (2016) ²⁷	None

Abbreviations: NASH-CRN, Nonalcoholic Steatohepatitis Clinical Research Network.

^aDetailed descriptions of each score are as follows: absent steatosis (grade 0), normal liver echotexture; mild steatosis (grade 1), slight and diffuse increase in fine parenchymal echoes with normal visualization of diaphragm and portal vein borders; moderate steatosis (grade 2), moderate and diffuse increase in fine echoes with slightly impaired visualization of diaphragm and portal vein borders; severe steatosis (grade 3), fine echoes with poor or no visualization of diaphragm, portal vein borders, and posterior portion of the right lobe.

^bNASH-CRN criteria were defined by Kleiner et al.²³

^cIt was not explicitly stated by Li et al²⁴ that the NASH-CRN criteria were used in the grading of the liver biopsy samples for fibrosis, ballooning, and steatosis; however, it appears from the baseline values (and their similarity to those reported for the other 3 studies), that the NASH-CRN criteria were most likely used.

excluded, the reference lists of these publications were manually screened to ensure all relevant studies were identified.

Data extraction and assessment of study quality

The intervention studies were divided into 2 sets, and for each set, the assessment of study quality and the transcription of data (which were subsequently used in the meta-analyses), were conducted in duplicate.

H.Y.L. and S.F. transcribed data from one-half of the studies, and M.D. and S.W. transcribed data from the other half of the studies. The data were transcribed into Excel spreadsheets and included the units for the outcome measure (eg, triglycerides were presented in either milligrams per deciliter or millimoles per liter, depending on the publication), the number of individuals in each group on which the analysis was based, the baseline measure and its variability (either standard deviation, standard error of the mean, or 95%CI), the end-of-treatment measure and its variability, the change from baseline measure and its variability, and the results of statistical analyses, both within groups and between

groups. These data were captured for serological measures of liver function (aspartate aminotransferase [AST], alanine transaminase [ALT], γ -glutamyl transferase [GGT]); measures of liver fat, as assessed via magnetic resonance imaging (MRI) or magnetic resonance spectroscopy (MRS); steatosis score (as assessed via ultrasonography; see Table 2 for scoring^{14–22}); liver biopsy measures (fibrosis score, ballooning score, steatosis score, lobular inflammation score, NAFLD activity score—see Table 2 for scoring^{23–27}); blood lipid levels (total cholesterol, low-density lipoprotein cholesterol [LDL-C], high-density lipoprotein cholesterol [HDL-C], and triglycerides); measures of glycemic control (fasting blood glucose, fasting insulin, homeostatic model assessment of insulin resistance [HOMA-IR], adiponectin); measures of body mass/composition (body mass index [BMI], body weight, waist circumference); and other metabolic risk factors (systolic and diastolic blood pressure). The data entered by each individual were cross-checked by either H.Y.L. or K.M.V., and discrepancies were resolved by referring to the original publication.

Using the Modified Jadad scale,²⁸ H.Y.L. and S.F. appraised the quality of one-half of the studies, and

M.D. and S.W. appraised the quality of the other half of the studies. The quality appraisals were then consolidated by K.M.V., at which point any discrepancies were resolved by referring to the original publication and by discussion and consensus.

Statistical analysis

To determine the effects of n-3 LC-PUFAs on each of the liver-related outcomes and metabolic risk factors, the results of the studies were pooled in a meta-analysis. The effect size calculated for each study was the raw mean difference. The inverse of the variance was used as the weighting factor, and so the pooled effect was the weighted mean difference. For parallel studies, the raw mean difference for each liver-related outcome and metabolic risk factor was calculated as the change from baseline in the control group subtracted from the change from baseline in the n-3 LC-PUFA group. For crossover studies, the raw mean difference in the effect for each liver-related outcome and metabolic risk factor was calculated as the value at the end of the control phase subtracted from the value at the end of the n-3 LC-PUFA phase. In the majority of studies, variances for the raw mean differences were not reported; thus, variances were calculated using information provided in the publication (eg, using CIs or individual variances for the n-3 LC-PUFA and control groups). If, in parallel studies, variances for the changes from baseline were reported separately for the n-3 LC-PUFA and control groups, then a pooled variance for the raw mean difference was calculated. If, for parallel studies, variances were reported only for the baseline and end-of-treatment values, then these were used to calculate the variance for the change from baseline, using a correlation coefficient of 0.8. Similarly, for crossover studies, if variances were reported only for the end-of-treatment values, then the variance for the raw mean difference was calculated using a correlation coefficient of 0.8. The magnitude of the correlation coefficient has no bearing on the size of the pooled effect or the 95%CI; the pooled mean differences and 95%CIs have been demonstrated to be similar, whether the correlation coefficient used was 0.2, 0.5, or 0.8²⁹; 0.25, 0.5, or 0.75³⁰; or 0.5, 0.7, or 1.0.³¹

If, for the liver-related outcomes, data were missing from the publications or were reported in a manner not conducive to pooling in a meta-analysis, then the corresponding author of the paper was contacted a minimum of 3 times, and each time, a formal request for the data was made. For the meta-analyses, a random-effects model was used according to the methods described by DerSimonian and Laird,³² given that random-effects

models take into consideration the variability in response both within and between studies.

Some of the identified studies had multiple comparisons (eg, in the study by Chen et al,³³ participants were randomly allocated to receive either placebo or 1 of 2 doses of seal oil). Each comparison between seal oil and placebo, hereafter referred to as a stratum, was considered a separate trial; however, the sample size of the control group was divided evenly among the comparisons to avoid inflating the weight of the study, as has been done previously.³⁴

The weighted mean differences and accompanying 95%CIs were determined using Comprehensive Meta-analysis Software (version 2.2.064). A *P* value of < 0.05 was considered statistically significant, and *P* values of < 0.10 were considered nearly significant. Publication bias was assessed according to the trim and fill method developed by Duval and Tweedie.³⁵ With this method, asymmetry is searched for within the funnel plot. If the asymmetry is determined to be caused by the presence of small studies (with large variances) in which large effect sizes were reported, with an unbalanced number of small studies showing a small effect, then those “missing” studies are imputed, and the pooled effect size is recalculated.

Subgroup analyses were conducted to evaluate the influence of age (ie, children vs adults), dose (adults only; ie, EPA + DHA < 3 g/d vs ≥ 3 g/d), duration of intervention (ie, ≤ 6 mo vs > 6 mo), and study quality (ie, quality rating using the modified Jadad scale score of < 6 vs ≥ 6) on each of the liver-related outcomes and metabolic risk factors. The subgroup analyses were conducted only if there were at least 3 data points for each comparison; otherwise, insufficient data precluded the subgroup analyses. Per the information registered with PROSPERO, there were plans to also conduct subgroup analyses to evaluate the influence of baseline disease severity (NAFL vs NASH) as well as the presence vs the absence of metabolic syndrome on each of the liver-related outcomes and metabolic risk factors. In most of the studies, a liver biopsy (which is required to differentiate NAFL from NASH) was not conducted, and so it is not known whether the study was comprised predominantly of NAFL patients or NASH patients. Furthermore, there were too few studies in which the participants were defined as having metabolic syndrome, and so these sensitivity analyses were not possible. Assessments of publication bias were not conducted for any of the subgroup analyses, as these were intended to be exploratory only. Forest plots and results of sensitivity analyses for the liver-related outcomes are reported within the manuscript. Forest plots and results of sensitivity analyses for all other outcomes are reported in the Supporting Information online.

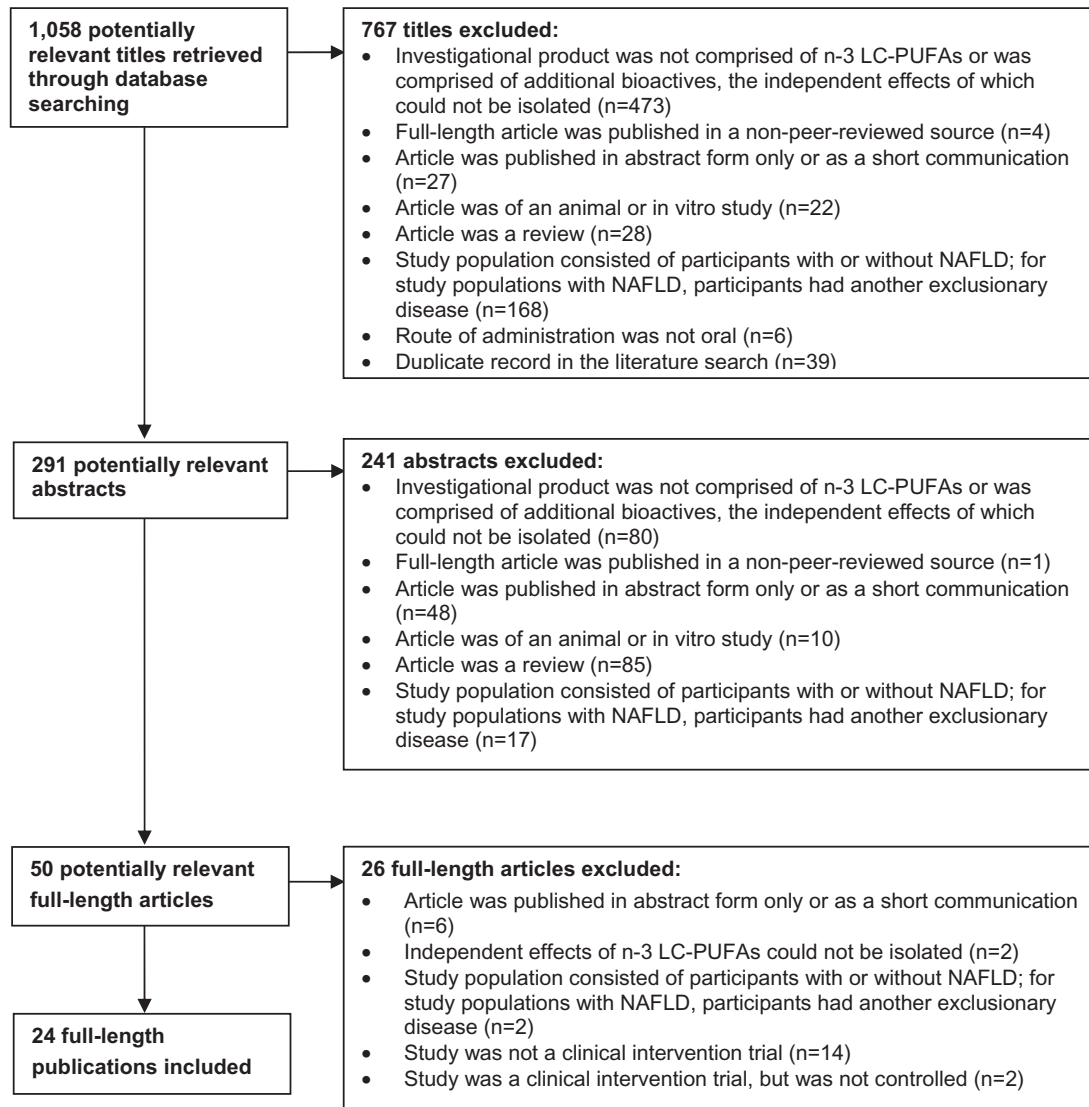


Figure 1 Flow diagram of the literature search process. Abbreviations: NAFLD, nonalcoholic fatty liver disease; LC-PUFAS, long-chain polyunsaturated fatty acids.

RESULTS

Literature search

As shown in Figure 1, the literature search resulted in the identification of 1058 titles, and abstracts were retrieved for 291 records. Of the 291 abstracts, 50 were considered potentially relevant, and their full-length versions were retrieved. One of the 50 publications was published in Chinese³³; this article was officially translated, and the publication was determined to meet the eligibility criteria for study inclusion. Of the remaining 49 publications, 26 were excluded; reasons for exclusion are provided in Figure 1. Thus, in total, 24 of the 50 full-text publications were included: 17 were of studies conducted in adults, and 7 were of studies conducted in

children. Three publications by Scorletti et al^{36–38} are kin studies (publications of different results but for the same study population) and were therefore considered collectively as 1 unique study in adults. Likewise, 3 publications by Nobili et al^{20–22} are kin studies and were also considered collectively as 1 unique study in children. The parallel study in adults by Al-Gayyar et al³⁹ could not be included in any of the meta-analyses because only end-of-treatment measures were provided for the control and n-3 LC-PUFA groups; likewise, the study in children by Spahis et al⁴⁰ could not be included in any of the meta-analyses because only baseline values were reported. Therefore, the evidence base comprises a total of 22 publications (and 18 unique studies): 16 publications (and 14 unique studies) in adults, and 6 publications (and 4 unique studies) in children.

Overview of studies conducted in adults

The key characteristics of the 14 unique studies conducted in adults are summarized in Table 3^{14–17,24–27,33,36–38,41–44}; more detailed summaries can be found in Table S1 in the Supporting Information online. All of the studies were parallel in design, except for the crossover studies by Vega et al⁴⁴ and Cussons et al.⁴¹ Of the 14 controlled intervention studies, 9 were described as randomized, double blinded, and placebo controlled; the placebo administered was soybean oil in 1 study,²⁵ olive oil in 2 studies,^{36–38,41} corn oil in 2 studies,^{26,42} and mineral oil in 1 study²⁷; in the remaining 3 randomized, double-blind, placebo-controlled studies, the placebo was not characterized.^{17,33,43} Of the 5 other controlled intervention studies, 3 were described as randomized but not double blinded,^{15,16,24} and 2 were described neither as randomized nor double blinded.^{14,44} In these 5 studies, the control group was not administered any product^{14,16} or was administered saline,²⁴ non-n-3 LC-PUFA-enriched olive oil,¹⁵ or an oil composed of 72% C18:1 *trans* fatty acid, 10% linoleic acid, and 12% palmitic acid.⁴⁴ Although the placebo oil in the study by Vega et al⁴⁴ was described as containing predominantly *trans* fatty acids, this is believed to be a typographical error.

There were some notable differences between the studies that were described as randomized, double blinded, and placebo controlled vs those that were either not randomized or not double blinded. Specifically, the latter studies were associated with smaller sample sizes, and the dose of EPA + DHA administered to the participants was either not reported or was generally lower; furthermore, the average quality rating of the latter studies was approximately half that of the former studies (3.6 vs 6.1), attributable, of course, to the lack of randomization or double blinding (quality ratings are discussed further in the section “Modified Jadad scale scores”).

The studies were conducted in the United States,^{25,26,43,44} China,^{17,24,33,42} Italy,^{14–16} Australia,⁴¹ Brazil,²⁷ and the United Kingdom.^{36–38} All of the studies included both male and female patients, except for the study by Cussons et al,⁴¹ which included only female participants (who also had polycystic ovarian syndrome). The participants were described as having NAFLD in 6 studies,^{14–16,33,41,44} NAFLD and hyperlipidemia/dyslipidemia in 2 studies,^{17,42} NAFLD and metabolic syndrome in 1 study,^{36–38} NASH in 4 studies,^{24,25,27,43} and NASH and type 2 diabetes mellitus in 1 study.²⁶ The effects of n-3 LC-PUFA supplementation on NAFLD were assessed using ultrasonography,^{14–17,33} biopsy/histology,^{24,26,27,43} MRS,^{36–38,41,44} or both biopsy and MRI.²⁵

The number of study participants ranged from 5 to 68 per group among the parallel studies and from 12 to 16 among the crossover studies. The mean age of the participants ranged from 35.3 to 57.5 years. All but 1 study³³ provided the mean BMI at baseline. On the basis of the mean BMI values, all study participants were generally overweight or obese. In 5 of the 14 studies, the participants were advised to follow a hypocaloric diet or a heart-healthy diet (eg, the American Heart Association Diet) and to increase their physical activity levels^{16,17,24–26}; in 1 additional study, the participants were said to have been given dietary recommendations, but no further details were provided.¹⁵

The source of EPA + DHA was described as fish oil in 5 studies,^{25,26,41,42,44} seal oil in 2 studies,^{17,33} an EPA or EPA + DHA ethyl ester in 3 studies,^{14,36–38,43} an enriched olive oil in 1 study,¹⁵ or an n-3 oil that also contained α -linolenic acid in 1 study.²⁷ In 2 studies, the source of the oil was not reported.^{16,24}

There were 16 strata among the 14 studies. A stratum is defined as a set of data from one control group or arm and an n-3 LC-PUFA group or arm. The daily intake of supplemental EPA + DHA was greater than 3 g/d in 7 strata (Chen et al³³ strata 1 and 2, Vega et al,⁴⁴ Zhu et al,¹⁷ Cussons et al,⁴¹ Scorletti et al,^{36–38} Dasarathy et al²⁶), between 1 g/d and < 3 g/d in 5 strata (Capanni et al,¹⁴ Sanyal et al,⁴³ strata 1 and 2, Argo et al,²⁵ Qin et al⁴²), and less than 1 g/d in 2 strata (Sofi et al,¹⁵ Nogueira et al²⁷). In 2 strata, the dose of EPA + DHA was not reported and could not be estimated from the information provided in the publication (Spadaro et al,¹⁶ Li et al²⁴). The duration of the supplementation period was 2 months in 2 studies,^{41,44} 3 months in 1 study,⁴² 6 months in 5 studies,^{16,17,24,27,33} 1 year in 5 studies,^{14,15,25,26,43} and 15 to 18 months in 1 study.^{36–38}

Biological measures of compliance were assessed in 7 of the 14 studies; these included the ratio of n-6 to n-3 fatty acids,^{14,25} the ratio of EPA to arachidonic acid,⁴³ or levels of EPA and DHA.^{27,36–38,42,44} These biological measures of compliance were assessed in various tissues, and in different lipid fractions, including plasma,^{14,27,42} serum,⁴³ plasma phospholipids,⁴⁴ red blood cells,^{36–38} and red blood cell phospholipids.²⁵

The outcomes assessed in the studies are classified as liver related, blood lipids, glycemic control, body weight/composition, or other in Table S1 in the Supporting Information online.

Overview of studies conducted in children

The key characteristics of the 4 unique studies conducted in children are summarized in Table 3;^{18–22,45} more detailed summaries can be found in Table S2 in

Table 3 Key study characteristics of omega-3 long-chain polyunsaturated fatty acid (n-3 LC-PUFA) supplementation trials conducted in adults and children with nonalcoholic fatty liver disease (NAFLD)

Reference	Country of study	Study design	Duration of study	Health status		Study population		Mean age (y) ^a	Mean BMI (kg/m ²) ^b	Control	Active	Investigational product		
				Initial count	Final count	EPA	DHA					ALA		
													No. of patients (gender distribution)	Final count
Studies in adults														
Argo et al (2015) ²⁵	USA	R, DB, PC, P	1 y	NASH	41 (NR)	34 (13 M, 21 F)	46.8±11.9	32.5±7.3	Soybean oil	Fish oil	1050	750	NR	
Capanni et al (2006) ¹⁴	Italy	C, OP, P	12 mo	NAFLD	56 (32 M, 24 F)	56 (32 M, 24 F)	57.5±10.5	28.5±4.5	None	EPA+DHA EE	375	625	NR	
Chen et al (2008) ³³	China	R, DB, PC, P	24 wk	NAFLD	46 (30 M, 16 F)	46 (30 M, 16 F)	46.0 ^d	NR	Placebo (NFS)	Seal oil	1440 or 1800	1680 or 2100	NR	
(strata 1 and 2)														
Cussons et al (2009) ⁴¹	Australia	R, PC, DB, X	8 wk	NAFLD+PCOS	12 (12 F)	12 (12 F)	35.3±6.7	38.2±7.4	Olive oil	Fish oil	1080	2240	NR	
Dasarathy et al (2015) ²⁶	USA	R, DB, PC, P	48 wk	NASH+T2DM	37 (8 M, 29 F)	37 (8 M, 29 F)	50.6±9.9	35.3±6.0	Corn oil	Fish oil	2160	1440	NR	
Li et al (2015) ²⁴	China	R, C, SB ^e , P	6 mo	NASH	78 (70 M, 8 F)	78 (70 M, 8 F)	51.9±7.8	27.9±1.6	Saline	PUFAs ^f (NFS)	NR	NR	NR	
Nogueira et al (2016) ²⁷	Brazil	R, DB, PC, P	6 mo	NASH	60 (NR)	50 (9 M, 41 F)	53.2±7.0	30.7±4.5	Mineral oil	n-3 oil (NFS)	195	150	600	
Qin et al (2015) ⁴²	China	R, DB, PC, P	3 mo	NAFLD+HypertL	80 (NR)	70 (51 M, 19 F)	45.2±10.8	26.2±3.4	Corn oil	Fish oil	728	516	NR	
Sanyal et al (2014) ⁴³	USA	R, DB, PC, P	12 mo	NASH	243 (95 M, 148 F)	174 (NR)	48.6±12.0	34.9±6.3	Placebo (NFS)	EPA EE	1800 or 2700	NR	NR	
(strata 1 and 2)														
Scorletti et al (2014, 2014, 2015) ³⁶⁻³⁸	UK	R, DB, PC, P	15-18 mo	NAFLD+MetSyn	103 (60 M, 43 F)	95 (55 M, 40 F)	51.3±10.3	33.1±5.1	Olive oil	EPA+DHA EE	1840	1520	NR	
Sofi et al (2010) ¹⁵	Italy	R, OP, PC, P	12 mo	NAFLD	11 (9 M, 2 F)	11 (9 M, 2 F)	54.5 ^g	29.3±4.0	Olive oil	Enriched olive oil	470	240	NR	
Spadaro et al (2008) ¹⁶	Italy	R, P, C, OP	6 mo	NAFLD	40 (NR)	36 (19 M, 17 F)	50.7±11.5	30.6±4.1	None	PUFAs (NFS)	NR	NR	NR	
Vega et al (2008) ⁴⁴	USA	PC, OP, X	4 wk (placebo) 8 wk (n-3 PUFAs)	NAFLD	22	16 (12 M, 4 F)	50.0±10.0	36.2±8.5	Oil (72% C18:1 <i>trans</i> FA, 10% LA, and 12% PA)	Fish oil	4626	2151	NR	
Zhu et al (2008) ¹⁷	China	R, DB, PC, P	24 wk	NAFLD+HypertL	144 (NR)	134 (97 M, 37 F)	44.5±11.1	26.2±2.9	Placebo (NFS)	Seal oil	2160	2520	NR	
Studies in children														
Boyras et al (2015) ¹⁸	Turkey	R, PC, DB, P	12 mo	NAFLD+OB	138 (73 M, 65 F)	108 (55 M, 53 F)	13.7±3.6	28.5±4.2	Placebo (NFS)	PUFAs (NFS)	380	200	NR	
Janczyk et al (2015) ¹⁹	Poland	R, PC, DB, P	24 wk	NAFLD +OB/OW	76 (65 M, 11 F)	64 (NR)	13.0 ^h	28.7 ⁱ	Sunflower oil	Fish oil	178, 355, or 533 ^j	267, 534, or 800	NR	
Nobili et al (2011, 2013, 2013) ²⁰⁻²²	Italy	R, PC, DB, P	24 mo	NAFLD	60 (25 M, 35 F)	60 (25 M, 35 F)	11.7 ^k	25.7 ^l	Germ oil	Algal oil	NR	250 or 500	NR	
(strata 1 and 2)														
Pacifico et al (2015) ⁴⁵	Italy	R, PC, DB, P	6 mo	NAFLD+OB/OW	58 (NR)	51 (30 M, 21 F)	10.9±2.7	28.2±4.9	Germ oil	Algal oil	NR	250	NR	

Abbreviations: ALA, α -linolenic acid; BMI, body mass index; C, controlled; DB, double-blinded; DHA, docosahexaenoic acid; EE, ethyl ester; EPA, eicosapentaenoic acid; F, females; FA, fatty acid; HyperL, hyperlipidemia/dyslipidemia; LA, linoleic acid; M, males; MetSyn, metabolic syndrome; NASH, nonalcoholic steatohepatitis; NFS, not further specified; NR, not reported; OB, obese; OP, open label; OW, overweight; P, parallel; PA, palmitic acid; PC, placebo-controlled; PCOS, polycystic ovary syndrome; PUFA = polyunsaturated fatty acid; R, randomized; SB, single-blinded; T2DM, type 2 diabetes mellitus; X, crossover.

^aIf mean age was reported separately for control and active groups, a weighted average was calculated and standard deviations were pooled.

^bIf mean BMI was reported separately for control and active groups, a weighted average was calculated and standard deviations were pooled.

^cPatients who met the inclusion criteria but refused treatment were monitored as controls without therapy.

^dNo standard deviation or standard error of the mean was reported.

^eIn the study by Li et al,²⁴ it is specifically stated that "All working staff involved in evaluating parameters were blinded to the information about both groups." Thus, it appears this study was single blinded, even though it was not explicitly labeled as such.

^fParticipants consumed 50 mL of PUFAs per day with a 1:1 ratio of EPA:DHA in their daily diet; details not further specified.

^gAge was reported as the median and interquartile range; the median was assumed to approximate the mean, and the weighted mean was calculated.

^hAge was reported as the median and interquartile range; the median was assumed to approximate the mean, and the weighted mean was calculated.

ⁱBMI was reported as the median and interquartile range; the median was assumed to approximate the mean, and the weighted mean was calculated.

^jThe dose of EPA and DHA was dependent on body weight.

^kAge was reported as the median and interquartile range; the median was assumed to approximate the mean, and the weighted mean was calculated.

^lBMI was reported as the median and interquartile range; the median was assumed to approximate the mean, and the weighted mean was calculated.

Table 4 Results of scoring with the modified Jadad scale

Reference	Score (/8)	Allocation concealment	Intention-to-treat analysis	Sample size justification
Studies in adults				
Argo et al (2015) ²⁵	6.5	Yes	No	Yes
Capanni et al (2006) ¹⁴	3.0	No	Yes	Yes
Chen et al (2008) ³³	5.0	No	Yes	No
Cussons et al (2009) ⁴¹	5.0	No	Yes	Yes
Dasarathy et al (2015) ²⁶	7.0	Yes	Yes	Yes
Li et al (2015) ²⁴	3.5	No	Yes	No
Nogueira et al (2016) ²⁷	7.0	No	No	Yes
Qin et al (2015) ⁴²	7.0	Yes	No	Yes
Sanyal et al (2014) ⁴³	6.5	Yes	No	Yes
Scorletti et al (2014, 2014, 2015) ^{36–38}	8.0	Yes	Yes	Yes
Sofi et al (2010) ¹⁵	3.5	No	No	Yes
Spadaro et al (2008) ¹⁶	6.0	No	No	Yes
Vega et al (2008) ⁴⁴	2.0	No	No	No
Zhu et al (2008) ¹⁷	3.0	No	Yes	No
Studies in children				
Boyraz et al (2015) ¹⁸	4.5	No	No	No
Janczyk et al (2015) ¹⁹	7.0	Yes	No	Yes
Nobili et al (2011, 2013, 2013) ^{20–22}	8.0	No	Yes	Yes
Pacifico et al (2015) ⁴⁵	8.0	No	No	Yes

the [Supporting Information online](#). The 4 studies, described as randomized, double blind, and placebo controlled, were all parallel in design. All 4 studies included both male and female pediatric patients with NAFLD. The effects of n-3 LC-PUFA supplementation on NAFLD were assessed using ultrasonography in the studies by Boyraz et al,¹⁸ Janczyk et al,¹⁹ and Nobili et al.^{20–22} Details on the scoring are provided in [Table 2](#). It should be noted that liver biopsies were performed in addition to ultrasonography in the study by Nobili et al,^{20–22} but only at baseline. Baseline steatosis, hepatocellular ballooning, inflammation, and fibrosis were scored using the Non-alcoholic Steatohepatitis Clinical Research Network (NASH-CRN) criteria.²³ The proportion of children with NASH was not reported, but, on the basis of the NASH-CRN scores for ballooning and inflammation, it appears that the majority of children had NASH. In the remaining study,⁴⁵ the effects of n-3 LC-PUFA supplementation were assessed using MRI; as in the study by Nobili et al,^{20–22} liver biopsies were performed in addition to MRI, but only at baseline. On the basis of the NASH-CRN scoring criteria,²³ 65% of the children in the study by Pacifico et al⁴⁵ had NASH.

The studies were conducted in Turkey,¹⁸ Poland,¹⁹ and Italy.^{20–22,45} The children in the studies ranged in age from a mean of 10.9 to 13.7 years and, on the basis of the mean BMI, were generally overweight. The number of children per group ranged from 20 to 56. DHA from algal oil was administered in 2 of the 4 studies at doses of 250 or 500 mg/d for 6 or 24 months.^{20–22,45} In the remaining 2 studies, fish oil was administered for 6 months, with daily EPA + DHA intakes of 580 mg/d¹⁸ or 450 to 1300 mg/d, depending on the child's body

weight.¹⁹ Across 3 of the studies, the placebo was either germ oil^{20–22,45} or sunflower oil¹⁹; in 1 study, the placebo was not characterized.¹⁸ In all studies, the children were prescribed or were encouraged to follow a calorically restricted diet and increase their energy expenditure. Biological measures of compliance, assessed as the content of DHA in whole blood, were reported in 2 of the 4 studies.^{20–22,45}

The outcomes assessed in the studies are classified as liver related, blood lipids, glycemic control, body weight/composition, or other in [Table S2](#) in the [Supporting Information online](#).

Modified Jadad scale scores

The quality ratings ranged from 2 to 8 among the studies in adults and from 4.5 to 8 among the studies in children ([Table 4](#)^{14–22,24–27,88,36–38,41–45}). Moreover, only in the studies by Scorletti et al^{36–38} and Dasarathy et al²⁴ was there evidence of allocation concealment, justification for the selected sample size, and an intention-to-treat analysis. Overall, the studies in children were generally associated with higher quality ratings than the studies in adults.

Meta-analyses

Liver-related outcomes. The effects of n-3 LC-PUFA supplementation on liver enzymes (ie, AST, ALT, and GGT), liver fat content (assessed via MRI or MRS), and steatosis score (assessed via ultrasonography), corrected for the effects in the control group, are shown in [Figures 2 through 6](#). When all of the available data were pooled, there were statistically significant reductions in

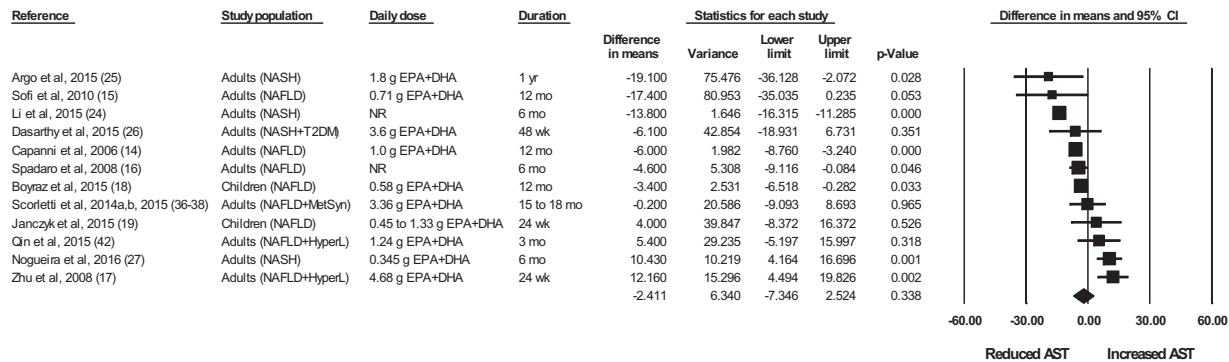


Figure 2 Effects of n-3 LC-PUFAs vs a control on AST levels in patients with NAFLD. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in serum AST levels with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -2.41 IU/L (95%CI, -7.35 to 2.52 IU/L; $P=0.338$). Using the trim and fill method of Duval and Tweedie,³⁵ 1 study was found to be missing to the right of the mean effect. With this study imputed, the pooled effect is -1.56 IU/L (95%CI, -6.47 to 3.35 IU/L). *Abbreviations:* AST, aspartate aminotransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HyperL, hyperlipidemia/dyslipidemia; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; MetSyn, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NR, not reported; T2DM, type 2 diabetes mellitus.

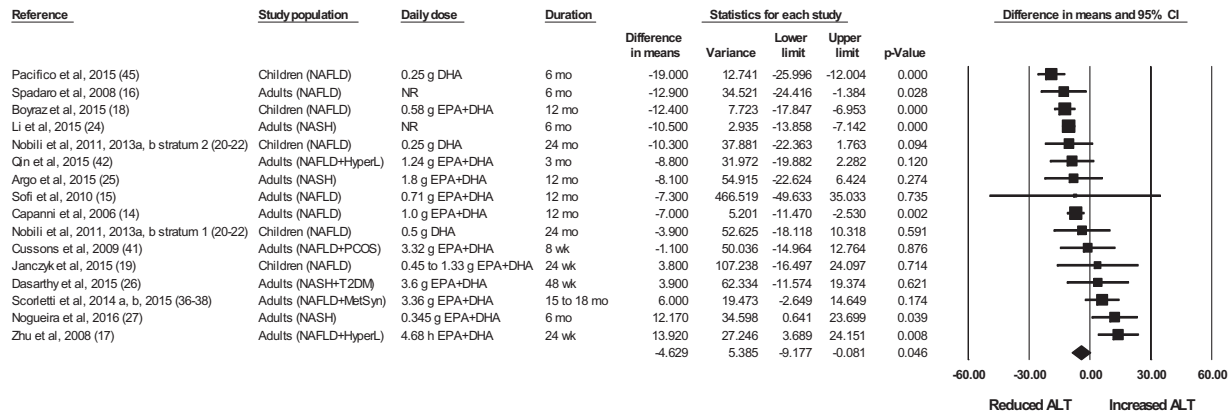


Figure 3 Effects of n-3 LC-PUFAs vs a control on ALT levels in patients with NAFLD. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in serum ALT levels with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -4.63 IU/L (95%CI, -9.18 to -0.08 IU/L; $P=0.046$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* ALT, alanine aminotransferase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HyperL, hyperlipidemia/dyslipidemia; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; MetSyn, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NR, not reported; PCOS, polycystic ovarian syndrome; T2DM, type 2 diabetes mellitus.

ALT, GGT, liver fat content, and steatosis score. For AST, the pooled reduction was statistically significant only when results from studies longer than 6 months were pooled (Table 5³⁵). The pooled reduction in ALT was statistically significant when data from the children (but not the adult) studies were pooled, when data from the higher-dose (but not the lower-dose) adult studies were pooled, and when data from the longer-duration (but not the shorter-duration) studies were pooled (Table 5). The pooled reduction in GGT was statistically or nearly statistically significant when data from the

adult studies were pooled (there were insufficient data points to conduct a separate analysis for children), when results from the lower-dose adult studies were pooled (there were insufficient data points to permit an analysis of the higher-dose adult studies), when the results from the shorter-duration (but not the longer-duration) studies were pooled, and irrespective of the quality rating of the study (Table 5). The pooled reduction in liver fat content was nearly statistically significant when data from the adult studies were pooled (there were insufficient data points to permit an

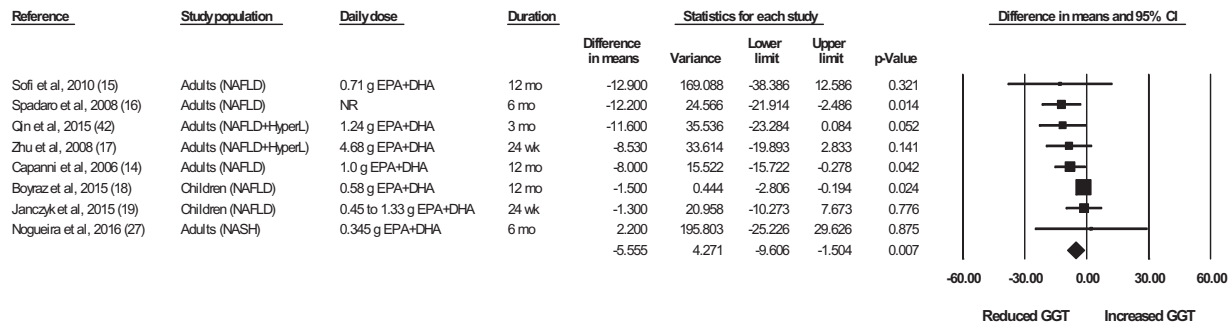


Figure 4 Effects of n-3 LC-PUFAs vs a control on GGT levels in patients with NAFLD. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in serum GGT with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -5.56 IU/L (95%CI, -9.61 to -1.50 IU/L; $P=0.007$). Using the trim and fill method of Duval and Tweedie,³⁵ 1 study was found to be missing to the right of the mean effect. With this study imputed, the pooled reduction in serum GGT levels with n-3 LC-PUFAs vs a control is -5.16 IU/L (95%CI, -8.92 to -1.40 IU/L). *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GGT, γ -glutamyl transferase; HyperL, hyperlipidemia/dyslipidemia; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NR, not reported.

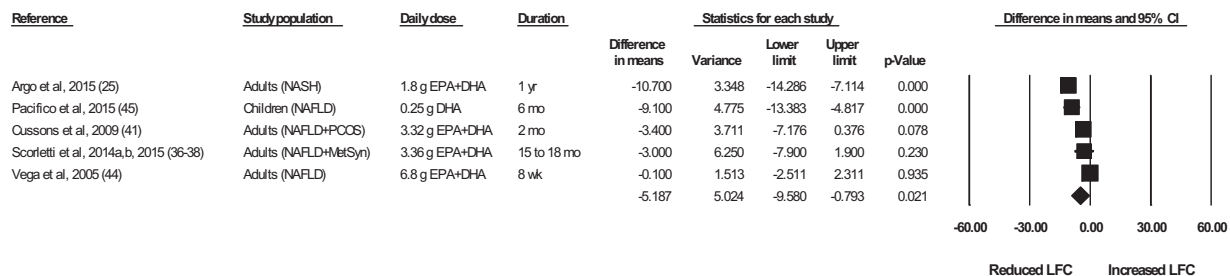


Figure 5 Effects of n-3 LC-PUFAs vs a control on liver fat content, assessed using magnetic resonance imaging or magnetic resonance spectroscopy, in patients with NAFLD. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in liver fat content with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -5.19% (95%CI, -9.58 to -0.79% ; $P=0.021$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; LFC, liver fat content; MetSyn, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PCOS, polycystic ovarian syndrome.

analysis of the studies in children) and when data from the higher-quality studies were pooled (Table 5). For steatosis score, there were statistically significant reductions when the results for adults and children were pooled separately, when the results of shorter-duration and longer-duration studies were pooled separately, and when the results of studies with lower- and higher-quality ratings were pooled separately (Table 5).

Liver biopsies in NASH patients were conducted at baseline and at the end of treatment in a total of 5 studies (representing 6 strata) (Sanyal et al,⁴³ strata 1 and 2, Argo et al,²⁵ Dasarathy et al,²⁶ Li et al,²⁴ Nogueira et al²⁷). In the studies by Argo et al²⁵ and Nogueira et al,²⁷ the end-of-treatment values were not reported in the publication; however, the authors were contacted for the data, and the data were made available for the

meta-analyses. In the study by Sanyal et al⁴³ (strata 1 and 2), the liver biopsy results were not reported in a manner that permitted their inclusion in the meta-analyses. The authors were contacted a minimum of 3 times, and requests for means and standard deviations were made, but the required data were not provided. Thus, data for 4 of the studies (representing 4 strata) were available for pooling in meta-analyses.²⁴⁻²⁷ As shown in Figures 7 through 11, relative to a placebo, n-3 LC-PUFA supplementation had no effect on the liver fibrosis score, hepatocellular ballooning score, steatosis score, lobular inflammation score, or the NAFLD activity score. Most of the sensitivity analyses could not be conducted because there were too few data sets (Table 6). Of note, all studies in which a liver biopsy was conducted at baseline and at end of treatment included adults with NASH.

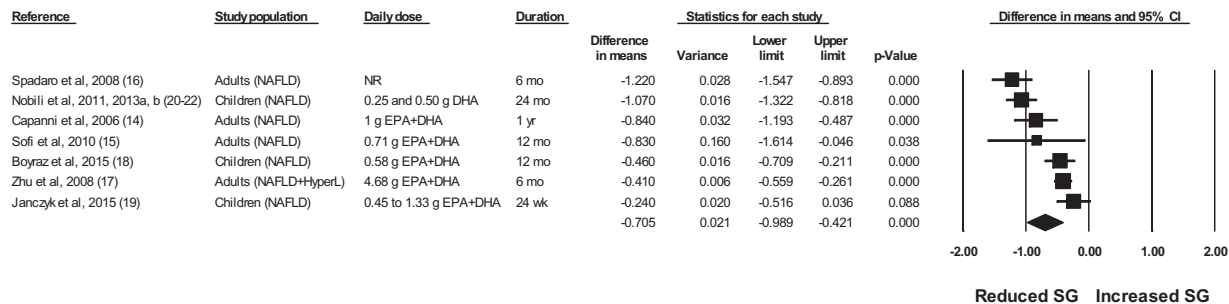


Figure 6 Effects of n-3 LC-PUFAs vs a control on the grade of steatosis, assessed using ultrasonography, in patients with NAFLD. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in the grade of steatosis with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -0.71 (95%CI, -0.99 to -0.42 ; $P < 0.001$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the pooled effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; HyperL, hyperlipidemia/dyslipidemia; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NAFLD, nonalcoholic fatty liver disease; NR, not reported; SG, steatosis grade.

Blood lipids. Changes from baseline in total cholesterol, LDL-C, HDL-C, and triglycerides with n-3 LC-PUFA supplementation, corrected for changes from baseline in the control group, are presented in [Figures S1 through S4](#), respectively, in the [Supporting Information online](#). Significant improvements with n-3 LC-PUFA supplementation were noted for all of the blood lipid parameters. Based on the sensitivity analyses ([Table S3](#) in the [Supporting Information online](#)), all of the pooled effects were favorable (ie, reductions in total cholesterol, LDL-C, and triglycerides and an increase in HDL-C), though they varied with regard to their statistical significance. All the improvements were significant when the analyses were restricted to studies conducted in adults; only for triglycerides was it possible to conduct an analysis restricted to studies conducted in children. When data from the 3 children strata were pooled, the reduction in triglycerides was of borderline significance ($P = 0.056$). When lipid data from studies 6 months or shorter in duration were pooled, the pooled effects were favorable and statistically significant for total cholesterol, LDL-C, HDL-C, and triglycerides; in contrast, when lipid data from studies longer than 6 months were pooled, the pooled effects were significantly favorable only for LDL-C and HDL-C. In general, statistically significant or near-significant improvements in blood lipid levels were observed, whether pooling the results of studies with a low-quality or a high-quality rating.

Glycemic control. Fasting blood glucose, fasting insulin, and adiponectin levels were unaffected by the supplementation of NAFLD patients with n-3 LC-PUFAs ([Figures S5–S7](#) in the [Supporting Information online](#), respectively). These observations generally persisted in the sensitivity analyses ([Table S4](#) in the [Supporting](#)

[Information online](#)), except that fasting insulin was significantly reduced when the results from studies with a lower quality rating were pooled (there were insufficient data points to conduct a separate analysis for higher-quality studies). Insulin resistance was significantly improved from baseline with the supplementation of NAFLD patients with n-3 LC-PUFAs relative to the change from baseline in the control/placebo group (-0.54 ; 95%CI, -0.93 to -0.14 ; $P = 0.008$; [Figure S8](#) in the [Supporting Information online](#)). This result was based on the pooling of data from 8 strata (5 of which were from studies in adults and 3 of which were from studies in children), with a supplementation duration ranging from 8 weeks to 24 months, and an EPA + DHA intake of 0.25 to 3.6 g/d. Based on the sensitivity analyses ([Table S4](#) in the [Supporting Information online](#)), the improvement in HOMA-IR was evident in children, but not in adults, and in the studies with a lower (but not a higher) quality rating.

Body weight/composition and other metabolic risk factors. Body mass index was significantly reduced from baseline with the supplementation of NAFLD patients with n-3 LC-PUFAs relative to the change from baseline in the control/placebo group (-0.85 kg/m²; 95%CI, -1.44 to -0.26 kg/m²; $P = 0.005$; [Figure S9](#) in the [Supporting Information online](#)). Supplementation with n-3 LC-PUFAs had no significant effects on body weight, waist circumference, systolic blood pressure, or diastolic blood pressure ([Figures S10–S13](#), respectively, in the [Supporting Information online](#)), and these null effects persisted in all of the sensitivity analyses. It should be noted that the BMI meta-analysis was based on 10 strata (8 strata from studies in adults and 2 strata

Table 5 Sensitivity analyses: liver enzymes and imaging measures^a

	Liver enzymes			Imaging measures	
	Aspartate aminotransferase (IU/L)	Alanine transaminase (IU/L)	γ -Glutamyl transferase (IU/L)	Liver fat content (assessed by MRI or MRS)	Steatosis score (assessed by ultrasonography)
All strata	n = 12 -1.56 (-6.47 to 3.35) ^b NS	n = 16 -4.63 (-9.18 to -0.08)	n = 8 -5.16 (-8.92 to -1.40) ^b	n = 5 -5.19 (-9.58 to -0.79)	n = 7 -0.71 (-0.99 to -0.42)
Study population	Adults n = 10 -2.89 (-8.93 to 3.14) P = 0.348	n = 11 -1.79 (-7.50 to 3.92) P = 0.538	n = 6 -9.52 (-14.22 to -4.82) P < 0.001	n = 4 -4.25 (-9.16 to 0.65) P = 0.089	n = 4 -0.81 (-1.26 to -0.36) P < 0.001
Children	n = 2; NA	n = 5 -11.69 (-17.66 to -5.73) P < 0.001	n = 2; NA	n = 1; NA	n = 3 -0.59 (-1.08 to -0.11) P = 0.017
EPA + DHA dose (g/d), adult studies only	< 3 g/d n = 5 -3.38 (-13.51 to 6.75) P = 0.513	n = 5 -3.41 (-11.63 to 4.81) P = 0.416	n = 4 -8.76 (-14.85 to -2.67) P = 0.005	n = 1; NA	n = 2; NA
	\geq 3 g/d n = 3 2.77 (-7.95 to 13.49) P = 0.613	n = 4 6.85 (0.98 to 12.73) P = 0.022	n = 1; NA	n = 3 -1.57 (-3.84 to 0.70) P = 0.174	n = 1; NA
Supplementation duration (months)	\leq 6 mo n = 6 1.90 (-8.17 to 11.96) P = 0.712	n = 8 -3.66 (-11.76 to 4.45) P = 0.377	n = 5 -7.45 (-12.48 to -2.41) P = 0.004	n = 3 -3.96 (-9.06 to 1.13) P = 0.127	n = 3 -0.61 (-1.09 to -0.13) P = 0.014
	> 6 mo n = 6 -5.17 (-8.20 to -2.14) P = 0.001	n = 8 -5.42 (-10.58 to -0.27) P = 0.039	n = 3 -3.78 (-9.04 to 1.48) P = 0.159	n = 2; NA	n = 4 -0.79 (-1.13 to -0.46) P < 0.001
Jadad score	< 6/8 n = 5 -4.89 (-11.78 to 2.01) P = 0.165	n = 6 -5.13 (-11.38 to 1.11) P = 0.107	n = 4 -4.32 (-9.0 to 0.367) P = 0.071	n = 2; NA	n = 4 -0.54 (-0.74 to -0.34) P < 0.001
	\geq 6/8 n = 7 -0.20 (-6.68 to 6.29) P = 0.952	n = 10 -4.25 (-11.55 to 3.06) P = 0.254	n = 4 -7.25 (-13.69 to -0.81) P = 0.027	n = 3 -7.04 (-14.58 to 0.50) P = 0.067	n = 3 -0.84 (-1.44 to -0.25) P = 0.006

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; MRI, magnetic resonance imaging; MRS, magnetic resonance spectroscopy; n, number of strata; NA, not applicable; NS, not significant.

^aResults in boldface type are statistically or nearly statistically significant ($P < 0.10$).

^bUsing the trim and fill method of Duval and Tweedie,³⁵ 1 study was found to be missing to the right of the mean effect. The missing study was imputed. The results presented are adjusted for publication bias.

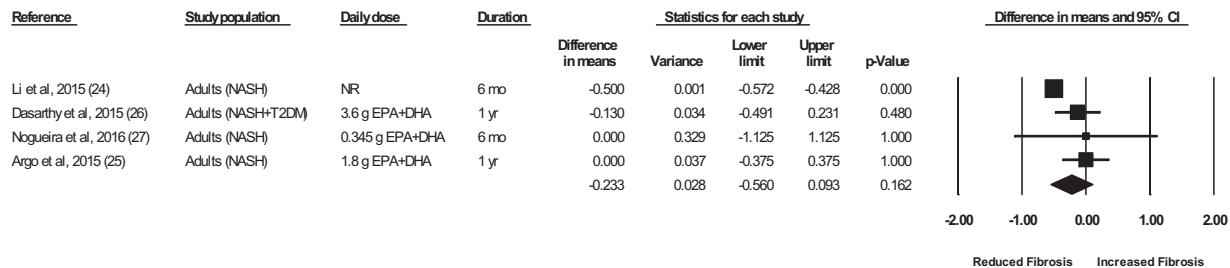


Figure 7 Effects of n-3 LC-PUFAs vs a control on the fibrosis score, assessed histologically, in patients with NASH. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in the fibrosis score with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -0.23 (95%CI, -0.56 to 0.093 ; $P=0.162$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NASH, nonalcoholic steatohepatitis; NR, not reported; T2DM, type 2 diabetes mellitus.

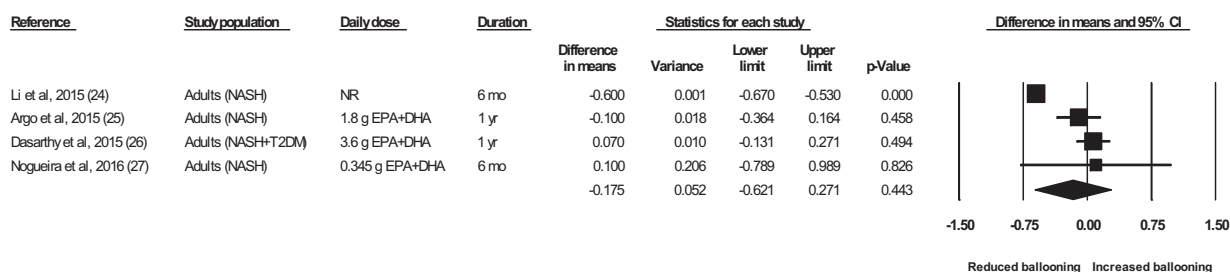


Figure 8 Effects of n-3 LC-PUFAs vs a control on the hepatocellular ballooning score, assessed histologically, in patients with NASH. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in the hepatocellular ballooning score with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -0.18 (95%CI, -0.62 to 0.27 ; $P=0.443$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NASH, nonalcoholic steatohepatitis; NR, not reported; T2DM, type 2 diabetes mellitus.

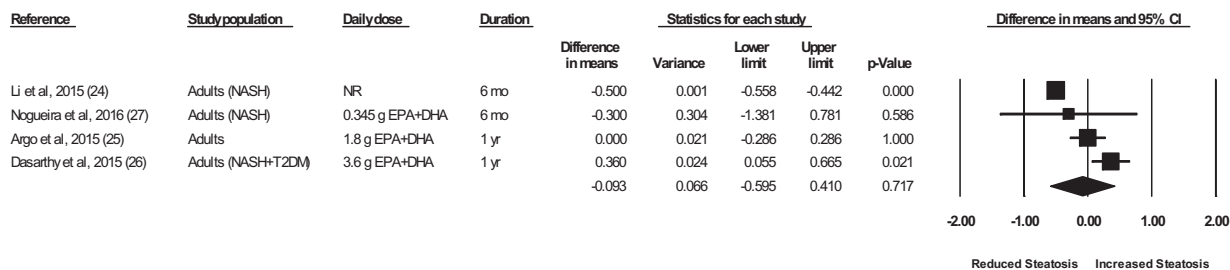


Figure 9 Effects of n-3 LC-PUFAs vs a control on the steatosis score, assessed histologically, in patients with NASH. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in the steatosis score with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -0.09 (95%CI, -0.60 to 0.41 ; $P=0.717$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NASH, nonalcoholic steatohepatitis; SS, steatosis score; T2DM, type 2 diabetes mellitus.

from studies in children), while the body weight meta-analysis was based on 6 strata (4 strata from studies in adults and 2 strata from studies in children). The reductions in BMI were generally the greatest for the 2 strata from the studies in children, and without these strata,

the pooled effect on BMI was not statistically significant ($P=0.059$) (Table S5 in the Supporting Information online).

An overall summary of the meta-analysis results is provided in Table 7.^{25,35}

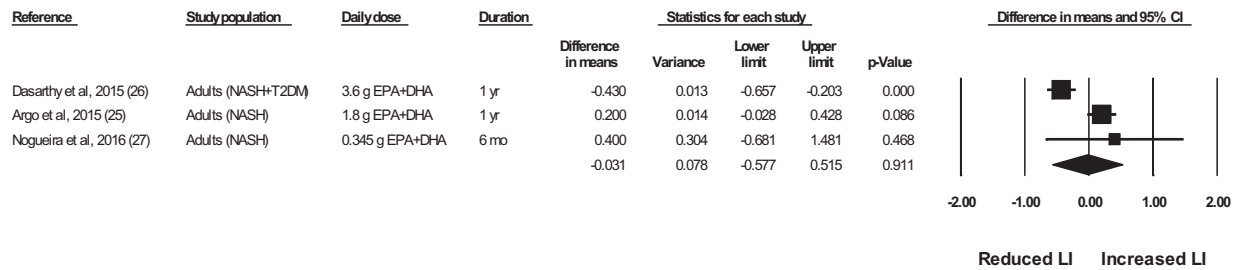


Figure 10 Effects of n-3 LC-PUFAs vs a control on the lobular inflammation score, assessed histologically, in patients with NASH. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in the lobular inflammation score with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is -0.03 (95%CI, -0.58 to 0.52 ; $P=0.911$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; LI, lobular inflammation; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

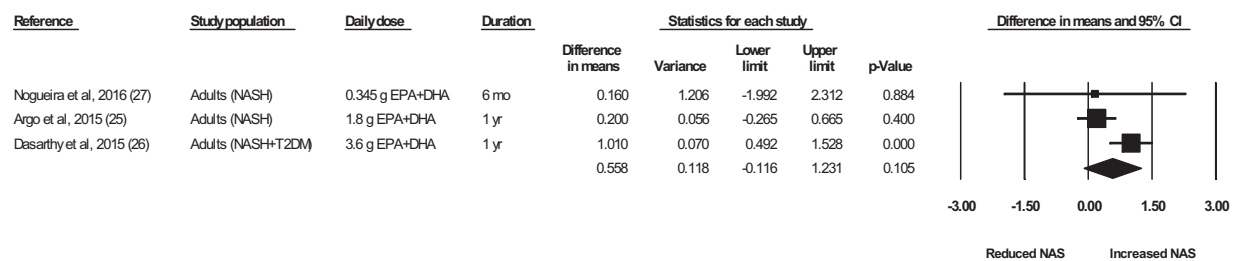


Figure 11 Effects of n-3 LC-PUFAs vs a control on NAS, assessed histologically, in patients with NASH. A random-effects model was used to calculate the pooled estimate of the differences in means and the accompanying 95%CI. Studies were weighted by the inverse of their variance; the area of each symbol is proportional to the weight of the study. The diamond represents the pooled effect. The pooled change from baseline in NAS with intake of n-3 LC-PUFAs, corrected for changes from baseline in the control group, is 0.56 (95%CI, -0.12 to 1.23 ; $P=0.105$). Using the trim and fill method of Duval and Tweedie,³⁵ no studies were found to be missing to the right of the mean effect. *Abbreviations:* DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n-3 LC-PUFAs, omega-3 long-chain polyunsaturated fatty acids; NAS, nonalcoholic fatty liver disease activity score; NASH, nonalcoholic steatohepatitis; T2DM, type 2 diabetes mellitus.

DISCUSSION

Nonalcoholic fatty liver disease has become a disease of public health significance in Westernized countries, afflicting both adults and children. Currently, there is no pharmacological therapy for NAFLD, and diet and exercise represent the first line of treatment in NAFLD management. Intrahepatic fat content is significantly improved with exercise (either aerobic or resistance), even in the absence of weight loss.^{46–50} Weight loss also is effective in improving NAFLD, with benefits dependent on the amount of weight lost: with a loss in body weight of 3% to 5%, steatosis is improved; with a loss of 5% to 7%, hepatic inflammation is improved; and with a loss of 7% to 10%, there may be a regression of fibrosis and a remission of NAFLD/NASH.^{5,51} Most patients, however, have difficulty with long-term changes in exercise and diet. For example, Pugh et al⁵² noted significant improvements in liver fat content in patients with NAFLD following a 16-week supervised exercise

program; however, 12 months after the cessation of exercise supervision, all exercise-induced benefits, including the reduction in liver fat content, had reverted to baseline. Likewise, Dudekula et al⁵³ reported that most patients cannot achieve the required degree of weight loss or have trouble with the long-term maintenance of body weight after weight loss.

Adjunctive treatments to exercise and weight loss are needed for the management of NAFLD. The meta-analysis results presented herein demonstrate the usefulness of n-3 LC-PUFAs in the dietary management of patients with NAFLD.

Effects of n-3 LC-PUFA supplementation on liver enzymes and on liver fat content and steatosis score assessed via imaging in patients with NAFLD

Based on the meta-analyses, n-3 LC-PUFA supplementation of patients with NAFLD is associated with significant reductions in both serum ALT and serum GGT

Table 6 Sensitivity analyses: liver biopsy measures

	Fibrosis	Hepatocellular ballooning score	Steatosis score	Lobular inflammation score	NAFLD activity score
All strata	n = 4 -0.23 (-0.56 to 0.093) P = 0.162	n = 4 -0.18 (-0.62 to 0.27) P = 0.443	n = 4 -0.09 (-0.60 to 0.41) P = 0.717	n = 3 -0.03 (-0.58 to 0.52) P = 0.911	n = 3 0.56 (-0.12 to 1.23) P = 0.105
Study population	Adults n = 4 -0.23 (-0.56 to 0.093) P = 0.162	n = 4 -0.18 (-0.62 to 0.27) P = 0.443	n = 4 -0.09 (-0.60 to 0.41) P = 0.717	n = 3 -0.03 (-0.58 to 0.52) P = 0.911	n = 3 0.56 (-0.12 to 1.23) P = 0.105
EPA + DHA dose (g/d), adult studies only	Children n = 0; NA	n = 0; NA	n = 0; NA	n = 0; NA	n = 0; NA
	< 3 g/d n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA
	≥ 3 g/d n = 1; NA	n = 1; NA	n = 1; NA	n = 1; NA	n = 1; NA
Supplementation duration (months)	< 6 mo n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA
	> 6 mo n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA	n = 2; NA
Jadad score	< 6/8 n = 1, NA	n = 1, NA	n = 1, NA	n = 0; NA	n = 0; NA
	≥ 6/8 n = 3	n = 3	n = 3	n = 3	n = 3
	-0.06 (-0.32 to 0.19) P = 0.620	0.01 (-0.15 to 0.17) P = 0.895	0.14 (-0.17 to 0.45) P = 0.379	-0.03 (-0.58 to 0.52) P = 0.911	0.56 (-0.12 to 1.23) P = 0.105

Abbreviations: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; n, number of strata; NA, not applicable.

(favorable effects), with no effects on serum AST, findings that are generally consistent with the meta-analyses conducted by He et al.¹¹ Further, based on the meta-analyses, liver fat content and steatosis score, assessed via MRI/MRS or ultrasonography, respectively, were both significantly improved with n-3 LC-PUFA supplementation. The pooled reduction in liver fat content of approximately 5.2% is clinically meaningful; likewise, the pooled reduction of approximately 0.7 in steatosis score also is clinically meaningful. As steatosis is scored using a 4-grade scale, the pooled reduction equates to an improvement approaching an entire grade. The improvements in steatosis score were very robust—pooled reductions were statistically significant when data from all studies were pooled as well as when data from studies in adults and children, from longer and shorter studies, and from higher-quality and lower-quality studies were pooled separately.

Effects of n-3 LC-PUFA supplementation on liver histological outcomes in patients with NAFLD

On the basis of the meta-analysis results, liver histology parameters (ie, fibrosis score, hepatocellular ballooning score, steatosis score, lobular inflammation score, and NAFLD activity score) are unaffected by supplementation with n-3 LC-PUFAs. Most of the liver histology meta-analyses were based on 4 clinical studies in NASH patients,^{24–27} 3 of which showed no benefit of n-3 LC-PUFA supplementation on any of the liver histology outcomes assessed.^{25–27} It is difficult to draw definitive conclusions from these studies, given that the dose of EPA + DHA administered was very low (0.345 g/d),²⁷ the studies were small,^{25,26} measures of compliance were not assessed,²⁶ physical activity levels were not assessed,^{26,27} or there were unexpected improvements in the placebo group in NAFLD activity score and steatosis score, implicating changes in diet or exercise as potential confounding variables.²⁶ In the study by Argo et al²⁵ the patients in the n-3 LC-PUFA group tended to have more severe NAFLD relative to patients in the placebo group at baseline; moreover, baseline intakes of fructose were significantly greater in the n-3 LC-PUFA group relative to the placebo group ($P = 0.01$). The latter observation is notable, given that high intakes of dietary fructose negatively affect the progression of NAFLD.⁵⁴ The disconnect between the higher-quality ratings of these studies (scores ranged from 6.5 to 8) and their methodological limitations illustrates the challenges in using a quality-appraisal tool in assessing study quality.

Only in the study by Li et al²⁴ was the n-3 LC-PUFA supplementation (50 mL/d with a 1:1 ratio of EPA to DHA) of patients with NASH associated with

Table 7 Effects of omega-3 long-chain polyunsaturated fatty acids on liver-related outcomes and metabolic risk factors in patients with nonalcoholic fatty liver disease (NAFLD): results of meta-analyses^a

Parameter	No. of strata	No. of participants		Pooled effect	Publication bias	Adjusted pooled effect
		Adults	Children			
Liver-related outcomes						
Serological measures						
AST	12	600	172	-2.41 IU/L (95%CI, -7.35 to 2.52 IU/L; <i>P</i> = 0.338)	Yes ^b	-1.56 IU/L (95%CI, -6.47 to 3.35 IU/L)
ALT	16	612	283	-4.63 IU/L (95%CI, -9.18 to -0.08 IU/L; <i>P</i> = 0.046)	No	NA
GGT	8	356	172	-5.55 IU/L (95%CI, -9.61 to -1.50 IU/L; <i>P</i> = 0.007)	Yes ^b	-5.16 IU/L (95%CI, -8.92 to -1.40 IU/L)
Imaging measures						
Liver fat content ^c	5	153	51	-5.19% (95%CI, -9.58 to -0.79; <i>P</i> = 0.021)	No	NA
Steatosis score ^d	7	237	225	-0.71 (95%CI, -0.99 to -0.42; <i>P</i> < 0.001)	No	NA
Fibrosis score	4	199	0	-0.23 (95%CI, -0.56 to 0.093; <i>P</i> = 0.162)	No	NA
Hepatocellular ballooning score	3	199	0	-0.18 (95%CI, -0.62 to 0.27; <i>P</i> = 0.443)	No	NA
Steatosis score	4	199	0	-0.09 (95%CI, -0.60 to 0.41; <i>P</i> = 0.717)	No	NA
Lobular inflammation score	3	121	0	-0.03 (95%CI, -0.58 to 0.52; <i>P</i> = 0.911)	No	NA
NAFLD activity score	3	121	0	0.56 (95%CI, -0.12 to 1.23; <i>P</i> = 0.105)	No	NA
Blood lipids						
Total cholesterol	11	507	159	-8.2 mg/dL (95%CI, -15.8 to -0.60 mg/dL; <i>P</i> = 0.035)	No	NA
LDL-C	9	447	108	-7.2 mg/dL (95%CI, -11.4 to -3.1 mg/dL; <i>P</i> = 0.001)	No	NA
HDL-C	11	486	159	3.1 mg/dL (95%CI, 1.5 to 4.8 mg/dL; <i>P</i> < 0.001)	No	NA
Triglycerides	15	563	168	-24.8 mg/dL (95%CI, -36.7 to -12.9 mg/dL; <i>P</i> < 0.001)	Yes ^b	-23.4 mg/dL (95%CI, -35.1 to -11.7 mg/dL)
Glycemic control						
Fasting blood glucose	9	298	159	-0.80 mg/dL (95%CI, -2.78 to 1.18 mg/dL; <i>P</i> = 0.427)	No	NA
Fasting insulin	5	94	108	-1.48 mIU/L (95%CI, -4.68 to 1.72 mIU/L; <i>P</i> = 0.364)	No	NA
HOMA-IR	8	130	168	-0.54 (95%CI, -0.93 to -0.14; <i>P</i> = 0.008)	No	NA
Adiponectin	3	115	0	0.49 µg/mL (95%CI, -0.30 to 1.27 µg/mL; <i>P</i> = 0.223)	No	NA
Body weight, body composition						
BMI	10	418	159	-0.85 kg/m² (95%CI, -1.44 to -0.26 kg/m²; <i>P</i> = 0.005)	No	NA
Body weight	6	236	159	-0.64 kg (95%CI, -1.59 to 0.31 kg; <i>P</i> = 0.184)	Yes ^b	-0.57 kg (95%CI, -1.51 to 0.37 kg)
Waist circumference	5	236	51	-1.5 cm (95%CI, -3.5 to 0.5 cm; <i>P</i> = 0.133)	Yes ^f	-0.39 cm (95%CI, -2.1 to 1.3 cm)
Other						
Systolic BP	5	177	159	-4.0 mmHg (95%CI, -15.0 to 7.0 mmHg; <i>P</i> = 0.476)	No	NA
Diastolic BP	5	177	159	-0.43 mmHg (95%CI, -2.4 to 1.5 mmHg; <i>P</i> = 0.668)	No	NA

Abbreviations: ALT, alanine transaminase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; GGT, γ -glutamyl transaminase; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; LDL-C, low-density lipoprotein cholesterol; NA, not applicable.

^aPooled effects represent the changes from baseline in the omega-3 long-chain polyunsaturated fatty acids group, corrected for the corresponding changes from baseline in the control group. Results in boldface type are statistically significant (*P* < 0.05).

^bOne study was found to be missing to the right of the pooled effect, using the trim and fill method of Duval and Tweedie.³⁵

^cAll study participants were NAFLD patients.

^dAll study participants were NAFLD patients, except for those in the study by Argo et al,²⁵ which included NASH patients.

^eAll study participants were NASH patients.

^fThree studies were found to be missing to the right of the pooled effect, using the trim and fill method of Duval and Tweedie.³⁵

significant improvements in histological outcomes (ie, steatosis score, necroinflammatory score, fibrosis score, and hepatocellular ballooning score) when compared with a placebo. This study was larger than those by Argo et al²⁵ and Dasarathy et al,²⁶ and participants in the placebo and n-3 LC-PUFA groups were well matched at baseline. The patients in the study by Li et al²⁴ were instructed to consume a low-fat, low-cholesterol, low-carbohydrate diet and to engage in moderate physical exercise (30 min/d, 5 d/wk). Dietary intakes were not monitored, and there were no measures of biological compliance; however, physical activity levels were monitored and were demonstrated to increase to the same degree in both groups.

Sanyal et al⁴³ (strata 1 and 2) evaluated the effects of EPA (administered at either 1.8 or 2.7 g/d in ethyl ester form for 12 mo) on histological outcomes in NASH patients, but this study could not be included in the meta-analyses because the data were not reported in a manner that permitted their inclusion in the meta-analysis and the research group failed to provide the requested data after at least 3 requests. No improvements in any of the histological outcomes were noted. However, in no other study was EPA administered in isolation. In the studies in children by Pacifico et al⁴⁵ and Nobili et al,^{20–22} DHA, when administered alone, was shown to improve liver fat content (assessed via imaging) in populations that included NASH patients, indicating that DHA may be required for efficacy. Likewise, Scorletti et al^{36–38} demonstrated that the reduction in liver fat content (assessed via imaging) was a function of the enrichment of red blood cells with DHA but not EPA. In an analysis of the total patient population (irrespective of the treatment received), Nogueira et al²⁷ demonstrated that lobular inflammation score (assessed histologically) was significantly and inversely correlated with plasma DHA. Thus, it appears that DHA may be required for efficacy. If this is indeed the case, then it is reasonable that the study by Sanyal et al⁴³ was a null study, in that only EPA was administered.

In the study in children conducted by Nobili et al,^{20–22} NAFLD was graded via liver histology in all children at baseline^{20–22} and again after 18 months, but only in the 20 children randomized to receive DHA at 250 mg/d.⁵⁵ Because histological analysis was not conducted in the context of a controlled intervention study, the results reported by Nobili et al⁵⁵ could not be included in the liver histology meta-analyses; however, the results are relevant for discussion, particularly since reports of histological evaluation of pediatric patients before and after n-3 LC-PUFA supplementation are limited. Histological analysis was performed by a single, blinded pathologist. Among the 20 children with

NAFLD—12 (60%) of whom had NASH—there were significant improvements after DHA supplementation in several histological outcomes, including steatosis score, hepatocellular ballooning score, lobular inflammation score, NAFLD activity score, and a pediatric-specific histology score, the Pediatric NAFLD Histological Score. Only fibrosis was unaffected by DHA supplementation. All children in the Nobili et al^{20–22,55} study were prescribed an energy-reduced diet and encouraged to exercise. Without liver biopsy results for the placebo group, it is not possible to definitively attribute the improvements in liver histology to DHA supplementation. However, steatosis grade, assessed via ultrasonography, was significantly improved with DHA supplementation (either 250 or 500 mg/d) vs the placebo by 6 months and remained so at 24 months (Nobili et al²⁰), indicating that improvements from baseline observed in liver histology in the 250 mg/d DHA group (Nobili et al⁵⁵) are likely attributable to DHA supplementation and not just to changes in diet or exercise.

As it stands, there is evidence from only 2 studies that n-3 LC-PUFA supplementation improves histological outcomes in patients with NASH (Nobili et al,⁵⁵ Li et al²⁴). Unfortunately, the study by Li et al²⁴ has a lower quality rating of 3.5 out of 8, and in the study by Nobili et al,⁵⁵ the liver histology assessments were not conducted in a controlled manner (ie, histology was assessed before and after n-3 LC-PUFA supplementation, rather than before and after n-3 LC-PUFA vs placebo supplementation). Thus, based on the available studies, no definitive conclusions on the effects of n-3 LC-PUFA supplementation on histological outcomes in patients with NAFLD can be made. It should be noted, however, that of all the studies in which effects of n-3 LC-PUFAs on histological outcomes were assessed, only in the studies by Li et al²⁴ and Nobili et al^{20,55} were significant benefits on triglyceride levels noted, in favor of the n-3 LC-PUFA groups. Since n-3 LC-PUFAs are used pharmacologically to treat hypertriglyceridemia, it is surprising that effects on triglyceride levels were not observed in any of the other studies, particularly those by Argo et al,²⁵ Dasarathy et al,²⁶ and Sanyal et al,⁴³ in which the doses administered were within the therapeutic range for triglyceride lowering (in the study by Sanyal et al,⁴³ the EPA ethyl ester used had previously been demonstrated to improve hyperlipidemia, including hypertriglyceridemia and hypercholesterolemia, in Japanese populations and is still used for this purpose in Japan).

Although histological examination by biopsy remains the gold standard in the grading of NAFLD, it is losing popularity, given that a liver biopsy is invasive, provides information on only 1/50 000 of the liver, and is associated with potential (and sometimes

life-threatening) complications to the patient, errors in sampling, and complexity in pathological interpretation (reviewed by Golabi et al⁴⁶). Indeed, the study by Scorletti et al^{36–38} was planned to include a liver biopsy at the end of supplementation period; however, this plan was changed because 2 liver biopsy-associated deaths (unrelated to the study) occurred in the hospital that year. In the studies in children by Nobili et al^{20–22} and Pacifico et al⁴⁵, it was noted that liver biopsies are ethical when conducted for diagnostic purposes, but of questionable ethics for the subsequent monitoring of NAFLD.

Imaging via MRI/MRS and ultrasonography is increasingly being used to assess liver fat content and steatosis score, respectively, in patients with NAFLD. The MRI method used by Pacifico et al⁴⁵ is similar to that used by Argo et al²⁵ and has been previously validated against histological findings confirming hepatic steatosis; specifically, strong and statistically significant correlations were reported between the hepatic fat fraction (assessed via MRI) and the histological grade of steatosis, such that mild (grade 1), moderate (grade 2), and severe (grade 3) steatosis were associated with hepatic fat fractions of 8.7% (95%CI, 6.0% to 11.6%), 21.6% (95%CI, 15.3% to 27.0%), and 39.7% (95%CI, 34.4% to 45.0%), respectively.⁵⁶ Moreover, the degree of fibrosis and inflammation had no impact on the accuracy of MRI in the assessment of the degree of steatosis. Likewise, MRS is a noninvasive and well-validated method for quantifying liver fat content, and results of MRS correlate well with findings of steatosis assessed via liver histology.^{57,58} Ultrasonography is the most economical and well-tolerated imaging technique that permits the grading of steatosis across all severities (mild to severe).^{59,60} As reviewed here, there is strong evidence that hepatic fat content (assessed via MRI or MRS) and steatosis score (assessed via ultrasonography) are significantly improved with n-3 LC-PUFA supplementation.

Effects of n-3 LC-PUFAs on cardiometabolic risk factors in patients with NAFLD

Based on the results of the meta-analyses presented herein, n-3 LC-PUFA supplementation of patients with NAFLD is associated with significant improvements in total cholesterol, LDL-C, HDL-C, and triglycerides. These findings are aligned with the results of a previous meta-analysis of 7 randomized controlled studies evaluating the effects of dietary n-3 LC-PUFAs in adults with NAFLD.¹¹ The current meta-analysis includes all 7 of the randomized controlled trials^{15–17,24,25,33,36–38} included in the earlier meta-analysis by He et al¹¹ as well as another 4 randomized controlled trials in adults,^{26,27,41,42} 2 nonrandomized controlled trials in

adults,^{14,44} and 4 randomized controlled trials in children.^{18–22,45}

No effects of n-3 LC-PUFA supplementation on fasting blood glucose, fasting insulin, or adiponectin were observed; however, a significant reduction in HOMA-IR (-0.54 ; 95%CI, -0.93 to -0.14 ; $P = 0.008$) was found. When data from adult and pediatric studies were analyzed separately, the significant reduction in HOMA-IR was evident only for children. There was also a significant reduction in BMI (-0.85 kg/m²; 95%CI, -1.44 to -0.26 kg/m²; $P = 0.005$) but no significant effects on body weight, waist circumference, systolic blood pressure, or diastolic blood pressure. It should be noted that, once the studies in children were removed from the BMI meta-analysis, the reduction in BMI was attenuated and was no longer significant, indicating that the pooled reduction in BMI when all studies were included may have been driven by a reduction in BMI in children, who, owing to increases in height during the study, may have experienced reductions in BMI.

Increased requirement for n-3 LC-PUFAs in patients with NAFLD

It has been demonstrated in several observational studies that patients with NAFLD have reduced relative hepatic levels of EPA and DHA compared with individuals who do not have NAFLD.^{61–63} Of note, Elizondo et al⁶³ observed that, compared with controls, patients with NAFLD had significantly elevated relative levels of Osbond acid in hepatic and red blood cell phospholipids. Osbond acid, or docosapentaenoic acid of the n-6 series (C22:5n-6), increases only in states of DHA deficiency.^{64,65} The production of Osbond acid is believed to be a compensatory mechanism for conserving the fluidity of membrane phospholipids when there is insufficient DHA.⁶⁶ Thus, there is some indication from the study by Elizondo et al⁶³ that patients with NAFLD may be deficient in DHA.

The etiology of the depressed hepatic levels of EPA and DHA in patients with NAFLD is unclear. The activity of $\Delta 5$ desaturase has been found to be significantly lower in patients with NAFLD than in controls.^{40,67–70} A reduction in the activity of D5D essentially means that EPA (20:5n-3) and, therefore, by default, DHA (22:6n-3) cannot be adequately synthesized from their precursor, 20:4n-3, thereby increasing the requirement for preformed dietary sources of EPA and DHA. Puri et al⁷¹ suggested there may be derangements in peroxisomal function in patients with NAFLD, given that DHA synthesis occurs in peroxisomes. Araya et al⁶² observed that levels of elaidic acid (*trans* 18:1n-9) in adipose tissue were significantly increased in NAFLD

patients vs controls. *Trans* fatty acids are potent inhibitors of $\Delta 6$ desaturase,⁷² a rate-limiting enzyme in the α -linolenic acid desaturation/elongation pathway. It is unclear why levels of *trans* fatty acids are increased in patients with NAFLD; while dietary intakes of these fatty acids could be greater in patients with NAFLD, the possibility of de novo synthesis of *trans* fatty acids by the human microbiome in NAFLD patients has never been investigated and cannot be ruled out. Finally, allelic variants in the genes that code for the desaturase enzymes have been strongly associated with NAFLD risk.⁷³

Both EPA and DHA are important modulators of hepatic gene expression, and, via their interactions with SREBP-1 and PPAR, they have a role in reducing hepatic lipogenesis and increasing hepatic fatty acid β -oxidation (reviewed in the Introduction). They also undergo metabolism in the cytochrome P450, cyclooxygenase, and lipoxygenase pathways (in direct competition with arachidonic acid) to eicosanoids such as prostaglandins, leukotrienes, thromboxanes, protectins, and resolvins.^{74,75} Without sufficient levels of EPA and DHA, the balance in the liver is tipped in favor of lipogenesis over lipolysis, and there may be inadequate production of anti-inflammatory mediators via the cytochrome P450, cyclooxygenase, and lipoxygenase pathways.^{74,75}

CONCLUSION

Given the results reported here of significant improvements in patients with NAFLD in liver fat content, steatosis score, and several cardiometabolic risk factors following supplementation with n-3 LC-PUFAs, adult and pediatric patients with NAFLD should be encouraged to increase their intakes of n-3 LC-PUFAs. Supplementation with n-3 LC-PUFAs should be encouraged in the context of increased physical activity and caloric restriction, which effectively reduce the progression of NAFLD and currently constitute the first line of treatment for patients with NAFLD.^{46,51}

In children with NAFLD, dietary and lifestyle interventions are particularly important, given that pharmacological interventions to treat NAFLD, when or if they become available, may be less desirable because of potential adverse effects. In addition, since NAFLD is a progressive disease most often associated with comorbidities, it is particularly concerning when the disease manifests in children. Despite the limited number of studies conducted specifically in children with NAFLD, it is clear from the sensitivity analyses reported herein that n-3 LC-PUFA supplementation in children significantly reduces serum ALT, steatosis score, triglycerides, and HOMA-IR. Liver fat content was assessed in only 1

controlled study in children.⁴⁵ In that study, liver biopsies were conducted at baseline, and MRI was used to assess liver fat content at baseline and at the end of the 6-month DHA (250 mg/d) intervention phase. There was a significant reduction in liver fat content in DHA-supplemented children vs controls; of note, 65% of the children had NASH. Other cardiometabolic risk factors may also be favorably impacted by n-3 LC-PUFA supplementation, but there were simply too few strata to conduct pooled analyses of results for these variables specifically in children with NAFLD.

Based on the dosages administered across the studies, effective daily intakes appear to be 250 mg of DHA in children and approximately 3.0 g of EPA + DHA in adults. The minimum effective intake of n-3 LC-PUFAs is not known, nor is it clear whether EPA is even necessary for clinical efficacy, given that efficacy has been reported in children with supplements containing only DHA. Additional studies are required to resolve these important questions. It is strongly recommended that diet and physical activity be closely monitored in future studies to ensure results are not confounded by unbalanced changes in these variables. Moreover, it is imperative that biological measures of compliance be assessed to ensure participants in the active intervention group consume the n-3 LC-PUFA supplements and those in the control group do not increase their intakes of n-3 LC-PUFAs (eg, from marine sources).

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Declaration of interest. All of the authors are employees of Intertek Scientific & Regulatory Consultancy. Pronova BioPharma Norge AS (Lysaker, Norway), part of BASF AS, is a client of Intertek Scientific & Regulatory Consultancy.

Supporting Information

The following Supporting Information is available through the online version of this article at the publisher's website.

Appendix S1 PRISMA checklist

Table S1 Key study characteristics of intervention studies in which the effects of n-3 LC-PUFAs in adults with NAFLD were assessed

Table S2 Key study characteristics of intervention studies in which the effects of long-chain n-3 LC-PUFA supplementation in children with NAFLD were assessed

Table S3 Sensitivity analyses—blood lipids

Table S4 Sensitivity analyses—measures of glycaemic control

Table S5 Sensitivity analyses—other metabolic risk factors

Figure S1 The effects of n-3 LC-PUFAs vs a control on total cholesterol (TC) levels in patients with NAFLD

Figure S2 The effects of n-3 LC-PUFAs vs a control on LDL-C levels in patients with NAFLD

Figure S3 The effects of n-3 LC-PUFAs vs a control on HDL-C levels in patients with NAFLD

Figure S4 The effects of n-3 LC-PUFAs vs a control on triglyceride (TG) levels in patients with NAFLD

Figure S5 The effects of n-3 LC-PUFAs vs a control on fasting blood glucose (FBG) levels in patients with NAFLD

Figure S6 The effects of n-3 LC-PUFAs vs a control on fasting insulin (FIN) levels in patients with NAFLD

Figure S7 The effects of n-3 LC-PUFAs vs a control on adiponectin levels in patients with NAFLD

Figure S8 The effects of n-3 LC-PUFAs vs a control on HOMA-IR in patients with NAFLD

Figure S9 The effects of n-3 LC-PUFAs vs a control on BMI in patients with NAFLD

Figure S10 The effects of n-3 LC-PUFAs vs a control on body weight (BW) in patients with NAFLD

Figure S11 The effects of n-3 LC-PUFAs vs a control on waist circumference (WC) in patients with NAFLD

Figure S12 The effects of n-3 LC-PUFAs vs a control on systolic blood pressure (SBP) in patients with NAFLD

Figure S13 The effects of n-3 LC-PUFAs vs a control on diastolic blood pressure (DBP) in patients with NAFLD

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