Docosahexaenoic acid for the treatment of fatty liver: Randomised controlled trial in children

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Adolescents;
Non-alcoholic fatty liver disease

Abstract  Background and aim: Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children. We tested whether dietary supplementation with docosahexaenoic acid (DHA) can decrease liver fat content in children with NAFLD.

Methods and results: We performed a randomized controlled trial of DHA supplementation (250 mg/day and 500 mg/day) vs. placebo in 60 children with NAFLD (20 children per group). The main outcome was the change in liver fat as detected by ultrasonography after 6, 12, 18 and 24 months of treatment. Secondary outcomes were changes in triglycerides, alanine transaminase (ALT), body mass index (BMI) and homeostasis model assessment of insulin resistance (HOMA). The odds of more severe versus less severe liver steatosis decreased to the same degree at 6 months in children treated with DHA 250 mg/day and DHA 500 mg/day vs. placebo and persisted virtually unmodified for 24 months (OR ≤ 0.02, p ≤ 0.05 for all time points). Triglycerides were lower in the DHA groups than in the placebo group at any time point and ALT was lower in these groups from month 12 onwards. HOMA was lower in the DHA 250 mg group vs. placebo at months 6 and 12.

Conclusion: DHA supplementation improves liver steatosis in children with NAFLD. Doses of 250 mg/day and 500 mg/day of DHA appear to be equally effective in reducing liver fat content.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children living in Western countries [1]. Although there is a general consensus that lifestyle changes should be the first step of NAFLD treatment in children as in adults [2–4], there is mounting interest in N-3 long-chain polyunsaturated fatty acids (LCPUFA) as potential treatment for liver steatosis [5,6]. Dietary N-3 LCPUFA lower blood triglycerides and have anti-inflammatory and insulin-sensitizing properties [5] which may explain why they are effective in reducing liver fat content [6]. We have recently reported the 6-month results of a randomized controlled trial (RCT) aimed at testing the efficacy of docosahexaenoic acid (DHA) on liver steatosis in children with NAFLD [7].

In the present paper, we report the long-term results of such RCT by showing the effect of docosahexaenoic acid (DHA) on liver steatosis in children with NAFLD [7].

Methods

Trial design

The present RCT, registered as NCT00885313 on www.clinicaltrials.gov, was performed on 60 consecutive children followed as outpatients at the Liver Research Unit of the Bambino Gesù Children's Hospital (Rome, Italy) [7].

Children were eligible for the study if they had: 1) age <18 years, 2) persistently elevated serum alanine transaminase (ALT ≥ 40 U/L), 3) diffusely hyperechogenic liver at ultrasonography, and 4) liver biopsy consistent with NAFLD. Children were excluded from the study if they had any of the following: 1) viral liver disease, 2) autoimmune liver disease, 3) Wilson’s disease, 4) α-1-antitrypsin deficiency, 5) celiac disease, 6) alcohol consumption (any quantity), 7) use of parenteral nutrition, 8) use of drugs known to induce fatty liver, and 9) previous use of N3-LCPUFA.

Twenty patients were treated with DHA 250 mg/day, 20 with DHA 500 mg/day and 20 with placebo. A balanced low-calorie diet was prescribed and physical activity was suggested to all patients as described in detail elsewhere [8]. Reinforcement of lifestyle changes was made by the first author at all visits.

Owing to the lack of data on the effects of DHA on liver steatosis in children, we choose doses of DHA known to be associated with physiologically relevant effects [7]. The presently suggested dietary intake of DHA for individuals at cardiovascular risk ranges between 250 and 500 mg/day [9], i.e. the two doses that we tested in this study. In detail, we hypothesized that the administration of placebo, DHA 250 mg/day and DHA 500 mg/day would increase whole blood DHA of a mean (standard deviation) of 0.0 (0.7%), 0.5 (0.7%) and 1.0 (0.7%), respectively. To detect this difference as significant at an alpha level of 0.05 with a power of 95%, 16 subjects per group were needed (analysis of variance with Monte Carlo simulation) [7]. To control for possible losses at follow-up, we enrolled 20 subjects per group.

A computer-generated randomization list assigned participants in equal number to DHA 250 mg/day, DHA 500 mg/day (39% DHA oil obtained from Schyzochitrium, Martek Biosciences Corporation, Columbia, MD, USA) and placebo (290 mg linoleic acid supplied with germ oil, Gel-fipharma International, Lodi, Italy). A statistician, who did not perform the final analysis, generated the allocation sequence and assigned participants to the treatment groups. DHA and placebo pills were of the same number (1), size, appearance, taste and provided about 7 kcal of energy/pill (DMF, Lainate, Italy). Pills were stored at the hospital pharmacy and dispensed at the baseline visit and every 2 months thereafter. Participants, investigators and outcome assessors were blinded to the treatment for all the duration of the study.

The main outcome was the change in liver fat content as detected by ultrasonography after 6, 12, 18 and 24 months of treatment. Secondary outcomes were the changes in fasting triglycerides, ALT, HOMA and body mass index (BMI) after 6, 12, 18 and 24 months of treatment.

Compliance to the study treatment was evaluated by pill count at each visit, review of medication records, and direct interview of patients by the first author. Adverse events were defined as those injuries related to or caused by the study treatment. At each visit, parents were specifically asked about adverse events, and the first author checked for any association between treatment and adverse events.

The trial was approved by the Ethical Committee of the Bambino Gesù Children’s Hospital and written informed consent was obtained from the parents or legal guardians of the children.

Liver ultrasonography

Liver ultrasonography was performed by an experienced radiologist using an Acuson Sequoia C512 scanner equipped with a 1SL8 transducer (Universal Diagnostic Solutions, Oceanside, CA). Absent steatosis (grade 0) was defined as normal liver echo-texture; mild steatosis (grade 1) as slight and diffuse increase in fine parenchymal echoes with normal visualization of diaphragm and portal vein borders; moderate steatosis (grade 2) as moderate and diffuse increase in fine echoes with slightly impaired visualization of diaphragm and portal vein borders; and severe steatosis (grade 3) as fine echoes with poor or no visualization of diaphragm, portal vein borders and posterior portion of the right lobe [10,11].

Clinical and laboratory evaluation

Weight and height were measured following standard guidelines [12]. Body mass index (BMI) was calculated and converted to standard deviation scores (SDS) using the Centers for Disease Control 2000 reference data [13]. Glucose was measured by standard methods and insulin by means of radioimmunoassay (MYRIA Technogenetics, Milan, Italy). Blood fatty acids, including DHA, were analyzed in a drop of whole blood absorbed on a strip and trans-methylated for gas-chromatography analysis [14].

Statistical analysis

Most variables were not normally distributed and all are reported as medians and interquartile range (IQR). IQR
was calculated as the difference between the 75th and 25th percentile. The effect of treatment on the main ordinal outcome (liver steatosis coded as 0, 1, 2 and 3) and the secondary continuous outcomes (triglycerides, ALT, BMI and HOMA) was evaluated using an ordinal generalized linear model (OGLM) and linear models employing treatment (0 = placebo, 1 = DHA 250 mg/day, 2 = DHA 500 mg/day; discrete), time (0, 6, 12, 18 and 24 months; discrete), a treatmentXtime interaction (discreteXdiscrete) and the baseline value of the outcome as predictors [15,16]. Repeated measures were taken into account by specifying cluster confidence intervals (CI) for each patient. The OGLM allows to directly control for heteroscedasticity and defaults to the proportional odds logistic regression model when the parallel-lines assumption is met [17,18]. The odds-ratio (OR) obtained from such model is a measure of the odds of more severe vs. less severe steatosis [19]. Between-group comparisons at each time point (6, 12, 18 and 24 months) were performed using pre-specified contrasts with Wald tests and Bonferroni’s correction for multiple comparisons. Probabilities estimated from the OGLM and values estimated from the linear models were plotted to aid the clinical interpretation of the results. The analysis was intention to treat and there were no missing data. Statistical significance was set to \( p < 0.05 \) and all statistical tests were two-tailed. Statistical analysis was performed using Stata 12.1 (Stata Corp, College Station, TX, USA) together with the user-written oglm package [20].

**Results**

Table 1 reports the baseline measurements of the 60 children aged 6–16 years that were enrolled into the study. As we reported in detail elsewhere [7], absolute increases of serum DHA equal to 0.65% (95%CI 0.30–1.01%) and 1.15% (95%CI 0.87–1.43%) were obtained at 6 months in the DHA 250 mg/day and 500 mg/day groups vs. placebo, respectively, matching the expectations we had when calculating sample size.

Fig. 1 plots the OR of more severe vs. less severe steatosis in the DHA groups vs. placebo. The OR was very small (\( < 0.02 \)) at any visit starting from month 6 (\( p < 0.05 \) for all values). Even if 95%CI were wider for the DHA 500 mg/day group, there is no doubt that there was a considerable reduction in the progression to more severe steatosis in the DHA groups as compared to placebo. On the contrary, no difference in such progression was noted between the DHA 250 mg/day and 500 mg/day groups (\( p > 0.05 \)).

Fig. 2 plots the probability of liver steatosis as estimated from the OGLM whose OR were reported in Fig. 1. This is a cumulative probability, i.e. the probability of the four degrees of liver steatosis (0, 1, 2, and 3) sums to 1 at any time point. To interpret these data, one should start considering that all children had degree 2 or 3 liver steatosis at baseline (Table 1 and panels 2 and 3 of Fig. 2). As expected from Fig. 1, all changes were evident at 6 months and were stable thereafter. No patient in the placebo group had his/her fatty liver cleared while this happened for 10% and 15% of patients treated with DHA 500 and 250 mg respectively (Panel 0 of Fig. 1). Likewise, the number of patients "reverting" to stage 1 steatosis was no more than 20% in the placebo group but between 40% and 50% in the DHA groups (Panel 1 of Fig. 2). The probability of moderate steatosis increased up to 45% in the placebo group while it was at least 10% lower in the DHA groups (Panel 2 of Fig. 2). Lastly, the probability of degree 3 steatosis decreased only from 60% to 40% in the placebo group but from 60% to less than 10% in the DHA groups (panel 3 of Fig. 2).

Fig. 3 depicts the values of the secondary outcomes, i.e. triglycerides, ALT, BMI and HOMA. As for the main outcome, these changes were always comparable in the DHA 250 mg/day and DHA 500 mg/day groups (\( p > 0.05 \)). Triglycerides

![Figure 1](image_url)  
**Figure 1** Odds ratio of more severe vs. less severe liver steatosis during the study. Odds ratios and 95% confidence intervals were obtained from an ordinal generalized model. Abbreviations: DHA = docosahexaenoic acid.

### Table 1 Baseline measurements of the children. Values are median and interquartile range (IQR) for continuous variables and counts for categorical variables. IQR is calculated as the difference between the 75th and 25th percentiles.

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>DHA 250 mg/day</th>
<th>DHA 500 mg/day</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Gender (M/F)</td>
<td>8/12</td>
<td>8/12</td>
<td>9/11</td>
</tr>
<tr>
<td>Age (years)</td>
<td>13 (4)</td>
<td>11 (3)</td>
<td>11 (2)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>57 (24)</td>
<td>55 (15)</td>
<td>54 (15)</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.51 (0.28)</td>
<td>1.48 (0.20)</td>
<td>1.50 (0.18)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.1 (5.1)</td>
<td>26.6 (4.9)</td>
<td>24.4 (3.6)</td>
</tr>
<tr>
<td>BMI (SDS)</td>
<td>1.76 (0.81)</td>
<td>1.81 (1.02)</td>
<td>1.63 (1.12)</td>
</tr>
<tr>
<td>DHA (%)</td>
<td>1.58 (0.41)</td>
<td>1.25 (0.44)</td>
<td>1.21 (0.32)</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>78 (37)</td>
<td>70 (25)</td>
<td>57 (27)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>89 (39)</td>
<td>90 (38)</td>
<td>89 (25)</td>
</tr>
<tr>
<td>(mg/dL)</td>
<td>87 (9)</td>
<td>86 (9)</td>
<td>81 (13)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>11 (6)</td>
<td>12 (15)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>2.2 (1.3)</td>
<td>2.4 (3.2)</td>
<td>1.9 (1.5)</td>
</tr>
<tr>
<td>HOMA</td>
<td>0/0/8/12</td>
<td>0/0/8/12</td>
<td>0/0/8/12</td>
</tr>
<tr>
<td>Liver Steatosis-US</td>
<td>0/1/2/3</td>
<td>0/1/2/3</td>
<td>0/1/2/3</td>
</tr>
</tbody>
</table>

Abbreviations: DHA = docosahexaenoic acid; BMI = body mass index; SDS = standard deviation scores; ALT = alanine transaminase; HOMA = homeostasis model assessment of insulin resistance; US = ultrasonography.
were lower in the DHA groups than in the placebo group at any time point and ALT was lower in these groups from month 12 onwards ($p < 0.05$). BMI changes were always comparable in the placebo, DHA 250 mg and DHA 500 mg groups ($p > 0.05$). HOMA was lower in the DHA 250 mg group vs. placebo at months 6 and 12 ($p < 0.05$) but this was not found for the DHA 500 mg group.

**Discussion**

In this RCT, we found that a previously reported decrease in liver fat obtained at 6 months [7] persisted virtually unchanged after 24 months of supplementation with DHA. Moreover, DHA doses of 250 mg/day and 500 mg/day appeared to be equally effective in reducing liver steatosis. Changes in triglycerides and HOMA went in the expected direction of a decrease in DHA-supplemented children but their estimates were imprecise and larger sample sizes are needed to adequately estimate these effects. We found no long-term effect of any dose of DHA on BMI and only a modest effect on ALT, which is compatible with available studies [6]. Also changes in weight and height, from which BMI is computed, were comparable between groups (data not shown). Contrarily to some trials [21] and in agreement with other trials [22], we found no effect of DHA on weight loss.

**Figure 2** Probability of liver steatosis during the study. The depicted mean probabilities were obtained from the ordinal generalized linear model whose OR are plotted in Fig. 1.

**Figure 3** Changes in the secondary outcomes during the study. Abbreviations: ALT = alanine transaminase; BMI = body mass index; HOMA = homeostasis model assessment of insulin resistance.
However, we were interested in BMI not as main outcome but as potential confounder of the DHA-NAFLD relationship [6], something which is ruled out by our findings.

Although this is the first RCT evaluating the effects of DHA supplementation in pediatric NAFLD, it is not without limitations. While we performed liver biopsy at baseline to diagnose NAFLD [7], we used ultrasonography to evaluate the changes of liver steatosis. This was done because of the recent demonstration from our group that ultrasonography offers an accurate assessment of liver fat as compared to liver biopsy [11] and because of the ethical impossibility of performing repeated biopsies during the study. Also, while we performed an oral glucose tolerance test (OGTT) at the baseline and 6-month visits to evaluate insulin sensitivity through calculation of the insulin sensitivity index [7, 10], we did not repeat the OGTT, again for ethical reasons, at further visits. Thus, although we found a decreasing trend for HOMA levels in the DHA 250 mg/day group, we could not test the long-term persistence of the clinically relevant decrease in ISI that we detected at 6 months [7].

Liver steatosis has been reported to regress frequently in the general population [23] and is especially sensitive to weight loss and physical activity [6]. Our RCT has many repeated measures, helping to evaluate the timing of the effect of DHA on liver steatosis. This effect appears to be at a peak at 6 months but it may be present sooner [6]. Further RCT should consider performing repeated measures between 0 and 6 months to allow a better characterization of the effect of DHA. Even if further studies in all ages of life are clearly needed to determine the optimal dose of N3-LCPUFA to reduce liver fat, our data agree with those available on adults in showing that higher doses are not necessarily more effective than lower doses [6]. Even more importantly, studies are needed to test the whether the reduction in liver fat achieved by DHA and other N3-LCPUFA is associated with hard clinical outcomes. Owing to the increasing evidence provided by cohort studies that fatty liver may be a predictor of type 2 diabetes mellitus and cardiovascular disease [3], intervention studies with N3-LCPUFA aimed at reducing liver fat content may be useful to test the existence of a cause–effect relationship between NAFLD and incident diabetes or cardiovascular disease.

In conclusion, DHA supplementation improves liver steatosis in children with NAFLD and doses of 250 mg/day and 500 mg/day appear equally effective.

References