Docosahexaenoic acid supplementation decreases liver fat content in children with non-alcoholic fatty liver disease: double-blind randomised controlled clinical trial

Valerio Nobili,[1] Giorgio Bedogni,[2,3] Anna Alisi,[1] Andrea Pietrobattista,[1] Patrizia Risé,[4] Claudio Galli,[4] Carlo Agostoni[3]

[1]Unit of Metabolic and Autoimmune Liver Diseases, "Bambino Gesù" Children's Hospital – IRCCS, Rome, Italy
[2]Clinical Epidemiology Unit, Liver Research Center, Basovizza, Trieste, Italy
[3]Department of Maternal and Pediatric Sciences, University of Milan, Fondazione IRCCS Ca'Granda Ospedale Maggiore Policlinico, Milan, Italy
[4]Department of Pharmacological Sciences, University of Milan, Milan, Italy

Correspondence to
Dr Valerio Nobili, Unit of Metabolic and Autoimmune Liver Diseases, "Bambino Gesù" Children's Hospital – IRCCS, Square S Onofrio 4, 00165 Rome, Italy; nobili66@yahoo.it

Trial identifier
NCT00885313 (http://www.clinicaltrials.gov).

Accepted 8 November 2010
Published Online First 12 January 2011

ABSTRACT

Objective To investigate whether dietary supplementation with docosahexaenoic acid (DHA) decreases liver fat content in children with non-alcoholic fatty liver disease (NAFLD).

Design, setting and patients We performed a randomised controlled trial of DHA supplementation (250 and 500 mg/day) versus placebo in 60 children with biopsy-proven NAFLD (20 children per group).

Main outcome measures The main outcome was the change in liver fat content as detected by ultrasonography after 6 months of treatment. Secondary outcomes were the changes in insulin sensitivity index, alanine transaminase, triglycerides and body mass index after 6 months of treatment.

Results Blood DHA increased in children supplemented with DHA (0.65%, 95% CI 0.30% to 1.10% for the DHA 250 mg group and 1.15%, 0.87% to 1.43% for the DHA 500 mg group). The odds of more severe versus less severe liver steatosis after treatment was lower in children treated with DHA 250 mg/day (OR = 0.01, 0.002 to 0.11, p <0.001) and DHA 500 mg/day (OR = 0.04, 0.002 to 0.46, p = 0.01) as compared to placebo but there was no difference between the DHA groups (p = 0.4). Insulin sensitivity index increased and triglycerides decreased to a similar degree in both DHA groups as compared to placebo but there was no effect on alanine transaminase and body mass index.

Conclusion DHA supplementation improves liver steatosis and insulin sensitivity in children with NAFLD.

What is already known on this topic

- N-3 long-chain polyunsaturated fatty acids (PUFA) have anti-inflammatory and insulin-sensitising properties in animal models.
- Dietary supplementation of N-3 PUFA reduces liver fat content in adults with non-alcoholic fatty liver disease.

What this study adds

- This is the first randomised controlled trial to test the efficacy of N-3 PUFA for the treatment of non-alcoholic fatty liver disease in children.
- Docosahexaenoic acid taken orally for 6 months decreases liver fat content and increase insulin sensitivity in children with non-alcoholic fatty liver disease with similar effects for doses of 250 and 500 mg/day.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in children and adolescents living in Western countries.[1 2] Because obesity is the main risk factor for NAFLD, there is a general consensus that lifestyle changes should be the first step of NAFLD treatment.[3 4] Effective drugs may be a reasonable second step but few randomised controlled trials (RCT) are available in children and no definitive conclusions can be presently reached.[5]

N-3 long-chain polyunsaturated fatty acids (LCPUFA) may be useful to control some of the metabolic stigmata of obesity.[6–8] Dietary N-3 LCPUFA lower blood triglycerides and have anti-inflammatory and insulin-sensitising effects.[9–11] These effects are of special interest for NAFLD, which is pathogenically linked to insulin resistance and involves inflammation in its advanced stages.[12] In adults, RCT and non-randomised clinical trials have shown that the supplementations of N-3 LCPUFA, including both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), improves the biochemical and ultrasonographic features of liver steatosis.[13 14]

We performed a RCT to test whether dietary supplementation with DHA decreases liver fat content in children with NAFLD.

METHODS

Study design and intervention

The study was performed on 60 consecutive children at the Liver Unit of the Bambino Gesù Pediatric Hospital (Rome, Italy). The entry criteria were: persistently elevated serum alanine transaminase (ALT ≥40 U/l), diffusely hyperechogenic liver at ultrasonography and liver biopsy consistent with NAFLD. The exclusion criteria were: viral liver disease, autoimmune liver disease, Wilson's disease, α-1-antitrypsin deficiency, coeliac disease, alcohol consumption, use of parenteral nutrition, use of drugs known to induce fatty liver or alterations of carbohydrate metabolism, and previous use of fish-oil supplements.

The main outcome was the change in liver fat content as detected by ultrasonography after 6 months of treatment. Secondary outcomes were the changes in insulin sensitivity index (ISI), ALT, triglycerides and body mass index (BMI) after 6 months of treatment. 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. Sample size was determined on the basis of the expected increase of blood DHA in the three groups. In the absence 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.[15 16] We hypothesised that the administration of placebo, DHA 250 mg and DHA 500 mg would increase blood DHA of a mean (SD) of 0.0 (0.7%), 0.5 (0.7%) and 1.0 (0.7%), respectively. To detect this difference as significant at an α level of 0.05 with a power of 95%, 16 subjects per group are needed (analysis of variance with Monte Carlo simulation). To control for possible losses at follow-up, we enrolled 20 subjects per group.

A computer-generated randomisation 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, Maryland, USA) or placebo (290 mg linoleic acid supplied with germ oil; Gelfipharma 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 similar appearance and taste and provided about 7 kcal of energy (DMF, Lainate, Italy). Pills were stored at the hospital pharmacy and dispensed at the baseline visit and every 2 months thereafter. Participants and investigators were blinded to the treatment for all the duration of the study. Compliance to the treatment was evaluated by pill count at every visit, review of the medication records and direct interview of participants. 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. The study was approved by the Ethical Committee of the Bambino Gesù Pediatric Hospital and written informed consent was obtained from the parents or legal guardians of the children.

Liver ultrasonography

Liver ultrasonography was performed by a single radiologist using a Siemens Acuson Sequoia C512 scanner with a 15L8 probe. The radiologist was blinded to the treatment group of the patients. 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 visualisation of diaphragm and portal vein borders; moderate steatosis (grade 2) as moderate and diffuse increase in fine echoes with slightly impaired visualisation of diaphragm and portal vein borders; and severe steatosis (grade 3) as fine echoes with poor or no visualisation of diaphragm, portal vein borders and posterior portion of the right lobe.[17 18]

Liver biopsy

Liver biopsies were performed at baseline and reviewed by a single pathologist who was blinded to the treatment group of the patients.[19] Steatosis, inflammation, ballooning and fibrosis were evaluated using the NASH Clinical Research Network criteria.[20]

Clinical and laboratory evaluation

Weight and height were measured following standard guidelines.[21] BMI was calculated and converted to standard deviation scores (SDS) using the CDC 2000 reference data.[22] A 2-h oral glucose tolerance test was performed following the recommendations of the World Health Organisation.[23] Glucose was measured by standard methods and insulin by means of radioimmunoassay (MYRIA Technogenetics, Milan, Italy). ISI was calculated from the oral glucose tolerance test as described by Matsuda and DeFronzo.[24] Blood fatty acids, including DHA, were analysed in a drop of whole blood absorbed on a strip and transmethylated for gas-chromatography analysis.[25]

Statistical analysis

As detected by a Shapiro–Wilk test performed in the whole sample (n = 60), only height and BMI were normally distributed. Thus, descriptive statistics were reported as medians and IQR. The effect of treatment on continuous (DHA, ISI, ALT, triglycerides, BMI) and ordinal outcomes (liver steatosis coded as 0 = absent; 1 = mild; 2 = moderate; 3 = severe) was evaluated using linear and generalised linear mixed models employing the treatment (0 = placebo, 1 = DHA 250 mg/day, 2 = DHA 500 mg/day), time (0 = baseline, 1 = 6-month follow-up), a treatment × time interaction, and the baseline value of the outcome as fixed-effect predictors and the patient as random effect.[26] Mixed linear regression was used to assess the effect of treatment on continuous outcomes and mixed proportional-odds ordinal logistic regression to assess the effect on liver steatosis.[27 28] The OR obtained from the proportional-odds model is a measure of the change in the odds from less severe to more severe steatosis. To verify the distributional assumptions made by the mixed linear regression model, we checked that the predicted random effects were normally distributed. Although kernel density plots showed that random effects were approximately normally distributed, a Shapiro–Wilk test of normality was statistically significant for all outcomes with the exception of ISI and triglycerides. Thus, we used robust 95% CI to relax distributional assumptions.[27] The distributional assumptions made by the mixed proportional-odds ordinal logistic regression model were checked by inspecting probability plots and by testing that there were no within-group differences in the changes over time in an ordinal model not making the proportional-odds assumption.[27 28] Robust 95% CI were calculated also for the proportional-odds ordinal logistic regression model. The analysis was intention to treat. Statistical significance was set to a p value <0.05 and all statistical tests were two-tailed. Statistical analysis was performed using Stata V.11.0 together with the GLLAMM package.[29]

RESULTS

The baseline clinical, ultrasonographic and histopathological features of the 60 children are given in table 1. All the children completed the study and there were no missing data. Compliance with the treatment was excellent in all groups and no adverse events were reported.

The effects of DHA supplementation are given in table 2. As expected, serum DHA increased more in the DHA 500 mg group than in the DHA 250 mg group (p = 0.002) and was higher in these groups than in the placebo group (p < 0.001).

Table 1 Baseline measurements of children randomised to placebo or DHA

		DHA	
	Placebo	**250 mg**	**500 mg**
N	20	20	20
Gender (M/F)	8/12	8/12	9/11
Age (years)	13 (4)	11 (3)	11 (2)
Weight (kg)	57 (24)	55 (15)	54 (15)
Height (m)	1.51 (0.28)	1.48 (0.20)	1.50 (0.18)
BMI (kg/m^2)	26.1 (5.1)	26.6 (4.9)	24.4 (3.6)
BMI (SDS)	1.76 (0.81)	1.81 (1.02)	1.63 (1.12)
N-3 PUFA (%)	2.4 (0.6)	2.1 (0.5)	2.0 (0.2)
N-6 PUFA (%)	25.6 (4.3)	26.2 (6.5)	27.3 (4.3)
N-3 PUFA (N-6 PUFA)	0.10 (0.02)	0.07 (0.02)	0.07 (0.01)
DHA (%)	1.58 (0.41)	1.25 (0.44)	1.21 (0.32)
ALT (U/l)	78 (37)	70 (25)	57 (27)
Triglycerides (mg/dl)	89 (39)	90 (38)	89 (25)
Glucose (mg/dl)	87 (9)	86 (9)	81 (13)
Insulin (μU/ml)	11 (6)	12 (15)	10 (10)
ISI	3.6 (2.6)	3.4 (2.1)	3.8 (2.2)
Liver steatosis-US (0/1/2/3)	0/0/8/12	0/0/8/12	0/0/8/12
Macrovesicular steatosis (0/1/2/3)	0/7/4/9	0/6/10/4	0/10/10/0
Microvesicular steatosis (no/yes)	6/14	8/12	7/13
Lobular inflammation (0/1/2)	4/13/3	6/11/3	4/14/2
Portal inflammation (0/1/2)	10/10/0	11/7/2	9/11/0
Ballooning (0/1/2)	3/6/11	5/10/5	3/9/8
Fibrosis (0/1a/1b/1c)	5/1/2/12	9/1/1/9	0/0/3/17

Values are median and IQR for continuous variables and counts for categorical variables.
ALT, alanine transaminase; BMI, body mass index; DHA, docosahexaenoic acid; ISI, insulin sensitivity index; PUFA, polyunsaturated fatty acids; SDS, standard deviation scores; US = ultrasonography.

Table 2 Effects of DHA supplementation

	Change vs baseline using placebo as comparator	
	DHA	
	250 mg	**500 mg**
DHA (%)	0.65*** (0.30 to 1.01)	1.15***,† (0.87 to 1.43)
ISI	1.2** (0.4 to 1.9)	1.2** (0.7 to 1.8)
ALT (U/l)	−10 (−23 to 2)	−1 (−14 to 13)
Triglycerides (mg/dl)	−14* (−27 to −2)	−16* (−29 to −3)
BMI (SDS)	0.03 (−0.07 to 0.13)	−0.11 (−0.35 to 0.13)

Values are regression coefficients (effect size) and 95% robust CI (effect variability, given in brackets).
*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ vs placebo. †$p = 0.002$ vs DHA 250 mg.
ALT, alanine transaminase; BMI, body mass index; DHA, docosahexaenoic acid; ISI, insulin sensitivity index; SDS, standard deviation score.

ISI increased ($p < 0.01$) and triglycerides decreased ($p < 0.05$) to a similar degree in both DHA groups as compared to placebo. No significant between-group changes in ALT and SDS of BMI were detected.

When the children were classified on the basis of the changes in liver fat observed during the study (–3/–2/–1/0/+1 units of liver steatosis), the following sequences were obtained: 0/0/7/12/1 for placebo, 1/8/10/1/0 for DHA 250 mg and 1/7/7/4/1 for DHA 500 mg. The odds of more severe versus less severe steatosis after treatment was much lower in children treated with DHA 250 mg/day (OR = 0.01, robust 95% CI 0.002 to 0.11, $p < 0.001$) and DHA 500 mg/day (OR = 0.04, 0.002 to 0.46; $p = 0.01$) than in those treated with placebo but there was no difference between the DHA groups ($p = 0.4$).

DISCUSSION

We have shown for the first time, using a RCT design, that the dietary supplementation of DHA for 6 months reduces liver fat content and increases insulin sensitivity in children with NAFLD. However, 250 mg/day of DHA were as effective as 500 mg/day in obtaining these outcomes.

Dietary polyunsaturated fatty acids (PUFA) of the N-6 and N-3 families are well-established negative regulators of hepatic lipogenesis.[30 31] In experimental models, N-3 PUFA reduce liver inflammation and steatosis, inhibit the production of eicosanoids from N-6 PUFA and trigger the formation of resolvins and protectins from N-3 PUFA.[32–34] Moreover, a DHA-enriched oil ameliorates hepatic steatosis in obese animals by modifying the genetic expression of key enzymes.[35] DHA downregulates hepatic triglyceride accumulation by decreasing the transcriptional activity of the sterol regulatory element binding protein-1 and by activating the peroxisome proliferator–activated receptor-mediated pathway of lipid catabolism.[36 37] It is also of interest that N-3 PUFA-depleted rats which are given an intravenous infusion of fish oil have a greater extraction of DHA as compared to EPA.[38]

In human intervention studies, N-3 PUFA are commonly administered as both EPA and DHA.[39 40] In the present study, we focused on DHA alone because of its increasingly reported anti-inflammatory and insulin-sensitising effects.[41 42] In our study, the administration of DHA was associated with improved insulin sensitivity, with a concomitant reduction in plasma triglycerides. The insulin-sensitising effect of DHA may be related to its ability to upregulate genes involved in insulin sensitivity, glucose transport and intracellular signalling.[34] However, this effect is not universal and may be peculiar for patients with NAFLD.[43] On the basis of the available experimental and clinical evidence, we hypothesise that the reduction of lipid accumulation promoted by DHA in adults and children with NAFLD can improve liver metabolism and insulin resistance owing to local and global effects mediated by DHA or its by-products on gene expression.

The main limitation of our study is the use of ultrasonography to quantify liver steatosis. Although we plan to perform liver biopsies after a longer follow-up time, there are many ethical constraints in performing repeated liver biopsies in children. However, we recently performed a validation study of ultrasonography versus biopsy for the quantification of liver fat. In 208 consecutive children, the association between the two methods was very good (Spearman's r = 0.80, 95% CI 0.71 to 0.88, $p < 0.001$) (submitted for publication). A second limitation is that 6 months are not enough to judge the long-term efficacy of a treatment for liver steatosis but our study is projected to continue for other 18 months with a final liver biopsy. A third limitation is that we did not evaluate the possible contribution of fatty-acid desaturase polymorphisms in determining the response to DHA.[44]

In conclusion, our RCT shows beneficial effects of DHA on liver steatosis and insulin sensitivity in children with NAFLD. Further paediatric RCTs are needed to confirm this very encouraging finding. Management of children with NAFLD might consider incorporating DHA as part of a multifaceted approach.

Acknowledgements The authors are grateful to Professor Claudio Tiribelli, Liver Research Center, Basovizza, Trieste, Italy for useful suggestions and critical review of the manuscript.

Funding The study was supported by the 'Bambino Gesù' Children Hospital.

Competing interests None.

Contributions Study guarantor: VN; study concept and design: VN, GB, CA; acquisition of clinical data: AA, AP; analysis and processing of fatty acid data: PR, CG; statistical analysis: GB; interpretation of data: GB, VN, CA; drafting of the manuscript: VN, GB, CA; obtained funding: VN; administrative, technical or material support: DMF (Lainate, Italy) provided DHA and placebo pills.

Ethics approval This study was conducted at Pediatric Hospital Bambino Gesù, Rome, Italy.

Provenance and peer review Not commissioned; externally peer reviewed.

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