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Randomized control trials

Omega-3 polyunsaturated fatty acids in treating non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled trial

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SUMMARY

Background: & aims: Few clinical trials have addressed the potential benefits of omega-3 polyunsaturated fatty acids (PUFAs) on non-alcoholic steatohepatitis (NASH). We evaluated the effects of supplementation with omega-3 PUFAs from flaxseed and fish oils in patients with biopsy-proven NASH.
Methods: Patients received three capsules daily, each containing 0.315 g of omega-3 PUFAs (64% alpha-linolenic [ALA], 16% eicosapentaenoic [EPA], and 21% docosahexaenoic [DHA] acids; n-3 group, $n = 27$) or mineral oil (placebo group, $n = 23$). Liver biopsies were evaluated histopathologically by the NASH activity score (NAS). Plasma levels of omega-3 PUFAs were assessed as a marker of intake at baseline and after 6 months of treatment. Secondary endpoints included changes in plasma biochemical markers of lipid metabolism, inflammation, and liver function at baseline and after 3 and 6 months of treatment.
Results: At baseline, NAS was comparable between the groups ($p = 0.98$). After intervention with omega-3 PUFAs, plasma ALA and EPA levels increased ($p \leq 0.05$). However in the placebo group, we also observed increased EPA and DHA ($p \leq 0.05$), suggesting an off-protocol intake of PUFAs. NAS improvement/stabilization was correlated with increased ALA in the n-3 group ($p = 0.02$) and with increased EPA ($p = 0.04$) and DHA ($p = 0.05$) in the placebo group. Triglycerides were reduced after 3 months in the n-3 group compared to baseline ($p = 0.01$).
Conclusions: In NASH patients, the supplementation of omega-3 PUFA from flaxseed and fish oils significantly impacts on plasma lipid profile of patients with NASH. Plasma increase of these PUFAs was associated with better liver histology. (ID 01992809)

1. Introduction

Non-alcoholic fatty liver disease (NAFLD[1]) encompasses a large spectrum of conditions, ranging from simple steatosis to steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma [1]. Mechanisms driving the transition from simple steatosis to NASH and the later degrees of NAFLD are multifactorial and seem to involve oxidative stress, lipotoxicity, insulin resistance, and central inflammatory signalling pathways [2,3]. Maintaining a proper body weight and a healthy lifestyle seem to provide benefits for controlling NASH development [4].

Because dietary and lifestyle modifications often fail or cannot be implemented effectively, new pharmacologic agents, designed

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[1] AA, arachidonic acid; ALA, alpha-linolenic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; CRP, C-reactive protein; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FA, fatty acid; GGT, gamma-glutamyl transferase; HDL, high-density lipoprotein; IL-6, interleukin-6; LDL, low-density lipoprotein; MS, metabolic syndrome; n-3, omega-3; n-6, omega-6; NAFLD, nonalcoholic fatty liver disease; NAS, NASH activity score; NASH, nonalcoholic steatohepatitis; PUFA, polyunsaturated fatty acids.

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and studied with the main goal of improving hepatic histopathology, are needed. In this regard, polyunsaturated fatty acids (PUFAs) play a role in lipid metabolism and inflammation and are potential candidates. Patients with NASH present a higher liver ratio of omega-6 (n-6) to omega-3 (n-3) PUFAs compared to healthy controls, suggesting a possible role for low n-3 PUFA or high n-6 PUFA content in the physiopathology of this disease [5].

Omega-3 PUFAs have biological properties of special interest to the treatment of NASH. They include the essential alpha-linolenic acid (ALA), present in some vegetable sources, and the eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, abundantly found in cold-water marine fish. These three n-3 PUFAs may influence hepatic lipid metabolism, adipose tissue function, and immune response through anti-inflammatory effects [6,7].

Due to its lower unsaturation, ALA is less vulnerable to oxidation than EPA and DHA. In addition, ALA can be endogenously converted into EPA and, to a lesser extent, into DHA [8]. Several studies have demonstrated various cardioprotective, glycaemic and lipid-lowering benefits for ALA, but n-3 PUFAs supplementation in NASH was mainly evaluated using EPA and DHA as source [9–12].

Experimentally, n-3 PUFAs were associated with a positive impact on NAFLD treatment by reducing hepatic steatosis, improving insulin sensitivity, and reducing inflammatory markers [13]. Despite these findings, only few randomized trials have addressed the effect of n-3 PUFA supplementation on liver histology in patients with NAFLD or NASH, and those studies suffer limitations including, poor study design, small samples, lack of omega-3 intake markers and use of techniques that measure only indirectly liver histology, mainly ultrassonography [14–19]. We performed a double-blind, randomized, and controlled study assessing the effect of oral supplementation of n-3 PUFAs derived from flaxseed and fish oils on the treatment of NASH, with emphasis on liver histology.

2. Materials and methods

2.1. Ethical considerations

This study was performed according to the ethical standards of the World Medical Association's Declaration of Helsinki. The protocol was approved by institutional ethics board review and registered on www.ClinicalTrials.gov (ID 01992809). Written informed consent was obtained from each patient prior to trial participation.

2.2. Patients and experimental design

Adult outpatients (18–75 years old) attending the Division of Clinical Gastroenterology and Hepatology of the Hospital das Clínicas of the University of São Paulo School of Medicine (Sao Paulo, Brazil) from April 2009 to 2011 were screened for eligibility. Criteria for inclusion were men and women with a proven histological diagnosis of NASH. Criteria for exclusion were: a history of any other acute or chronic liver or biliary disease; substance abuse, especially intake of >100 g alcohol/week; use of hepatotoxic drugs (e.g. corticosteroids, high-dose oestrogens, methotrexate, tetracycline, calcium channel blockers, amiodarone, or tamoxifen); neurologic or psychiatric dysfunctions; any allergy or food intolerance, including intolerance to any ingredient of the supplemental capsules; and refusal to give informed consent.

A randomization sequence with two branches of 40 patients each (1:1 allocation) was generated by computer (GraphPad statistical software; QuickCalcs, La Jolla, CA–USA) before initiation of the study by an independent dietician (MCGD), to assign participants to either the n-3 or the placebo group. With the exception of this independent dietician, investigators and clinical staff remained blinded to each study participant's assignment until the end of the statistical analysis phase of the trial. Included patients were enrolled in the study by two trained investigators (CPMSO and MAN) following this randomization sequence.

Treatment was performed in a double-blind manner. Identical capsules containing omega-3 fatty acids or mineral oil were packed in identical bottles and labelled with 10 different codes (5 for each treatment). The n-3 group received appropriate labelled bottles with capsules containing 0.315 g of n-3 PUFAs (64% ALA, 16% EPA, and 21% DHA; Table 1) and instructions to ingest 3 capsules daily, comprising a total daily intake of 0.945 g of n-3 PUFAs. The placebo group received proper labelled bottles with identical capsules, each containing 2 mL of mineral oil, with identical intake instructions. Two capsules of each labelled bottle (5 omega-3 and 5 mineral oil) were kept sealed by MCGD in order to certify the blinding procedures after the study end.

The intervention was implemented for 6 continuous months and, at the end, the patients were readmitted for a new liver biopsy. Each patient's plasma lipid profile was analysed to assess treatment compliance. The primary endpoint was a change in liver histopathology compared to baseline (before treatment), according to the NASH activity score (NAS), after 6 months of treatment. Secondary endpoints included changes over baseline scores in biochemical and anthropometric data after 3 and 6 months of treatment.

2.3. Analysis of plasma fatty acids

Plasma lipid gas chromatography was performed using an Agilent 7890A GC chromatograph System and J&W DB-23 columns of 60 m × 250 μm × 0.15 μm (Agilent Technologies, Santa Clara, CA, EUA) with plasma samples obtained at baseline and after 6 months of treatment. Methyl esters of fatty acids (FAs) were obtained as previously described [20]. Chromatographic conditions were set as previously described [21], with some specific adaptations to optimize study samples according to parameters of selectivity, linearity, precision, accuracy, and limits of detection and quantification (injection split mode = 50:1, injector temperature = 250 °C, detector temperature = 280 °C, and chromatographic column described above). The column was operated at an initial temperature of 80 °C for 1 min, followed by an increase to 230 °C for 5 min, with a total run time of 45 min. To obtain the calibration curve, FAs were assessed by external standardization using reference standards (Sigma–Aldrich; St. Louis, MO, USA).

2.4. Histopathologic analysis

All liver biopsy slides were stained with hematoxylin and eosin. Two liver pathologists, experts in NAFLD scoring (VAFA & BC),

Table 1
Composition of each capsule offered to the omega-3 group.

Component	Amount[a]	Total intake/day
Calories (kcal)	8	24
Carbohydrates (g)	0	0
Proteins (g)	0	0
Total fat (g)	1	3
Saturated fat (g)	0	0
Monounsaturated fat (g)	0	0
Polyunsaturated fat (g)	0.315	0.945
Omega-3 EPA (g)	0.065	0.195
Omega-3 DHA (g)	0.050	0.15
Omega-3 ALA (g)	0.200	0.6
Cholesterol (g)	0	0

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; ALA: alpha-linolenic acid.
[a] Data provided by the manufacturer (Amway; Buena Park, CA, USA).

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