Nutrition, Metabolism & Cardiovascular Diseases (2015) **25**, 734–741

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

A double-blind, placebo-controlled randomized trial to evaluate the efficacy of docosahexaenoic acid supplementation on hepatic fat and associated cardiovascular risk factors in overweight children with nonalcoholic fatty liver disease

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Received 5 February 2015; received in revised form 3 April 2015; accepted 16 April 2015
Available online 25 April 2015

KEYWORDS
Docosahexaenoic acid;
NAFLD;
Liver fat;
Visceral adipose tissue;
Epicardial adipose tissue;
Cardiac function;
Children

Abstract *Background and Aims:* Very little information is available on whether docosahexaenoic acid (DHA) supplementation has a beneficial effect on liver fat and cardiovascular disease (CVD) risk factors in children with nonalcoholic fatty liver disease (NAFLD). In a double-blind, placebo-controlled randomized trial we investigated whether 6-month treatment with DHA improves hepatic fat and other fat depots, and their associated CVD risk factors in children with biopsy-proven NAFLD.
Methods and Results: Of 58 randomized children, 51 (25 DHA, 26 placebo) completed the study. The main outcome was the change in hepatic fat fraction as estimated by magnetic resonance imaging. Secondary outcomes were changes in visceral adipose tissue (VAT), epicardial adipose tissue (EAT), and left ventricular (LV) function, as well as alanine aminotransferase (ALT), triglycerides, body mass index-standard deviation score (BMI-SDS), and insulin sensitivity. At 6 months, the liver fat was reduced by 53.4% (95% CI, 33.4–73.4) in the DHA group, as compared with 22.6% (6.2–39.0) in the placebo group ($P = 0.040$ for the comparison between the two groups). Likewise, in the DHA group VAT and EAT were reduced by 7.8% (0–18.3) and 14.2% (0–28.2%), as compared with 2.2% (0–8.1) and 1.7% (0–6.8%) in the placebo group, respectively ($P = 0.01$ for both comparisons). There were no significant between-group changes for LV function as well as BMI-SDS and ALT, while fasting insulin and triglycerides significantly decreased in the DHA-treated children ($P = 0.028$ and $P = 0.041$, respectively).
Conclusions: DHA supplementation decreases liver and visceral fat, and ameliorates metabolic abnormalities in children with NAFLD.

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http://dx.doi.org/10.1016/j.numecd.2015.04.003
0939-4753/

Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a range of liver histology severity and outcomes in the absence of chronic alcohol use. The mildest form is simple steatosis in which triglycerides accumulate within hepatocytes. A more advanced form of NAFLD, nonalcoholic steatohepatitis (NASH), includes inflammation and liver cell injury, progressive to cryptogenic cirrhosis. NAFLD has become the most common cause of chronic liver disease in children and adolescents [1]. The presence of NAFLD also has important implications regarding cardiovascular health. As described in adults, children and adolescents with fatty liver display metabolic abnormalities that are risk factors for cardiovascular disease(CVD), such as insulin resistance, glucose intolerance, and dyslipidemia [1,2]. Several studies, including pediatric subjects, have reported independent associations between NAFLD and markers of subclinical atherosclerosis, such as impaired flow-mediated vasodilation, increased carotid artery intima-media thickness and arterial stiffness, after adjusting for CVD risk factors and metabolic syndrome (MetS) [3,4]. Thus NAFLD has emerged as the hepatic component of MetS and a strong risk factor for the development of atherosclerotic disease, even at a very early age [5,6]. More recent work has identified NAFLD as a risk factor also for myocardial insulin resistance, altered cardiac energy metabolism, abnormal left ventricular (LV) structure, and impaired diastolic function [7–9].

Currently, only weight loss and increased physical activity decrease hepatic fat, and to date there are no evidenced-based guidelines, and no approved pharmacologic therapy for the treatment of NAFLD in children [1]. Growing evidence suggests that n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) may have a beneficial role on many cardio-metabolic risk factors, including NAFLD [10,11]. In adults, randomized and non-randomized clinical trials have shown that the supplementation of n-3 LC-PUFAs, including both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), leads to amelioration of liver fatness [12,13]. In children, Nobili et al. have recently reported that DHA supplementation improves liver steatosis and is able to reduce the levels of serum alanine aminotransferase (ALT) and triglycerides, and to improve insulin sensitivity [14,15]. However, very little information is available on whether DHA treatment has a significant benefit on cardiovascular health in children with NAFLD. In a double-blind, parallel-group, placebo-controlled randomized trial we investigated in children and adolescents with NAFLD, the impact of 6-month n-3 LC-PUFAs supplementation on hepatic fat and other fat depots [i.e. abdominal visceral adipose tissue (VAT), abdominal subcutaneous adipose tissue (SAT), and epicardial adipose tissue (EAT)], and their associated CVD risk factors including LV dysfunction. We focused only on the effects of DHA supplementation, which has been suggested to have greater benefit for CVD risk factors than EPA [16].

Methods

The study protocol was approved by the local Ethics Committee (Policlinico Umberto I Hospital, Rome, Italy) (2485/24.05.2012) and written consent was obtained from the next of kin, caretakers, or guardians on behalf of the children enrolled in this study, in accordance with principles of the Helsinki Declaration.

Study population

Patients were eligible for the study if they had at enrollment: 1) age $<$18 years; 2) body mass index (BMI) $>$ 85th percentile according to age- and gender-specific percentiles of BMI [17]; 3) persistently elevated aminotransferase levels; 4) magnetic resonance imaging (MRI)-diagnosed NAFLD [hepatic fat fraction (HFF) $\geq$ 5%] [18]; and 5) liver biopsy consistent with NAFLD. Secondary causes of steatosis including hepatic virus infections (hepatitis A–E and G, cytomegalovirus, and Epstein–Barr virus), autoimmune hepatitis, metabolic liver disease, α-1-antitrypsin deficiency, cystic fibrosis, Wilson's disease, hemochromatosis, and celiac disease were excluded after appropriate tests. Other exclusion criteria were smoking, and history of type 1 or type 2 diabetes, renal disease, total parenteral nutrition, alcohol intake, use of hepatotoxic medications, and previous use of n-3 LC-PUFAs.

Study design

The study was a double-blind, parallel-group, placebo-controlled randomized trial performed at the Hepatology outpatient Clinic of the Department of Pediatrics, Sapienza University of Rome, Italy, between May 2012 and September 2014. Participants were randomly assigned to DHA supplementation [250 mg/day (39% DHA algae oil; Dietetic Metabolic Food – DMF, Limbiate, MB, Italy)] or placebo (290 mg linoleic acid supplied with germ oil; IBSA, Lodi, Italy). A randomization list (in a 1:1 ratio to treatment with DHA or placebo) was generated by an independent statistician who was blinded to participants' clinical data and did not perform the final analysis. DHA and placebo pills were of similar appearance and taste and provided about 7 kcal of energy (DMF, Limbiate, MB, Italy). Pills were stored at the hospital pharmacy and dispensed at the baseline visit and every month thereafter. All participants and research staff were blind to the group assignment. Compliance to treatment was encouraged by weekly phone calls and text messages and monitored by pill count and direct interview at every monthly visit. Blood DHA concentrations before and after treatment were used as an objective measure of compliance. Adverse events were defined as those injuries related to or caused by the treatments under study. At each visit, parents were specifically asked about adverse events, and the first author checked for any association between the adverse events and morbidity. A balanced low-calorie diet was