Estimation of Fish and ω-3 Fatty Acid Intake in Pediatric Nonalcoholic Fatty Liver Disease

David E. St-Jules, Corilee A. Watters, Elizabeth M. Brunt, Lynne R. Wilkens, Rachel Novotny, Patricia Belt, and Joel E. Lavine, for the Nonalcoholic Steatohepatitis Clinical Research Network

ABSTRACT

Aims: Fish and ω-3 fatty acids are reported to be beneficial in pediatric nonalcoholic fatty liver disease (NAFLD), but no studies have assessed their relation to histological severity. The objectives of this study were to evaluate the dietary intake of fish and ω-3 fatty acids in children with biopsy-proven NAFLD, and examine their association with serological and histological indicators of disease.

Methods: This was a cross-sectional analysis of 223 children (6–18 years) who participated in the Treatment of Nonalcoholic Fatty Liver Disease in Children trial or the NAFLD Database study conducted by the Nonalcoholic Steatohepatitis Clinical Research Network. The distribution of fish and ω-3 fatty acid intake was determined from responses to the Block Brief 2000 Food Frequency Questionnaire, and analyzed for associations with serum alanine aminotransferase, histological features of fatty liver disease, and diagnosis of steatohepatitis after adjusting for demographic, anthropometric, and dietary variables.

Results: The minority of subjects consumed the recommended 8 ounces of fish per week (22/223 [10%]) and 200 mg of long-chain ω-3 fatty acids per day (12/223 [5%]). Lack of fish and long-chain ω-3 fatty acid intake was associated with greater portal (P = 0.03 and P = 0.10, respectively) and lobular inflammation (P = 0.09 and P = 0.004, respectively) after controlling for potential confounders.

Conclusions: Fish and ω-3 fatty acid intake was insufficient in children with NAFLD, which may increase susceptibility to hepatic inflammation. Patients with pediatric NAFLD should be encouraged to consume the recommended amount of fish per week.

Key Words: adolescents, fatty acid, fish, nonalcoholic fatty liver disease, ω-3

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of dietary fish and ω-3 fatty acids in attenuating the progression of NAFLD. The purpose of this study was to evaluate the dietary intake of fish and ω-3 fatty acids and their relation to serum ALT and histological features of liver disease in pediatric NAFLD. We hypothesized that most pediatric patients with NAFLD would report fish and ω-3 fatty acids intakes that were below the recommended levels for children, and that lower intakes of fish and ω-3 fatty acids would be associated with higher serum ALT values and more severe histological indicators of liver disease.

METHODS

Study Population

This study was a cross-sectional analysis of data that was collected as part of the Treatment of Nonalcoholic Fatty Liver Disease in Children (TONIC) trial and the NAFLD Database study (13,14). The design of the TONIC trial has been described previously (13,15). Briefly, children (8–17 years) with biopsy-proven NAFLD were recruited among unsolicited referrals from September 2005 to September 2007 to 8 clinical centers of the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN, n = 229) including the University of California, San Diego, University of California, San Francisco, University of Washington, St. Louis University (in collaboration with Texas Children’s Hospital), Duke University Medical Center (in collaboration with Johns Hopkins University), Indiana University, Case Western Reserve University, and Virginia Commonwealth University (in collaboration with Children’s National Medical Center). Subjects who had liver cirrhosis, diabetes mellitus, and other liver diseases, or who were pregnant were excluded from the study (n = 56) (15). The NAFLD Database study included NAFLD patients age 2 years or older who were being treated at one of the NASH CRN clinical centers (n = 218) from October 2004 to February 2008, and contained 23 patients who participated in the TONIC trial.

Of the 368 children enrolled in TONIC or the NAFLD Database, 238 completed the Block Brief 2000 Food Frequency Questionnaire (FFQ) (16) within 6 months of the liver biopsy. Three subjects did not meet the definition of NAFLD because they had a liver steatosis grade indicating <5% macrovesicular steatosis, and were excluded from the study. Additional subjects were excluded because they were missing ≥1 variables of interest (n = 7), or did not report intake of fried and/or nonfried fish (n = 4). Finally, there was a 2-year-old who was identified as an outlier with respect to age, and was therefore not included in the study sample. Consequently, 223 (61%) children from the initial study population were deemed eligible and included in the study sample. Sensitivity analysis was performed for demographic and histological characteristics of children eligible and included in the study compared with children who were excluded; study subjects were more likely to be boys and to have more severe steatosis and hepatocyte ballooning (P < 0.05).

Assessment of Fish and ω-3 Fatty Acid Intake

Participants completed the FFQ at baseline and annually for the TONIC trial and NAFLD Database study. This semiquantitative FFQ approximates usual dietary intake based on reported consumption of 77 food items in the previous year. The food items are a composite of food lists developed for whites, Hispanics, and African Americans based on 24-hour dietary recall data from the National Health and Nutrition Examination Survey III (16). Visual displays of portion sizes were included in the FFQ to assist in estimation of quantity (16). This FFQ has been used previously to assess the relation between other dietary variables and histological features of pediatric NAFLD (17).

As part of the Block Brief 2000 FFQ, subjects reported their intake of fried and nonfried fish across 9 frequencies ranging from never to daily, and 4 serving sizes ranging from 1/4 cup to 2 cups. The responses for fried and nonfried fish were pooled together to estimate the overall frequency and amount of fish consumed. The nutrient content of the diet was calculated by NutritionQuest using the US Department of Agriculture Food and Nutrient Database for Dietary Studies, which provides a population-weighted nutrient composition for each food item based on national dietary intake data (16). Total ω-3 fatty acid consumption was analyzed as a nutrient density (grams per 1000 kcal) and in relation to ω-6 fatty acid intake. The ratio of ω-6 fatty acids to ω-3 fatty acids was used because the synthesis of long-chain ω-3 fatty acids (EPA and DHA) from α-linolenic acid (ALA; 18:3 ω-3), which makes up approximately 95% of total ω-3 fatty acids in the diet of children and adolescents in the United States, is thought to be dependent on ω-6 fatty acid intake because of shared metabolic pathways (18,19). Although nutrient density is often preferred over absolute intakes when using FFQ data, absolute intakes of EPA, DHA and long-chain ω-3 fatty acids were used in statistical analyses because they are concentrated in a limited number of sporadically consumed foods that are represented in the Block Brief 2000 FFQ (20).

Assessment of NAFLD

Liver disease was evaluated using serum alanine aminotransferase (ALT) and histological features of NAFLD. For the histological parameters, liver biopsies were graded centrally by NASH CRN pathologists for steatosis (<5%, 5%–33%, >33%–66%, or >66% macrovesicular steatosis), portal inflammation (none, mild, more than mild), lobular inflammation (<2 foci, 2–4 foci, or >4 foci at 20× field), hepatocellular ballooning (none, few, many), and fibrosis (none [0], zone 3 perisinusoidal, delicate [1A], zone 3 perisinusoidal, dense [1B], portal/peripheral [1C], perisinusoidal and portal/peripheral [stage 2], bridging fibrosis [stage 3], or cirrhosis [stage 4]) using standard scoring criteria (21). Zone 3 perisinusoidal (1A) and (1B), and portal/peripheral (1C) fibrosis were collapsed for analysis as stage 1, as were bridging and cirrhosis, as stage 3 to 4. Finally, subjects biopsies were diagnosed as “not NASH,” “borderline zone 1 pattern,” “borderline zone 3 pattern,” or “definite NASH,” as described previously (14).

Other Variables

Demographic and anthropometric data were obtained from participants in the TONIC trial and NAFLD Database study through structured interviews and questionnaires conducted at each of the NASH CRN clinical centers. For ethnicity, subjects were asked to identify themselves as American Indian, Asian, Pacific Islander, black or white, and as Hispanic or non-Hispanic. After examining the ethnicity distribution, responses were assigned as Hispanic, non-Hispanic white, and Other because of a limited representation from most ethnic groups. Height and weight were measured in duplicate to the nearest 0.1 cm and 0.1 kg, respectively, with subjects wearing lightweight clothing and no shoes. The average of height and weight were used to calculate body mass index (BMI) (kg/m²), and these values were converted in z scores for age and sex based on the Centers for Disease Control and Prevention growth charts (22).

Statistical Analysis

Demographic and dietary characteristics of subjects were summarized as frequency and percentage for categorical variables (sex and ethnicity), mean ± standard deviation for normally
distributed continuous variables (age), and median and interquartile range for non-normally distributed continuous variables (energy intake, total, fried and nonfried fish consumption, ω-3 fatty acid density and ω-6 to ω-3 fatty acid ratio of the diet, and EPA, DHA, and long-chain ω-3 fatty acid intake). Additionally, the proportion of subjects consuming the recommended amount of fish (≥2 servings and ≥8 oz/week), and long-chain ω-3 fatty acids (≥200 mg/day) was determined and reported as frequency and percentages (24,25). Continuous variables were designated as normally distributed based on the Shapiro-Wilk test, and visual analysis of frequency distribution graphs. The association between age and dietary fish and ω-3 fatty acid intake variables were analyzed using Spearman rank correlation coefficient. Differences in subject characteristics by sex and ethnicity were evaluated using χ² analysis, independent 2-sample t test and Wilcoxon rank sum test for categorical, and normally and non-normally distributed continuous variables, respectively.

A positive correlation has been observed between how often a food is consumed and the serving size that is eaten (26). To determine whether this pattern was present for dietary fish in this population, the relation between the frequency and the quantity of fried and nonfried fish intake was assessed. Subjects were grouped according to the reported frequency of fried and nonfried fish intake as a few times a year, monthly (1× per month and 2–3× per month), and weekly (1× per week, 2× per week, 3–4× per week, 5–6× per week and daily), and the corresponding portion size of fried and nonfried fish was compared across the frequency groups using the Mantel-Haenszel test for trend. Only a few subjects reported eating 2-cup servings of fried (n = 3) and nonfried (n = 4) fish, so a combined 1- to 2-cup serving was used in this analysis.

The association of serum ALT with total, fried, and nonfried fish consumption, ω-3 fatty acid density and ω-6 to ω-3 fatty acid ratio of the diet, and EPA, DHA and long-chain ω-3 fatty acid intake was assessed using Spearman rank correlation coefficient, and linear regression analysis adjusting for age, sex, BMI z score, and daily intake of energy, carbohydrates, protein, total, saturated, monounsaturated and polyunsaturated fat, sugar, fiber, cholesterol, vitamins A, C, and E, β-carotene, betaine, and choline. For the histological features of NAFLD, the differences in fish, long-chain ω-3 fatty acid, and ω-6 to ω-3 fatty acid ratio were examined across histology levels using the Kruskal-Wallis test for steatosis, portal and lobular inflammation, hepatocyte ballooning and fibrosis, and the Wilcoxon rank sum test to compare subjects diagnosed as having isolated steatosis to those with definite NASH. The associations with histological features and NASH diagnosis were also assessed using multivariate analysis of variance (MANOVA) adjusting for age, sex, ethnicity, BMI z score, and daily intake of energy, carbohydrates, protein, total, saturated, monounsaturated, and polyunsaturated fat, sugar, fiber, cholesterol, vitamins A, C, and E, β-carotene, betaine, and choline. In the linear regression and MANOVA, serum ALT and all of the dietary variables were log-transformed because they were non-normally distributed as per the Shapiro-Wilk test and analysis of frequency distribution graphs. Total, fried, and nonfried fish consumption were entered as log (fish intake + 1) before transformation to accommodate zero values.

All of the statistical tests were carried out using SAS version 9.2 (SAS Institute, Cary, NC), and graphs were constructed using Microsoft Excel for Macintosh 2011 version 14.2.5. The University of Hawaii Committee on Human Studies approved this research study.

RESULTS

The 223 subjects included in the study were mostly boys (171 [77%]), and Hispanic (128 [57%]) or non-Hispanic white (79 [35%]) with a mean age of 12.6 ± 2.6 years (Table 1). Consistent with the diagnosis of pediatric NAFLD, both BMI z score (2.31 ± 0.36) and serum ALT (85 U/L [interquartile range {IQR} 64–130]) were well above the normal ranges. The dietary variables of interest are summarized in Table 1. The median daily intake of 1616 kcal (IQR 1185–2351) was lower than would be expected based on the energy needs for this population, which is a common issue of dietary assessment using FFQs with moderate-size food lists; however, the macronutrient distribution appears probable (50.4%+8.3% carbohydrates, 16.2%+3.4% protein, 35.3%+6.9% fat), and was similar to what has been reported in other pediatric NAFLD studies (12,27).

Although there is no specific recommendation for fish intake in pediatric patients with NAFLD, most guidelines suggest consuming at least 2 servings, or 8 ounces of fish, per week (25). Only 33 of 223 (15%) of subjects reported consumption of fish ≥2 times per week, and <10% (22/223) achieved the target of 8 ounces per week (Table 1). Furthermore, nearly one-fourth (52/223, 23%) indicated that they never ate fish (Fig. 1). As seen in Figure 1, the distribution of fish consumption was typical of sporadically consumed foods with the mode located at the lowest level of intake, and a large right skew. This pattern can be partly attributed to the larger portion sizes consumed by regular fish eaters (supplemental Fig. 1 [http://links.lww.com/MPG/A242] and Figure 2 [http://links.lww.com/MPG/A243]). Among subjects who reported eating fish (n = 171), there was no correlation between fried and nonfried sources (Spearman ρ = −0.05, P = 0.55), suggesting that the 2 food items were distinct in the mind of the respondents (data not shown). As expected, fish intake was a major determinant of the long-chain ω-3 fatty acid intake variables were determined to be normally distributed based on the Shapiro-Wilk test and analysis of frequency distribution graph.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>12.6 ± 2.5</td>
</tr>
<tr>
<td>Sex, male (%)</td>
<td>171/223 (77)</td>
</tr>
<tr>
<td>Ethnicity (%)</td>
<td>79/223 (35)</td>
</tr>
<tr>
<td>Hispanic (%)</td>
<td>128/223 (57)</td>
</tr>
<tr>
<td>Other (%)</td>
<td>16/223 (7)</td>
</tr>
<tr>
<td>Energy, kcal/day</td>
<td>1616 (1186–2367)</td>
</tr>
<tr>
<td>Fish, oz/mo</td>
<td>4.4 (0.7–13.6)</td>
</tr>
<tr>
<td>≥8 oz/wk (%)</td>
<td>22/223 (10)</td>
</tr>
<tr>
<td>≥2 times/wk (%)</td>
<td>33/223 (15)</td>
</tr>
<tr>
<td>Fish, nonfried, oz/mo (range)</td>
<td>1.4 (0.0–12.1)</td>
</tr>
<tr>
<td>Fish, fried, oz/mo</td>
<td>1.5 (0.0–3.0)</td>
</tr>
<tr>
<td>ω-3 fatty acid, g/1000 kcal</td>
<td>0.71 (0.59–0.93)</td>
</tr>
<tr>
<td>ω-6: ω-3 fatty acid</td>
<td>9.0 (7.4–10.2)</td>
</tr>
<tr>
<td>Eicosapentaenoic acid, mg/day</td>
<td>9 (5–22)</td>
</tr>
<tr>
<td>Docosahexaenoic acid, mg/day</td>
<td>27 (16–48)</td>
</tr>
<tr>
<td>Long-chain ω-3 fatty acid, mg/day</td>
<td>43 (26–80)</td>
</tr>
<tr>
<td>≥200 mg/day (%)</td>
<td>12/223 (5)</td>
</tr>
</tbody>
</table>

Other ethnicities include non-Hispanic American Indian, Asian, Pacific Islander, Black, and Mixed. Categorical variables (sex, ethnicity, and proportion of subjects consuming ≥8 oz and ≥2 servings of fish per week and ≥200 mg of long-chain ω-3 fatty acids per day) are presented as frequency (percent), normally distributed continuous variables (age, body mass index z score, and proportion of energy from carbohydrates, protein, and fat) are presented as mean ± standard deviation, non-normally distributed continuous variables (energy intake, total, fried and nonfried fish consumption, ω-3 fatty acid density and ω-6 to ω-3 fatty acid ratio of the diet, and eicosapentaenoic acid, docosahexaenoic acid, and long-chain ω-3 fatty acid intake) are presented as median (interquartile range). Continuous variables were determined to be normally distributed based on the Shapiro-Wilk test and analysis of frequency distribution graph.
ω-3 fatty acid content of the diet (Spearman \( r = 0.73 \), \( P < 0.0001 \), data not shown). Given the relatively limited amount of fish consumed, it is not surprising that the diets were low in long-chain ω-3 fatty acids, with only 12 of 223 (5%) of subjects consuming ≥200 mg/day (Table 1).

The ω-3 fatty acid density of the diet was largely reflective of ALA intake, because ALA constituted 96% (IQR 94%–98%) of ω-3 fatty acid intake (data not shown). According to the dietary reference intakes, the acceptable macronutrient distribution range for ALA in children and adolescents is 0.6% to 1.2% of energy, which equates to 0.67 to 1.33 g/1000 kcal of ω-3 fatty acids when applying an Atwater factor of 9 kcal/g of ALA (25). The ω-3 fatty acid density of the diets observed here (0.72 g/1000 kcal [IQR 0.59–0.93 g/1000 kcal]) was concentrated around the lower end of the acceptable macronutrient distribution range (Fig. 2, Table 1). The ω-3 fatty acid intake was also low in relation to ω-6 fatty acids (median ω-6 to ω-3 ratio 9.0 (IQR 7.4–10.2) (Table 1). Although a desirable ω-6 to ω-3 fatty acid ratio has not been determined for pediatric NAFLD, the observed ratio of 9:1 greatly exceeds most references for disease prevention, which range from approximately 3:1 to 6:1 (19).

Despite greater reported energy intake in boys (1772 kcal [IQR 1369–2406 kcal]) compared with girls (1220 kcal [IQR 949–1860 kcal]) (\( P < 0.001 \)), there were no significant differences in total, fried, and nonfried fish consumption by sex (supplemental Table 1 [http://links.lww.com/MPG/A244]). Girls appeared to consume a more ω-3 fatty acid–rich diet than boys in relation to energy intake (0.77 g/1000 kcal [0.61–0.98 g/1000 kcal] vs 0.71 g/1000 kcal [0.58–0.88 g/1000 kcal], \( P = 0.05 \)), and ω-6 fatty acid intake (ω-6 to ω-3 ratio 8.6 [7.1–10.0]) vs 9.1 [7.6–10.3], \( P = 0.05 \) (supplemental Table 1 [http://links.lww.com/MPG/A244]). Larger disparities in fish and ω-3 fatty acid intake were observed between

![FIGURE 1. Frequency distribution of fish intake among fish consumers (n = 171). Fish intake is sum of fried and nonfried fish intake calculated from semiquantitative Food Frequency Questionnaire responses. An additional 52 (23.3%) subjects reported never consuming fish.](image)

![FIGURE 2. Frequency distribution of ω-3 fatty acid intake density (g/1000 kcal).](image)
ethic groups than between sexes (supplemental Table 2 [http://links.lww.com/MPG/A245]). Subjects from the Other ethnicity group consumed greater amounts of fish (12.8 oz/month [3.7–31.3 oz/month]) than Hispanics (4.4 oz/month [0.0–13.0 oz/month], $P = 0.03$) and non-Hispanic whites (3.3 oz/month [0.7–13.6 oz/month], $P = 0.02$). Additionally, non-Hispanic whites reported diets that were lower in ω-3 fatty acid content compared with subjects in the Hispanic and Other ethnicity groups (supplemental Table 2 [http://links.lww.com/MPG/A245]). Age was not associated with any of the fish or ω-3 fatty acid parameters measured (data not shown).

Higher fish and ω-3 fatty acid intakes were generally associated with lower ALT values, although none of the correlations were strong or statistically significant ($P > 0.05$); however, when additional factors including demographic, anthropometric, and dietary variables were accounted for in a linear regression model, the relations between serum ALT and long-chain ω-3 fatty acid intake tended toward significance ($P = 0.08$, data not shown).

The dietary fish and long-chain ω-3 fatty acid intake and ω-6 to ω-3 fatty acid intake ratio were also examined in relation to histological features of NAFLD. The main significant findings of these analyses were related to inflammation (Table 2). There appeared to be a protective effect of fish intake on hepatic inflammation that was significant for portal inflammation ($P = 0.02$) and tended toward significance for lobular inflammation ($P = 0.08$) (Table 2). Fish and long-chain ω-3 fatty acid consumption and ω-6 to ω-3 fatty acid ratio were not associated with the other histological parameters or a diagnosis of definite NASH (data not shown). Adjustment for demographic, anthropometric, and dietary variables did not influence the findings for fish intake and hepatic inflammation, but resulted in a stronger association between long-chain ω-3 fatty acid intake and lobular inflammation ($P = 0.004$, Table 2).

### DISCUSSION

There is emerging evidence that long-chain ω-3 fatty acids may be important mediators of NAFLD pathogenesis (7). The findings from this registry-based study offer insight into the dietary intake of fish and ω-3 fatty acids in children with documented NAFLD in the United States. This provides valuable contextual information and a useful perspective from which to examine the diet–disease relation.

Most of the subjects in this sample were not consuming the recommended amounts of fish and ω-3 fatty acids (Table 1) (24,25). The observed low intake of ω-3 fatty acids are consistent with the results of 3-day food records collected from 35 pediatric patients with NAFLD in Toronto, which reported average intakes of ALA that were less than two-thirds the adequate intake level (12). Together, these findings indicate that diet may be contributing to the low EPA and DHA content of cell membranes that has been observed in patients with NAFLD (9). Although this study did not have a control group for comparison, the frequency of fish consumption was less than that reported from a sample of >1000 adolescents from 5 public schools in Rhode Island, which had 36% of subjects who indicated eating fish at least once per week (vs 26%) and 17% who reported never eating fish (vs 23%) (28). Of interest, the Other ethnicity group reported approximately 3 times greater fish consumption than the Hispanic and non-Hispanic white subjects; however, this was a diverse group of non-Hispanic Indian, Asian, Pacific Islander, black, and Mixed ethnicities that contained only 16 subjects, which precluded meaningful subgroup analysis (supplemental Table 2 [http://links.lww.com/MPG/A245]).

Although NAFLD risk could not be determined from this analysis, it was possible to explore the relation of fish and ω-3 fatty acid consumption to serological and histological indicators of disease. The Toronto study noted a strong inverse relation between EPA and DHA intake and ALT (12). A similar, although non-significant, inverse association between fish and long-chain ω-3 fatty acid intake and ALT was observed in this sample. More important, lack of fish and long-chain ω-3 fatty acid consumption was associated with greater portal and lobular inflammation (Table 2). Although fish and long-chain ω-3 fatty acid intake were not associated with a diagnosis of definite NASH, inflammation is known to predispose to fibrosis and progressive liver disease (29). The failure to detect a relation between fish intake and definite NASH may be related to the fact that histologic parameters for steatohepatitis in pediatric biopsies are not as well understood as they are in adults (30).

There are several anti-inflammatory and proresolution mechanisms of EPA and DHA in fish that would support the observation of protective effects on both portal and lobular inflammation. Historically, the effects of long-chain ω-3 fatty acids on inflammation have been largely attributed to the shift from ω-6 to ω-3 fatty acid–derived eicosanoids (19). In recent years, additional EPA and DHA-derived lipid mediators, including protectins and resolvins, have been identified, and are thought to be important anti-inflammatory mediators (31). A series of experiments by Oh et al (32) also demonstrated that DHA suppresses activation of the

### TABLE 2. The relation between dietary fish and long-chain ω-3 fatty acid intake, and histological features of nonalcoholic fatty liver disease

<table>
<thead>
<tr>
<th>Histological parameter</th>
<th>Diagnosis</th>
<th>Crude</th>
<th>Adjusted</th>
<th>Portal inflammation</th>
<th>Crude</th>
<th>Adjusted</th>
<th>Lobular inflammation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>None</td>
<td>Crude</td>
<td>Adjusted</td>
<td>&lt;2 Foci/20× field</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mild</td>
<td>Crude</td>
<td>Adjusted</td>
<td>2–4 Foci/20× field</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>More than mild</td>
<td>Crude</td>
<td>Adjusted</td>
<td>&gt;4 Foci/20× field</td>
</tr>
<tr>
<td></td>
<td>Fish, oz/mo</td>
<td>11.7 (4.7–19.8)</td>
<td>57 (36–110)</td>
<td>4.4 (0.7–13.6)</td>
<td>48 (26–81)</td>
<td>4.4 (0.7–16.1)</td>
<td>40 (26–80)</td>
</tr>
<tr>
<td></td>
<td>LCn3, mg/day</td>
<td>2.9 (0.0–5.8)</td>
<td>33 (23–102)</td>
<td>3.0 (0.0–25.0)</td>
<td>40 (27–117)</td>
<td>3.0 (0.0–25.0)</td>
<td>40 (27–117)</td>
</tr>
</tbody>
</table>

Zones 1 and 3 refer to the periportal and centrilobular zones of the liver, respectively. Intake of fish and long-chain ω-3 fatty acids are reported for each level of the histological parameters as a median with interquartile range in parentheses. Crude analysis using the Kruskal-Wallis test to assess differences in dietary variables across levels of portal and lobular inflammation, and the Wilcoxon rank sum test to compare dietary variables of subjects diagnosed as having steatosis and definite nonalcoholic steatohepatitis. MANOVA controlling for age, sex, ethnicity, BMI z score, and intake of energy, carbohydrates, protein, total, saturated, monounsaturated and polyunsaturated fat, sugar, fiber, cholesterol, vitamins A, C, and E, β-carotene, betaine, and choline was used for the adjusted analysis. Dietary variables were determined to be non-normally distributed using the Shapiro-Wilk test and analysis of frequency distribution graphs, and were log-transformed for the MANOVA. Fish intake was entered as log (fish intake + 1) to accommodate zero values. LCn3 = long-chain ω-3 fatty acids; NASH = nonalcoholic steatohepatitis.

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nuclear factor-xB inflammatory pathway in macrophages through a G-protein–coupled receptor. CRP, an indicator of systemic inflammation, was measured in a subset of subjects in this study (n = 151). Although CRP was not associated with fish consumption, there was a weak inverse correlation with long-chain ω-3 fatty acid intake (Spearman ρ = −0.155, P = 0.058, data not shown).

Subjects consuming greater amounts of fish and long-chain ω-3 fatty acids did not have lower levels of hepatic steatosis (Table 2). The profound effects of EPA and DHA on lipid metabolism that have been reported in experimental animal models of obesity have been given as a major rationale for their importance in NAFLD (6). Moreover, this finding conflicts with the results of a double-blind, randomized trial of pediatric patients with NAFLD in Italy, which noted dramatic reductions in ultrasound liver steatosis grade among subjects that were receiving DHA compared with germ oil (11). One possible explanation for this discrepancy may be the dose of long-chain ω-3 fatty acids required to induce an effect. The DHA supplements in the Italian study were 5 times greater than the median intake of long-chain ω-3 fatty acids that was reported by our subjects (11). Alternatively, the effect of ω-3 fatty acids on hepatic steatosis may have been confounded by genetic factors. Recently, a study of obese adolescents noted that hepatic fat fraction detected by magnetic resonance imaging was associated with the ω-6 to ω-3 fatty acid ratio of the diet, but only among participants who were homozygous for the G allele of rs738409 in the PNPLA3 gene, which codes for adiponutrin (33).

In interpreting the results of this study, there are limitations that should be considered. The diet was assessed using an FFQ that was administered after subjects were diagnosed as having NAFLD. There was differential inclusion of subjects by sex and by level of steatosis and hepatocyte ballooning, which may have overestimated the association of including a more diseased population. The relatively low intake of sugar-sweetened beverages observed in the analysis of children in the NASH CRN database by Vos et al (17) suggests that respondents may have already modified their diets before filling out the FFQ, and/or were misreporting their intake to appear healthier. Although the NASH CRN Standards of Care for Pediatric Patients with Fatty Liver Disorders makes no reference to fish consumption, there are recommendations to limit fried fish, which may have prompted subjects to reduce the amount of fried fish they were eating or reporting. At the same time, most adolescents consider fish to be healthy, so overall consumption of fish may have actually increased (28). The validity of the Block Brief FFQ has not been evaluated in adolescents, but a moderate amount of error can be inferred based on the subject’s low energy intakes (Table 1); however, for the size and purpose of this study, an FFQ may be superior to short-term dietary assessment instruments such as 24-hour dietary recalls or food diaries for episodically consumed foods such as fish. Although data were collected on both fried and nonfried fish intake, the long-chain ω-3 fatty acid content varies considerably by species (34). Furthermore, no data were available on the use of supplements containing ω-3 fatty acids. Finally, care must be taken when attempting to extrapolate these findings to other pediatric NAFLD populations, because there appeared to be some selection bias.

The results of this study show that pediatric patients with NAFLD consume less than the recommended amount of fish and ω-3 fatty acids (24,25). Promoting the intake of fish may help to reduce both portal and lobular inflammation, but further research is needed to test this hypothesis and to determine the necessary amount and best sources. Based on the present fish consumption, dietary supplements may be a good option for increasing long-chain ω-3 fatty acid intake to recommended levels (25). Advances in food biotechnology may offer opportunities for alternative sources of ω-3 fatty acids in the future, but this remains to be seen (35). Until additional clinical trials evaluating the effectiveness of long-chain ω-3 fatty acid supplements on pediatric NAFLD are conducted, patients should be encouraged to increase fish intake to meet general health recommendations.

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Fish and ω-3 Fatty Acid Intake in Pediatric NAFLD

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