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ORIGINAL ARTICLE

Monitoring the efficacy of omega-3 supplementation on liver steatosis and carotid intima–media thickness: a pilot study

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Summary

Purpose

To determine the effects of omega-3 supplementation on liver fat and carotid intima–media thickness (IMT) and to assess accuracy of ultrasound (US) for grading liver steatosis.

Materials and Methods

In this one-way crossover pilot study, we assigned children with obesity and liver steatosis to receive 1.2 g daily of omega-3 supplementation vs. inactive sunflower oil for 24 or 12 weeks. Liver fat content was assessed by magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI) and US, and common carotid IMT by US. Statistical analysis included Chi-square, Student's *t*-tests, ANOVA tests and receiver operating characteristic (ROC) curves.

Results

Omega-3 supplementation was associated with a trend towards decrease in MRS-determined liver fat fraction (0.7% and 2.1% decrease in the 24-week and 12-week omega-3 group, respectively) compared with the sunflower oil group (1.0% increase). These changes were not significant, whether assessed by MRS (P = 0.508), MRI (P = 0.508) or US (P = 0.678). Using US, the area under the ROC curves were 0.964, 0.817 and 0.783 for distinguishing inferred steatosis grades 0 vs. 1–2–3, 0–1 vs. 2–3 and 0–1–2 vs. 3, respectively, indicating good accuracy of US-based fat grading. Omega-3 supplementation was associated with a decrease in US-determined IMT (0.05-mm decrease in the 24-week omega-3 group. A 0.015-mm increase was found in the 12-week omega-3 group, and a 0.007-mm decrease in the sunflower oil group (P = 0.003).

Conclusion

Omega-3 supplementation had no significant effect on liver fat fraction, but led to carotid IMT decrease in children with obesity and liver steatosis.

Keywords: Carotid intima–media thickness (IMT), liver steatosis, MRI-proton density fat fraction (PDFF), omega-3 fatty acids.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is found in 34.2% of children with obesity (1) and is the leading cause of chronic liver disease in pediatrics (2). Obesity and chronic liver steatosis are associated with early-stage atherosclerotic changes that predict future cardiovascular risk (3). Carotid intima–media thickness (IMT) has been shown to be already increased in children with obesity, especially those affected with type 2 diabetes (4,5).

The management of children with obesity is multifaceted and challenging. The main strategy consists on the institution of a balanced diet, accompanied by changes in physical activity (6). Maintaining a healthy lifestyle is

challenging to children with obesity. Pharmacological treatments are under-utilized for children and adults. While bariatric surgery is safe and effective for adolescents, it also is under-utilized (7). Laparoscopic adjustable gastric banding (LABG) for children with a BMI over 35 kg/m^2 (8) has proven to reduce hepatic steatosis (9), but is reserved only for very specific cases with severe medical complications secondary to obesity (10).

Omega-3 polyunsaturated fatty acids were found to act on multiple pathways implicated in the pathophysiology of hepatic steatosis. Their role in improving insulin resistance, decreasing dyslipidemia associated with hypertriglycidemia, and acting both as an anti-inflammatory and anti-oxidative agent are potential mechanisms that may help prevent or at least lower liver steatosis in children with obesity (11–14) and ameliorate their lipid profile (15). Omega-3 supplementation has shown promising results in decreasing liver steatosis in animal studies (16–19), pilot clinical investigations in adults (20,21) and randomized trials in adults (22–25). The effect of omega-3 supplementation on carotid IMT has also been studied in adults with conflicting results (26–29), leading Balk *et al*. to conclude to insufficient data in a review article (30).

To quantitate liver steatosis in a pediatric population, noninvasive imaging-based methods are preferable to liver biopsy because of poor acceptance and risks of complications (31,32). Magnetic resonance (MR) may be used as a surrogate biomarker of fat content for short-term trials when serial biopsies are not practical (33). MR spectroscopy (MRS) is accepted as the noninvasive reference standard for fat quantification in children and can be used for longitudinal monitoring of fat fraction (34–36). MR imaging permits evaluation of the entire liver parenchyma. Although ultrasound (US) allows qualitative fat evaluation, it remains less studied than MR for liver quantitative fat grading (37–39). However, it is a good tool for evaluation of artery wall thickness. Furthermore, this modality would enable assessment of liver steatosis and carotid IMT within the same imaging session (40).

The primary aim of this study was to determine the effects of omega-3 supplementation on liver steatosis and carotid IMT in children with obesity. A secondary objective was to assess the diagnostic accuracy of US for grading liver steatosis using MRS as the reference standard.

Materials and methods

Study design

This is an ancillary imaging pilot study to a prospective, doubled-blinded, one-way crossover randomized clinical trial registered as NCT02201160 on www.clinicaltrials.gov, approved and reviewed by the Clinical Research Ethics Committee of the Centre Hospitalier Universitaire (CHU) Ste-Justine in Montréal, Canada. All subjects provided written informed consent.

Participants

Between March 2009 and July 2011, children with obesity were pre-screened by the Metabolic Unit of the Nutrition Department from two pediatric institutions (CHU Ste-Justine and Montreal Children's Hospital).

Eligibility criteria

Inclusion criteria

Children with obesity and a body mass index (BMI) $> 95^{th}$ percentile were eligible to participate in this study if they were older than 8 years old (to allow MR without need for sedation), and showed hepatic steatosis on a baseline screening abdominal US. Contraceptive measures for female subjects of reproductive capacity were provided if required.

Exclusion criteria

Subjects were excluded if they had an identifiable secondary cause explaining their hepatic steatosis, had absolute contra-indications for MR or were taking any medication or supplementation that could interfere on the study results (anti-inflammatory drugs, medications for dyslipidemia and/or hypertension).

Eligible subjects who consented to the study were scheduled for an assessment by the participating pediatric hepatologist (F.A.).

Randomization

Children with obesity and liver steatosis were subsequently randomized. Investigators and study participants were blinded to the treatment allocation. Investigators were also blinded to imaging results until data analysis.

Interventions

Subjects were randomly assigned to either a daily dose 1.2 g of omega-3 or inactive sunflower oil (Nutrisanté Inc./Ponroy, Canada). The overall study lasted 9 months for each patient, divided in three distinct trimesters. During the first trimester, the subjects were randomly assigned either to omega-3 supplement or sunflower oil.

In the second trimester, the study was designed as a one-way crossover, with the subjects on omega-3 remaining on the same treatment, and the subjects on sunflower oil switching over to omega-3 supplementation. The last trimester was used as an observation period, with no treatments given to all groups.

Study visits

In total, four (4) clinical evaluations were scheduled: one at baseline (i.e. at the beginning of treatment), followed by visits at 3, 6 and 9 months after beginning the study. Each visit consisted in a half-day session to the Gastroenterology/Hepatology Unit, where the diverse inclusion and exclusion criteria were reviewed and the patient's nutritional and energetic status as well as anthropometric measurements was assessed. Subjects also underwent a complete physical examination, some biochemical testing, a carotid Doppler examination and a liver US and MR examination. Compliance was measured by pill count at every visit, review of the medication record and direct interview of the patients by the physician. All visits were similar.

Ultrasound fat grading

Semi-quantitative fat grading was performed by visual assessment using the scoring system adapted from Hamaguchi *et al*. (41). The scoring system was based on the presence or absence of liver–kidney contrast, US attenuation and vessel blurring. For each US study, a total steatosis score (from 0 to 6) was calculated as the unweighted sum of the three indices.

MR imaging fat quantification

All studies were acquired on a 1.5T clinical system (Avanto, Siemens Healthcare, Erlangen, Germany) with a body coil. Spoiled gradient-echo sequences with seven echoes were acquired during breath-holds. Sequence parameters were: flip angle, 20°; field of view, 350 mm (adapted to patient size); matrix, 256 × 166; section thickness, 10 mm; gap, 0 mm; receiver bandwidth, 780 Hz/pixel; voxel size, 2.5 mm × 2.5 mm × 10.0 mm; acceleration factor, none applied; number of averages, 1; repetition time (TR), 30 ms. The echo times (TE) were 2.3, 4.5, 6.8, 9.0, 11.3, 13.5 and 15.8 ms. Total acquisition time was typically 77 s for entire liver coverage.

MRI fat fraction sampling and calculation

The anonymized MR images acquired were analyzed by a radiology resident (M.-C.L., 3 years of experience) under the supervision of an abdominal radiologist (A.T., 9 years of experience). Rectangular regions of interest (ROI) of approximately 50 voxels were drawn in each of the nine (9) liver segments.

The MR imaging-proton density fat fraction (PDFF) maps were calculated using the method described by Yokoo *et al*. (42) This algorithm corrected for T2* and calculated PDFF in each pixel using all the echoes. The multi-frequency interference effects of multiple fat peaks were corrected using a triglyceride model of human liver fat (43).

MR spectroscopy

Using respiratory triggered PRESS MRI sequences with no fat or water saturation, three voxels were acquired for each study. The characteristics of these voxels were: 20 mm × 20 mm × 20 mm, spectral width, 1,000 Hz; TR, 3,000 ms; TE, 30 ms. Analysis of the spectroscopic data was performed by the same radiology resident (3 years' experience) using RDA file format on the AMARES algorithm (44) provided in the jMRUI software (45).

The model described by Hamilton *et al.* (43) was adopted for the spectroscopic analyses, depicting all the observed or measurable fat peaks by multiple Gaussian resonances. The 1.3-ppm and 2.1-ppm lipid peaks were expressed as the sum of 3 Gaussian resonances, the 0.9-ppm lipid peak represented by the sum of two Gaussian resonances and the 2.75-ppm peak as a single Gaussian resonance. Five Gaussians portrayed the different water and lipid peaks in the 4 to 6-ppm domain. A non-linear least-squares fitting approach estimated the T2-corrected peak areas using the input from the different echo times. Corrections were performed to the final estimated fat fraction depending on the presence of lipid peaks in the water resonance region (4–6 ppm) (43).

Carotid Doppler

Longitudinal images of the common carotid arteries were acquired by combination of 2-D mode and color Doppler examination using a high-resolution linear US transducer. Common carotid IMT was measured by calculating the mean value of three consecutive measurements of the deep wall thickness of the vessel, 10 mm below the carotid bulb (46).

Statistical analysis

Categorical variables were expressed as numbers and percentages. Continuous variables were expressed as

mean ± standard deviation (SD). Intra-individual comparisons between groups at baseline were analysed with the Student's *t*-test or Chi-square. Comparisons between groups were performed with mixed model repeated-measure analysis of variance (ANOVA) with two factors, one factor 'time' at four levels (visit 1, 2, 3 and 4), one factor 'group' with two levels (24-week omega-3 group, 12-week omega-3 group). In case of interaction, the specific contrasts were used to study separately the evolution of each group and to compare groups at each visit. Fat grading accuracy of US was assessed by receiver operating characteristic (ROC) curves. Estimates of diagnostic performance (sensitivity, specificity) were assessed for MRS-determined fat fraction thresholds of ≥6.4%, ≥17.4% and ≥22.1% according to thresholds derived from Tang *et al.* (47) for inferred steatosis grades 0 vs. 1–2–3, 0–1 vs. 2–3 and 0–1–2 vs. 3, respectively. *P* values < 0.05 were considered significant. All statistical analyses were performed by a biostatistician (M.C.) with statistical software (SPSS for Windows, version 22.0; IBM, Chicago, Ill).

Results

Study population

Between March 2009 and July 2011, 22 children with obesity and hepatic steatosis met eligibility criteria and consented to participate in the study. Three subjects only attended the first baseline visit before dropping out, and were therefore not included in the statistical analysis. In the 19 subjects showing baseline hepatic steatosis, 10 were randomized to the 24-week omega-3 group, and 9 to the 12-week omega-3 group. Ten subjects (45.5%) completed the entire study, which included being present for all clinical assessments and performing all necessary imaging studies. Of these 10 subjects, five were in the 24-week omega-3 group, and five were in the 12-week omega-3 group.

The total study population included 16 (84.2%) males and 3 (15.8%) females. The mean age was 13.7 ± 3.0 years. The mean BMI was 31.2 ± 5.3 kg/m^2. Baseline characteristics were similar between the groups, except for height and waist circumference (Table 1). At baseline, liver fat content was higher in the steatosis group 24-week omega-3 group than in the 12-week group, whether assessed by MRS, MRI or US. Carotid IMT was similar between both groups. No adverse events were reported during the course of the study.

Effect of omega-3 and sunflower oil on liver fat content

The liver mean fat fraction was not significantly affected by omega-3 or sunflower oil supplementation (Figure 1). In the 24-week omega-3 group, the MRS-determined liver fat fraction decreased by 0.7%, (22.4% at visit 1 to 21.7% at visit 3). In the 12-week omega-3 group, the liver fat fraction increased by 1.0% (15.4% at visit 1 to 16.4% at visit 2) during the sunflower oil period, and decreased by 2.1% (16.4% at visit 2 to 14.3% at visit 3) during omega-3 supplementation. None of the changes were significant as shown below.

MR spectroscopy and MR imaging

We did not observe an overall significant interaction between visit and group (F (3, 35.554) = 0.554, *P* = 0.649). Furthermore, we also did not observe a significant visit effect (F (3, 35.554) = 0.790, *P* = 0.508). Thus, liver fat fraction as measured by MRS and MRI was stable over the four visits in the two groups.

Ultrasound steatosis score

We did not observe an overall significant interaction between visit and group (*F* (3, 44.060) = 0.410, *P* = 0.747). Hence, the evolution of US steatosis scores between the two different regimens of omega-3 was not significantly different. We also did not observe a significant visit effect (*F* (3, 44.060) = 0.509, *P* = 0.678). Thus, liver fat fraction as measured by US steatosis score was stable over the four visits in the two groups.

Ultrasound fat grading accuracy

At baseline visit, the diagnostic performance estimates of semi-quantitative US for fat grading are provided in detail with 95% confidence intervals in Table 2. In summary, a ≥1.5 steatosis score threshold has a 0.964 area under the ROC curve, 85.7% sensitivity, 100.0% specificity, 100.0% positive predictive value (PPV) and 50.0% negative predictive value (NPV) for detecting MRS threshold ≥6.4% (which corresponds to inferred steatosis grade 0 vs. 1–2–3); a ≥2.5 steatosis score threshold has a 0.817 area under the ROC curve, 100.0% sensitivity, 55.6% specificity, 63.6% PPV and 100.0% NPV for detecting MRS threshold ≥ 17.4% (which corresponds to inferred steatosis grade 0–1 vs. 2–3); and a ≥2.5 steatosis score threshold has a 0.783 area under the ROC curve, 100.0% sensitivity, 50.0% specificity, 54.6% PPV and 100.0% NPV for detecting MRS threshold ≥ 22.1% (which corresponds to inferred steatosis grade 0–1–2 vs. 3) (Figure 2).

Effect of omega-3 on Ultrasound IMT

The baseline mean IMT was 0.60 ± 0.1 mm in both groups.

Table 1 Baseline characteristics

	Steatosis			
	24-week omega-3 (n = 10)	**12-week omega-3** (n = 9)	**Total** (n = 19)	***P-value*** (24-week omega-3 vs. 12-week omega-3)
Demographic				
Sex, n (%)				
Male	9 (90%)	7 (77.8%)	16 (84.2%)	0.582
Female	1 (10%)	2 (22.2)	3 (15.8%)	
Age (years)	14.4 ± 3.2	12.9 ± 2.6	13.7 ± 3.0	0.418
Weight (kg)	99.4 ± 29.1	74.4 ± 17.0	87.6 ± 26.8	**0.036**
Height (m)	1.7 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	**0.023**
Body mass index (kg/m^2)	32.6 ± 5.6	29.7 ± 4.9	31.2 ± 5.3	0.246
Systolic blood pressure (mm Hg)	122.6 ± 15.4	113.3 ± 15.3	118.4 ± 15.6	0.218
Diastolic blood pressure (mm Hg)	62.8 ± 8.5	56.6 ± 6.1	60.1 ± 8.0	0.092
Heart rate (bpm)	79.6 ± 6.3	86.6 ± 15.5	82.7 ± 11.5	0.260
Waist circumference (cm)	108.1 ± 16.6	92.8 ± 11.4	100.8 ± 16.1	**0.031**
Hip circumference (cm)	113.7 ± 14.4	103.9 ± 10.3	109.1 ± 13.3	0.105
Biochemical				
Fasting plasma glucose (mmol/L)	5.2 ± 0.4	5.3 ± 0.4	5.3 ± 0.4	0.617
Insulin (pmol/L)	187.9 ± 130.3	129.5 ± 61.0	158.7 ± 103.2	0.248
Alanine aminotransferase (U/L)	52.8 ± 16.4	54 ± 65.7	53.4 ± 46.5	0.958
Aspartate aminotransferase (U/L)	34.2 ± 6.4	37.3 ± 34.8	35.8 ± 24.4	0.798
Triglycerides (mmol/L)	1.5 ± 1.4	1.5 ± 0.4	1.5 ± 1.0	0.935
Liver MR				
Mean MRS (%)	21.9 ± 10.9	15.4 ± 12.5	18.6 ± 11.8	0.291
Mean MRI-PDFF (%)	26.8 ± 12.4	21.3 ± 18.6	24.1 ± 15.5	0.503
Liver ultrasound				
Liver-kidney contrast, n (%)				
0 = liver hypoechoic relative to kidney	0 (0%)	1 (11.1%)	1 (5.3%)	0.730
1 = mild hyperechoic liver relative to kidney	1 (10%)	2 (22.2%)	3 (15.8%)	
2 = moderate hyperechoic liver relative to kidney	2 (20%)	2 (22.2%)	4 (21.1%)	
3 = marked hyperechoic liver relative to kidney	7 (70%)	4 (44.4%)	11 (57.9%)	
Ultrasound deep attenuation, n (%)				
0 = no deep attenuation	3 (30%)	5 (55.60%)	8 (42.1%)	0.141
1= visible, blurred diaphragm	3 (30%)	4 (44.4%)	7 (36.8%)	
2 = undistinguishable diaphragm	4 (40%)	0 (0%)	4 (21.1%)	
Vessel blurring, n (%)				
0 = no vessel blurring	1 (10%)	5 (55.6%)	6 (31.6%)	0.057

Continues

Table 1. *Continued*

	Steatosis			
				P-value
	24-week omega-3 (n = 10)	**12-week omega-3** (n = 9)	**Total** (n = 19)	(24-week omega-3 vs. 12-week omega-3)
1 = narrowed and blurred vessels	9 (90%)	4 (44.4%)	13 (68.4%)	
Steatosis score	4.6 ± 1.7	2.9 ± 2.0	3.8 ± 2.0	0.058
Carotid ultrasound				
Carotid intima–media thickness (mm)	0.6 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.172

Note—Plus–minus values are means ± SD except where indicated. *MR*, magnetic resonance; *MRI-PDFF*, MR imaging proton density fat fraction; *MRS*, MR spectroscopy.

Carotid IMT showed a 0.05-mm decrease in the 24-week omega-3 group (Figure 3). The 12-week omega-3 group showed a 0.007-mm decrease during the sunflower oil period (between visits 1 and 2), and a 0.015-mm increase during omega-3 supplementation (between visits 2 and 3).

We did not observe significant interaction between visit and group (F (3, 29.846) = 2.899, P = 0.051). Hence, the evolution of IMT between the two steatosis groups was not significantly different. However, we did observe a significant visit effect (F (3, 29.846) = 5.868, P = 0.003), with carotid IMT significantly decreased between visit 4 as compared with visit 1 (P = 0.005) and visit 2 (P = 0.007). No significant group effect (F (1, 15.889) = 1.592, P = 0.225) was found.

Discussion

This prospective, double-blinded, one-way crossover randomized control trial compared the effect of treatment with 1.2 g daily of omega-3 versus sunflower oil on liver steatosis in children with obesity and baseline liver steatosis. Liver fat quantification was assessed by MRS as the reference standard, as well as by US and MRI. Carotid IMT was assessed by US.

In our study, omega-3 supplementation caused no significant effect on liver fat fraction as measured by MRS, MRI or US, and was not associated with a treatment duration effect [i.e. 24-week vs. 12-week supplementation]. Omega-3 supplementation was associated with a trend towards decrease in carotid IMT between visits (P = 0.003), and this effect was stronger with longer treatment intervals. However, IMT changes were not significantly different between the omega-3 and sunflower oil groups. The trend towards carotid IMT reduction associated with omega-3 supplementation should be explored in larger cohorts.

Our results also showed excellent or good accuracy for US-based fat grading, using MRS as the reference standard for fat quantification and for inferring steatosis grade. The area under the ROC curve was 0.964 for detection of mild-to-severe steatosis (≥6.4% by MRS), 0.817 for detection of moderate to severe steatosis (≥17.4% by MRS) and 0.783 for detection of severe steatosis (≥22.1 by MRS). These preliminary results suggest that a semi-quantitative approach may have a diagnostic accuracy similar to that of quantitative US (area under the curve (AUC) = 0.98) for detection of steatosis (≥6.0% by MRI proton density fat fraction) (48).

Nearly all clinical studies that have previously assessed the effect of omega-3 on liver steatosis have been

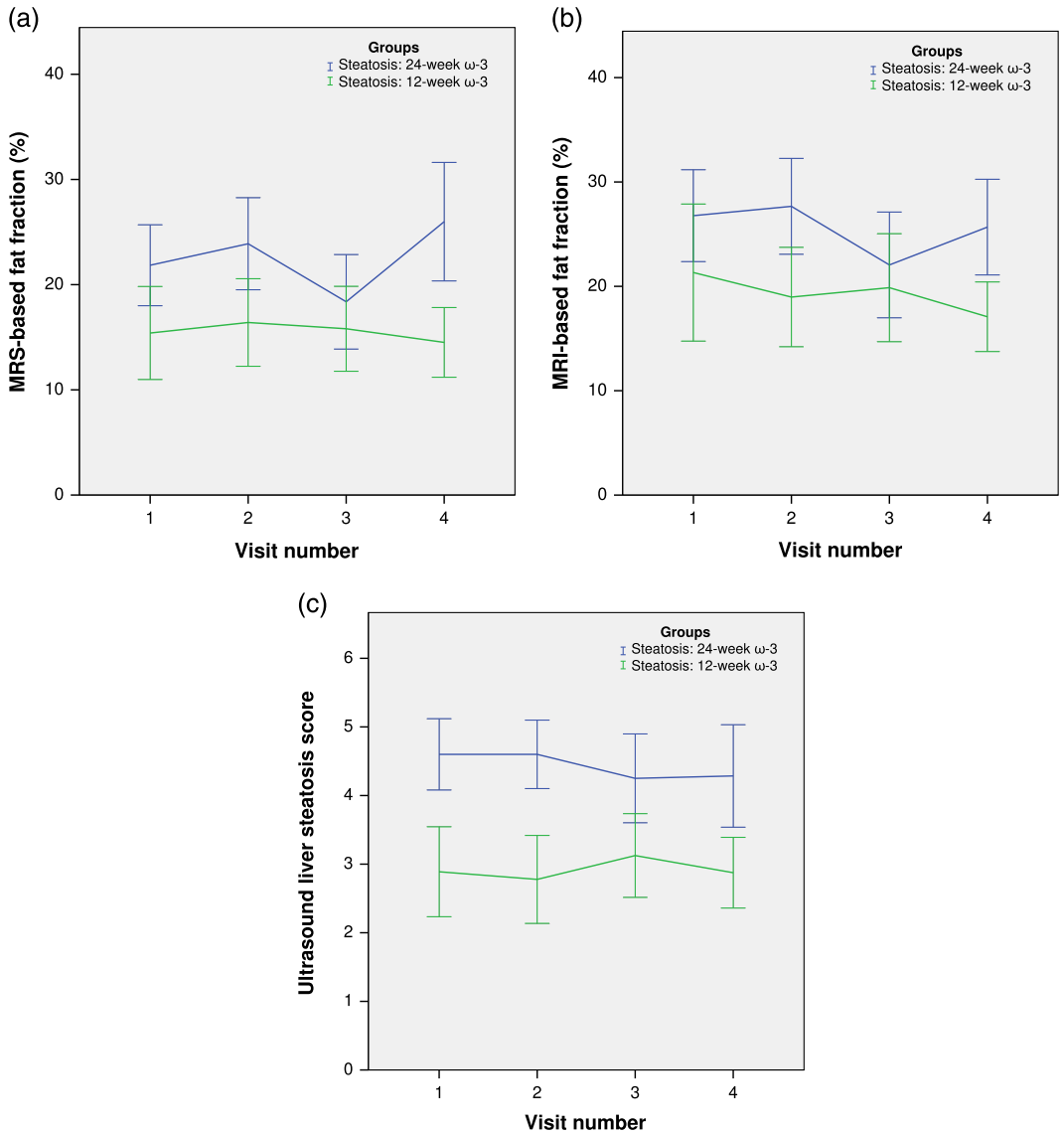

Figure 1 Changes in liver fat content as assessed by (a) fat fraction (%) measured by magnetic resonance spectroscopy (mean of 3 voxels), (b) fat fraction (%) measured with magnetic resonance imaging (mean of all liver segments) and (c) ultrasound qualitative liver steatosis score (ranging from 0 to 6). *Blue* = 24-week omega-3 group. *Green* = 12-week omega-3 group. Error bars indicate mean ± 1 SD.

performed on adult subjects (49). While these studies differ in study design (pilot clinical studies, randomised controlled trials [RCT] and systematic review), dosage (from 0.83 g daily (24) up to 9 g daily (50)), duration (from 8 weeks (23,50) to 12 months (20,21,24)) and technique for assessing liver steatosis (mostly US and MRI), most have been conducted on a small number of patients (at most 134 patients (51)) and have reported a beneficial effect of omega-3 supplementation. Available RCTs demonstrate some liver fatty regression in the majority of the patients after assessment with US (22,24,51) or MRS (23). A RCT study performed on a pediatric population (25) reported less odds of having severe hepatic steatosis after 6 months of omega-3 supplementation, with persisting beneficial effects up to 24 months (52). However, a more recent RCT in children showed no effect of omega-3 supplementation on liver steatosis on US (53). The result of our study is hence concordant with this RCT, which also studied a similar patient population.

Most available investigations studying the effect of omega-3 supplementation on carotid IMT are observational studies targeting Northern European populations, communities in small fishing villages in Japan and

Table 2 Diagnostic accuracy of liver steatosis assessment by semi-quantitative ultrasound compared to MRS (reference standard) at the baseline visit. Estimates of diagnostic performance are reported for points on ROC curves that maximize the Youden index

Inferred steatosis grade	MRS thresholds (%)	US steatosis score thresholds	AUC for US steatosis score	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
0 vs. 1–2–3	6.4	≥ 1.5	0.964 [0.864; 1.000]	85.7 [56.2; 97.5]	100.0 [19.8; 100.0]	100.0 [69.9; 100]	50.0 [15.0; 85.0]
0–1 vs. 2–3	17.4	≥ 2.5	0.817 [0.602; 1.000]	100.0 [56.1; 100.0]	55.6 [22.7; 84.7]	63.6 [31.6; 87.6]	100.0 [46.3; 100.0]
0–1–2 vs. 3	22.1	≥ 2.5	0.783 [0.558;1.000]	100.0 [51.7; 100.0]	50.0 [23.7; 76.3]	54.6 [24.6; 81.9]	100.0 [46.3; 100.0]

Note—Numbers in brackets are 95% confidence intervals. AUC, area under the receiver operating characteristic curve; MRS, magnetic resonance spectroscopy; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; US, ultrasound.

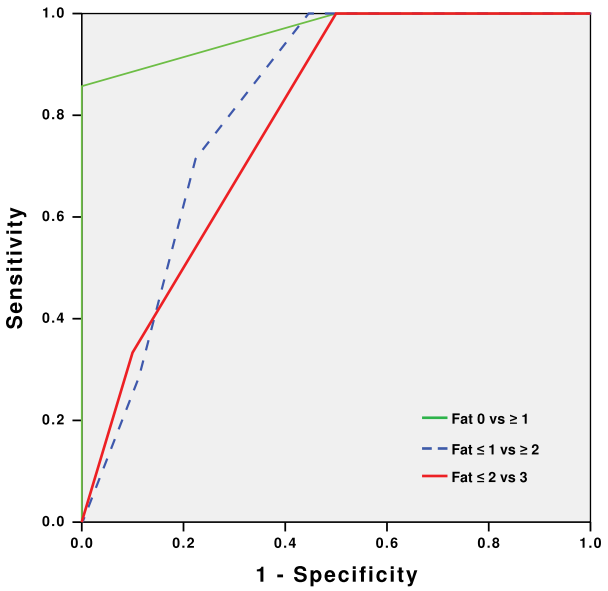

Figure 2 Receiver operating characteristic curve analysis of ultrasound for classification of liver fat grades compared to the magnetic resonance spectroscopy as the reference standard at 6.4% (inferred steatosis grades 0 vs. ≥ 1), 17.4% (inferred steatosis grades ≤ 1 vs. ≥ 2) and 22.1% fat fraction thresholds (inferred steatosis grades ≤ 2 vs. 3).

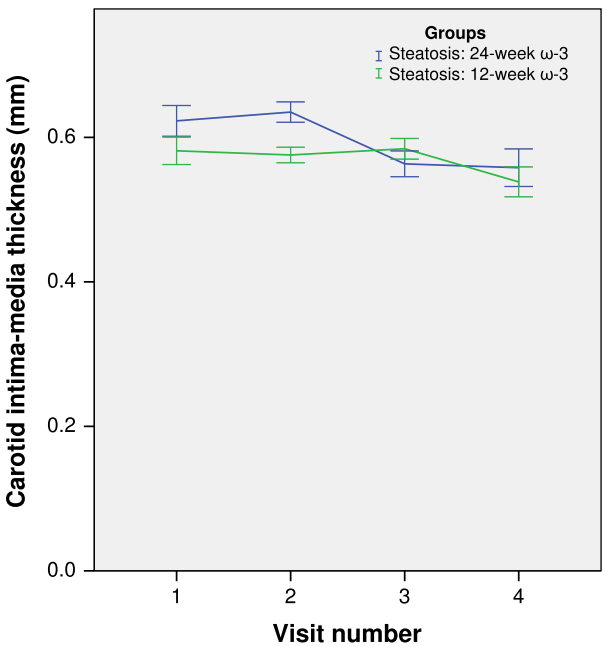

Figure 3 Variations through groups and visits of mean carotid intima–media thickness (IMT) as measured by ultrasound (mm). *Blue* = 24-week omega-3 group. *Green* = 12-week omega-3 group.

Native American populations (26,54,55), thought to have a higher baseline dietary consumption of marine oils. Most cross-sectional studies showed decreased carotid

IMT and lower plaque incidence (26,28,56). Results from RCTs previously showed no effect of omega-3 supplementation on carotid IMT (27,29). However, a recent study found a reduced progression of carotid IMT following omega-3 treatment (57). Only one RCT was performed on a pediatric population (55), and although showing promising preliminary results, no long-term benefits of omega-3 supplementation were found (58). Hence, the only systematic review available (30) concluded that because of scarcity of valid RCT, it was impossible to draw conclusion as to the effect of omega-3 on carotid IMT.

In our study, MRS was used as a surrogate reference standard for the estimation of liver fat fraction. While histological assessment remains the definitive reference standard for the measurement of liver fat fraction, it would have been unacceptable to submit asymptomatic children to repeated liver biopsies, exposing them to a painful experience and to the non-negligible risks of complications. Furthermore, as stated by Sanyal *et al.* (33), the spectrum of NAFLD is less well defined in children, and the histologic endpoints are more variable. As suggested, non-invasive imaging studies (MRI and MRS) might be acceptable in a pediatric population in order to monitor variations in steatosis in short-term clinical trials. While the thresholds used for dichotomization of steatosis severity were originally based on MRI-PDFF technique, they were transposed to MRS for this study because of the strong correlation between these MR-based techniques (47,59).

The main limitation of our study is the small number of subjects included in the statistical analysis, as only 22 subjects were recruited, and 10 subjects (45.5%) completed all four clinical visits and various imaging studies. Challenges in patient enrollment and significant drop-out rate may be explained by the length of the study. Also, the subjects were required to miss 4 days of school for this clinical study, which limits acceptance, both from parents and study participants. The small number of subjects included in this study might have been insufficient to detect a small benefit of Omega-3 supplementation over sunflower oil (type 2 error).

Conclusion

In conclusion, omega-3 supplementation had no significant effect on liver fat content but led to a decrease in carotid IMT in children with obesity and hepatic steatosis. Future and larger clinical trials in children with obesity are required to confirm the long-term effect of omega-3 supplementation on carotid IMT.

Conflict of Interest Statement

The authors declare no conflict of interest.

Authors' Contribution and Acknowledgements

Authors listed on the title page have participated in the conception and design of this work or the analysis and interpretation of the data, as well as the writing of the manuscript, and take public responsibility for it. We believe the manuscript represents valid work. We have reviewed the final version, and approve it for publication. Neither this manuscript nor one with substantially similar content under our authorship has been published or is being considered for publication elsewhere.

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