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ARTICLE

Safety and Efficacy of a Fish-Oil–Based Fat Emulsion in the Treatment of Parenteral Nutrition–Associated Liver Disease

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ABSTRACT

BACKGROUND. Parenteral nutrition–associated liver disease can be a progressive and fatal entity in children with short-bowel syndrome. Soybean-fat emulsions provided as part of standard parenteral nutrition may contribute to its pathophysiology.

METHODS. We compared safety and efficacy outcomes of a fish-oil–based fat emulsion in 18 infants with short-bowel syndrome who developed cholestasis (serum direct bilirubin level of >2 mg/dL) while receiving soybean emulsions with those from a historical cohort of 21 infants with short-bowel syndrome who also developed cholestasis while receiving soybean emulsions. The primary end point was time to reversal of cholestasis (3 consecutive measurements of serum direct bilirubin level of ≤2 mg/dL).

RESULTS. Among survivors, the median time to reversal of cholestasis was 9.4 and 44.1 weeks in the fish-oil and historical cohorts, respectively. Subjects who received fish-oil–based emulsion experienced reversal of cholestasis 4.8 times faster than those who received soybean emulsions and 6.8 times faster in analysis adjusted for baseline bilirubin concentration, gestational age, and the diagnosis of necrotizing enterocolitis. A total of 2 deaths and 0 liver transplantations were recorded in the fish-oil cohort and 7 deaths and 2 transplantations in the historical cohort. The provision of fish-oil–based fat emulsion was not associated with essential fatty acid deficiency, hypertriglyceridemia, coagulopathy, infections, or growth delay.

CONCLUSIONS. Parenteral fish-oil–based fat emulsions are safe and may be effective in the treatment of parenteral nutrition–associated liver disease.

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Key Words
ω-3, cholestasis, parenteral nutrition associated liver disease, fish oil, parenteral nutrition

Abbreviations
PN—parenteral nutrition
PNALD—parenteral nutrition–associated liver disease
EN—enteral nutrition
CHB—Children's Hospital Boston
SBS—short-bowel syndrome
IQR—interquartile range
CI—confidence interval

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PARENTERAL NUTRITION (PN) provides an alternative for patients unable to absorb adequate enteral nutrients, usually secondary to insufficient intestinal length or function. Diagnoses include motility disorders, intestinal resections, atresias, necrotizing enterocolitis, and inflammatory bowel disease. In 2002, the National Institute of Diabetes and Digestive and Kidney Diseases stated that 20 000 individuals in the United States were supported by PN for intestinal failure.[1] Before the PN era, these patients commonly died of malnutrition.[2]

PN contains macronutrients in their most elemental form and is commonly administered with fat emulsions to prevent essential fatty acid deficiency and to provide nonprotein calories. Although PN is life saving, it is associated with hepatic dysfunction, including biochemical (ie, elevated bilirubin and transaminases) and histologic alterations (ie, steatosis, steatohepatitis, cholestasis, fibrosis, and cirrhosis).[3] These abnormalities, which may worsen with prolonged administration, are more prevalent in the pediatric population.[4] Approximately 30% to 60% of children develop hepatic dysfunction while receiving long-term PN.[5]

Although the pathology of PN-associated liver disease (PNALD) has been well described,[6] its etiology, prevention, and treatment are poorly understood. Risk factors include prolonged PN use, prematurity, frequent surgical proce-

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TABLE 1 **Comparison of Parenteral Fat Emulsions (10 g of fat per 100 mL)**

Variable	Intralipid	Liposyn II	Omegaven
Oil			
Soybean	10	5	—
Safflower		5	—
Fish	—	—	10
Fats, %			
Linoleic	50	65	0.1–0.7
α-Linolenic	9	4	<0.2
EPA	—	—	1.3–2.8
DHA	—	—	1.4–3.1
Arachidonic acid	—	—	0.1–0.4
Glycerol	2.3	2.5	2.5
Egg phospholipid	1.2	1.2	1.2
Phytosterols, mg/L[26]	348 + 33	383	0

EPA indicates eicosapentaenoic acid; DHA, docosahexaenoic acid; —, no data.

dures, lack of enteral intake, and sepsis.[7] Early feeding may slow progression and, if not cirrhotic, cholestasis may be reversible once PN is discontinued and full enteral nutrition (EN) is established.[8] Partial EN may also be protective.[9] However, treating cholestasis by discontinuing PN is difficult, because it may result in starvation if EN is not absorbed. Other treatment options, including metronidazole, ursodeoxycholic acid, and choline, have had moderate success.[10] In refractory hepatic failure, liver with or without small-bowel transplantation remains the only option. Considering modern graft and patient 5-year survival rates for liver/small-bowel and small-bowel-alone transplants are in the range of 43% to 75% and 57% to 75% respectively, alternative medical and surgical strategies for short-bowel syndrome (SBS) and PNALD are needed.[11]

The etiology of PNALD is unknown, but a contributing factor may be the intravenous fat emulsion. Currently in the United States, fat emulsions are derived from either soybean/safflower or soybean oils, both rich in ω-6 fatty acids (Table 1). In a murine model, fish-oil emulsions prevented steatosis, a precursor of PNALD, as well as reversed preexisting disease, likely via improved triglyceride clearance coupled with antiinflammatory properties.[12] This emulsion contains ω-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid, an eicosanoid precursor. Increased synthesis of eicosapentaenoic acid-derived mediators may promote antiaggregatory and antiinflammatory effects. This emulsion also contains arachidonic acid, an ω-6 fatty acid. In a case series where 2 patients with cholestasis were treated with fish-oil–based fat emulsions, serum bilirubin levels normalized.[13] Patients tolerated this therapy well, and no adverse reactions attributed to its use were observed.

We further investigated the safety and efficacy of fish-oil–based emulsions in the reversal of cholestasis. As part of a compassionate treatment protocol, PN-dependent children with cholestasis had conventional soy-containing emulsions discontinued and replaced by fish-oil–based emulsions. Outcomes were compared with patients studied by Andorsky et al[14]

METHODS

Patients

Between September 2004 and August 2006, under a compassionate treatment protocol at Children's Hospital Boston (CHB), 18 infants receiving PN with soybean emulsions who developed cholestasis were treated with fish-oil emulsions and prospectively followed. Eligibility requirements included serum direct bilirubin level of ≥2 mg/dL and predicted PN duration of >30 days because of congenital or acquired gastrointestinal disease. Children with other liver diseases (ie, cystic fibrosis, inborn metabolic errors, and hepatitis C) were excluded.

A historical cohort of 30 infants with SBS, who were PN dependent for >90 days and treated at CHB from 1986 to 1996, was reviewed for comparison purposes.[14] Outcomes of 21 eligible subjects (2 consecutive direct bilirubin levels of >2 mg/dL) were included.

Study Treatment

In the fish-oil cohort, the soybean-based emulsion (Intralipid, Fresenius Kabi AG, Bad Homburg vdh, Germany) was discontinued, and treatment with fish-oil–based emulsion (Omegaven, Fresenius Kabi AG) was started. For the first 2 days of treatment, patients received fish-oil emulsions at 0.5 g/kg per day to assess tolerance and were progressed to a maintenance dosage of 1 g/kg per day over 12 hours. The 12-hour infusion time was used to minimize waste because of Centers for Disease Control and Prevention requirements that source containers of lipid emulsion be changed every 12 hours. Dosing was based on the previous use of fish-oil emulsions as monotherapy.[15] The comparison group received either Lyposin II (Hospira, Lake Forest, IL) or Intralipid, which are soybean/safflower and soybean emulsions, respectively. Doses ranged from 1 to 4 g/kg per day over 24 hours.

Study Outcomes

The primary study outcome was time to reversal of cholestasis, defined as time to the first of 3 consecutive serum direct bilirubin measurements of ≤2 mg/dL. Laboratory values were prospectively measured at approximately weekly intervals. The historical cohort had all of the available tests retrospectively recorded and measured approximately biweekly. Safety outcomes, including fatty acid and coagulation profiles, growth (weight-for-age z scores), and bloodstream infections, were systematically recorded only in the experimental group. Laboratory values and nutritional intake were retrospectively recorded ≤2 months before baseline, defined as the date that fish-oil emulsion began for the fish-oil cohort or as the date of the second of 2 consecutive direct bilirubin values of >2 mg/dL for the comparison group.

Statistical Analysis

Statistical significance of baseline differences between the 2 groups was assessed via t tests when reporting means (or Wilcoxon tests when reporting medians) and χ^2 tests when reporting proportions (or Fisher's exact

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TABLE 2 **Baseline Characteristics of Patients in the Fish-Oil and Historical Cohorts**

Variables	Fish Oil ($N = 18$)	Soybean ($N = 21$)	P[a]
Demographic			
Gender (male), *n* (%)	12 (67)	10 (48)	.23
Age, mean ± SD, wk	14 ± 7	14 ± 20	.85
Birth weight, mean ± SD, kg	2.03 ± 1.85	2.23 ± 1.00	.69
Gestational age, mean ± SD, wk	30 ± 4	34 ± 5	.03
Ethnicity, *n* (%)			
Black	5 (29)	6 (35)	.81[b]
White or Asian[c]	10 (59)	8 (47)	—
Latino	2 (12)	3 (18)	—
Clinical			
Diagnosis of gastroschisis, *n* (%)	4 (22)	5 (24)	>.99[b]
Diagnosis of intestinal atresia, *n* (%)	6 (33)	8 (38)	.76
Diagnosis of malrotation or midgut volvulus, *n* (%)	3 (17)	3 (14)	>.99[b]
Diagnosis of necrotizing enterocolitis, *n* (%)	10 (56)	8 (38)	.28
Other diagnosis, *n* (%)	3 (17)	1 (5)	.32[b]
No. of diagnoses, *n* (%)			
1	11 (61)	17 (81)	.17
≥2[d]	7 (39)	4 (19)	—
Laboratory			
Direct bilirubin, median (IQR), mg/dL	5.4 (3.4–7.7)	3.5 (3.2–5.5)	.13
Nutrition			
PN calories, median (IQR)[e]	69 (57–80)	84 (53–90)	.47
EN calories, median (IQR)[e]	6 (0–24)	22 (4–33)	.21
Daily soybean emulsion dose, median (IQR), g/kg per d	1.4 (0.2–2.1)	1.8 (1.2–2.3)	.25
Daily PN dextrose dose, median (IQR), g/kg per d	13 (10–17)	17 (11–19)	.31
Daily PN protein dose, median (IQR), g/kg per d	2.1 (1.7–2.8)	1.6 (1.3–2.1)	.03
Breast milk (yes), *n* (%)	4 (24)	2 (10)	.38[b]

— indicates no data. Baseline is defined as the date that treatment started for the fish-oil cohort and the date of the second of 2 consecutive direct bilirubin levels >2 mg/dL for the historical cohort.

[a] The *P* values for continuous variables were obtained via *t* test, when reporting means, and Wilcoxon test, when reporting medians. The *P* values for differences of proportions were obtained via Pearson's χ^2 tests.

[b] The *P* value was obtained by Fisher's exact test.

[c] There was only 1 Asian subject in the fish-oil group.

[d] Only 1 subject in the fish-oil group had 3 diagnoses.

[e] PN calories = daily PN energy/weight (kilogram); EN calories = daily EN energy/weight (obtained); daily PN energy = daily PN volume + daily PN dextrose × 0.001 × 3.4 + daily PN volume × daily PN protein × 0.001 × 4 + daily intralipid volume × 2 + daily Omegaven volume × 1.1; daily EN energy = daily EN volume × 0.0338 × EN caloric density.

tests when warranted). Primary analysis of efficacy of fish-oil–based emulsions was based on comparisons of time to reverse cholestasis while still receiving PN and included only those who did not die or undergo liver transplantation. Secondary analysis of efficacy included all of the patients, with censor criteria of death and transplantation. In both analyses, patients who did not reverse cholestasis by PN cessation or by end of follow-up were censored, and bilirubin levels were imputed by using linear interpolation, if ≤2 consecutive measurements were missing. Twenty-three bilirubin levels (of a total of 528 recorded measurements) were imputed. Four bilirubin levels were imputed in the fish-oil group and 19 in the soybean group. Results were comparable with and without imputation.

Kaplan-Meier survival curves were estimated for the time to reverse cholestasis using the product limit estimator and compared through log-rank tests. Crude and adjusted hazard ratios were estimated using proportional hazard models. In regression models, after including the treatment effect, variables were forced in for gestational age and direct bilirubin at enrollment, and these additional confounders were considered: gender, birth weight, diagnosis of necrotizing enterocolitis and/or atresia, multiple surgical diagnoses, baseline PN and EN energy intake (kilojoules per kilogram of body weight per day), and daily carbohydrate, fat, and protein intake (grams per kilogram per day). The additional confounders were retained if they appreciably affected the coefficient of treatment or if the *P* value for the likelihood ratio test was <.05. Survival analyses were also performed assuming that the PN effect could last ≤2 months by defining reversal of cholestasis as 3 direct bilirubin levels of ≤2 mg/dL ≤2 months after PN cessation. In these analyses, patients were censored 2 months after being weaned off PN. In patients receiving fish-oil emulsions, adverse events were summarized for the period before the beginning of therapy, 0 to 30 days after, and >30 days after. Primary analysis was based on comparisons between the pretherapy period and 30 days after.

All of the *P* values were 2 sided. Analyses were performed with SAS 9.1 (SAS Institute, Inc, Cary, NC) and S-plus 7 (Insightful, Seattle, WA).

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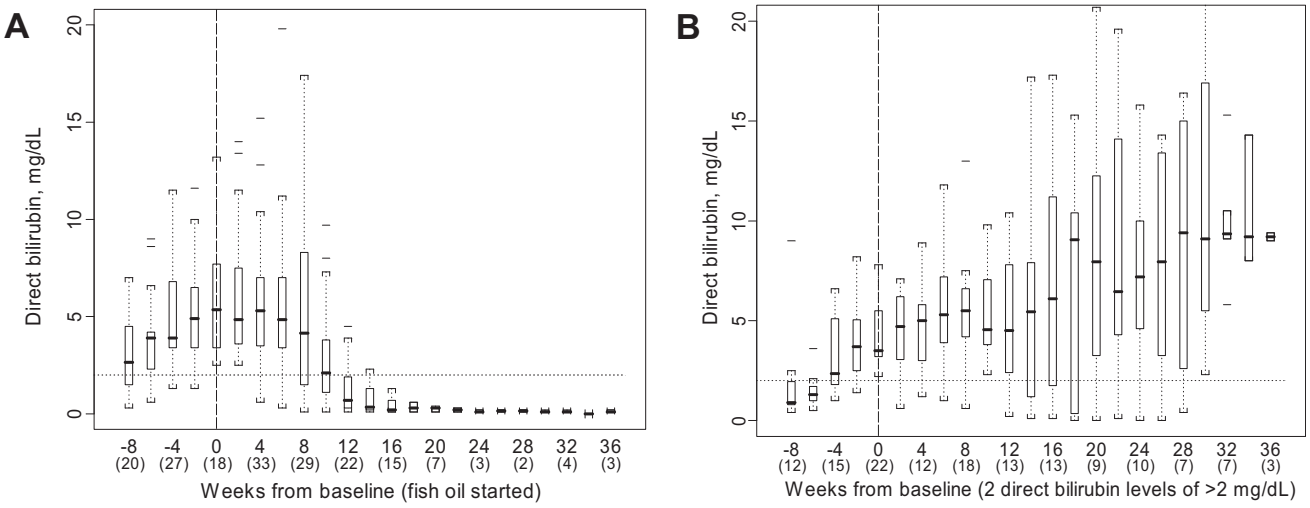

FIGURE 1

Direct bilirubin trajectories over time (weeks from baseline) for the fish-oil (A) and soybean (B) cohorts. Box plots represent the distribution of direct bilirubin levels in each 2-week interval plotted at the end of the interval, except for the bar at week 0, which represents only direct bilirubin measures at baseline. The number of observations contained in each 2-week interval is in parentheses. The solid bar within the box represents the median value; upper boundary of the box, the 75th percentile; lower boundary of the box, 25th percentile. Whiskers extend to the most extreme observation within 1.5 IQR units of the 25th and 75th percentiles.

Because Omegaven is not yet approved for use in the United States, Food and Drug Administration approval was obtained. This study was also approved by the CHB institutional review board. Informed consent was also obtained.

RESULTS

At baseline, several characteristics of the experimental group pointed to a more severe extent of illness compared with the historical cohort (Table 2), although differences were not statistically significant. These included shorter gestation, fewer enteral calories, and higher direct bilirubin level on enrollment. Nutritional intake at baseline was comparable; enteral intake was greater in the historical cohort. Results at baseline were comparable when analyzing only survivors. Of note, 81% of patients in the historical cohort were enrolled after 1992.

Patients were followed from baseline to last observation date (at or before PN cessation) for a median time of 18.4 weeks (interquartile range [IQR]: 8.7–36.4 weeks). Seven children in the fish-oil cohort and 14 children in the historical cohort never reversed cholestasis. Two of the 7 in the fish-oil cohort died, whereas 7 of 14 in the historical cohort died; 0 patients in the fish-oil cohort and 6 patients in the historical cohort died from liver-related causes. Patients who did not die or undergo transplantation were followed from baseline for a median time of 15.2 weeks (IQR: 8.1–25.0 weeks). Among survivors, the median time to PN cessation was 13.8 weeks (IQR: 7.6–36.4 weeks) in the fish-oil cohort and 22.9 weeks (IQR: 12.6–76.8 weeks) in the historical cohort. The risk of death was 11.1% and 33.3% for the fish-oil and soybean groups, respectively, but differences were not statistically significant (P of Fisher's exact test =.14). Times to death for the 2 patients in the fish-oil cohort were 6.7 and 10.7 weeks, and the median time to death in the historical cohort was 41.0 weeks (IQR: 29.0–84.6 weeks). Mortality in the soybean cohort was uniformly distributed over the years: 1 death in 1988, 2 in 1991, 1 in 1992, 1 in 1993, and 2 in 1997. Two children in the historical cohort (vs 0 in the fish-oil cohort) underwent liver transplantation and were among the deaths. Despite participation in this compassionate use protocol, liver or liver/small-bowel transplant options were made available to all of the subjects. Only 4 of the 18 subjects in the fish-oil cohort did not undergo a transplant evaluation. Of the 3 subjects who ultimately were listed for transplant, 2 were subsequently deactivated from the list, whereas 1 died of aspiration pneumonia.

In all 39 of the subjects, although levels of direct bilirubin at baseline were higher in the fish-oil cohort, trajectories of direct bilirubin were similar before (week −8 to 0) and ≤4 weeks after baseline (Fig 1). After this time, and particularly after week 8, direct bilirubin levels decreased in the fish-oil group but not in the historical cohort. In subjects who received higher mean doses of fish oil during therapy, that is, larger than the 80th percentile for mean dose in the fish oil group or between 0.87 and 0.92 mg/kg per day, the decaying trajectory was equivalent to subjects who received lower doses, that is, ≤0.87 mg/kg per day (Fig 2). In the soybean-emulsion group, subjects who received a mean dose of lipid from baseline to the end of PN equivalent to the higher dose of the fish oil took longer to reverse cholestasis. The mean dose of soybean oil these subjects received was below the 20th percentile of the mean daily soybean oil dose, that is, 0.67 to 0.98 mg/kg per day.

Among survivors, Kaplan-Meier-based estimates of the median time to reverse cholestasis were 9.4 weeks (IQR: 7.6–10.9 weeks) in the fish oil and 44.1 weeks (IQR: 10.9–45.6 weeks) in the historical cohort (P = .002; Fig 3). Patients in the fish-oil cohort who did not die had a 3.8 (hazard ratio: 4.8; 95% confidence interval

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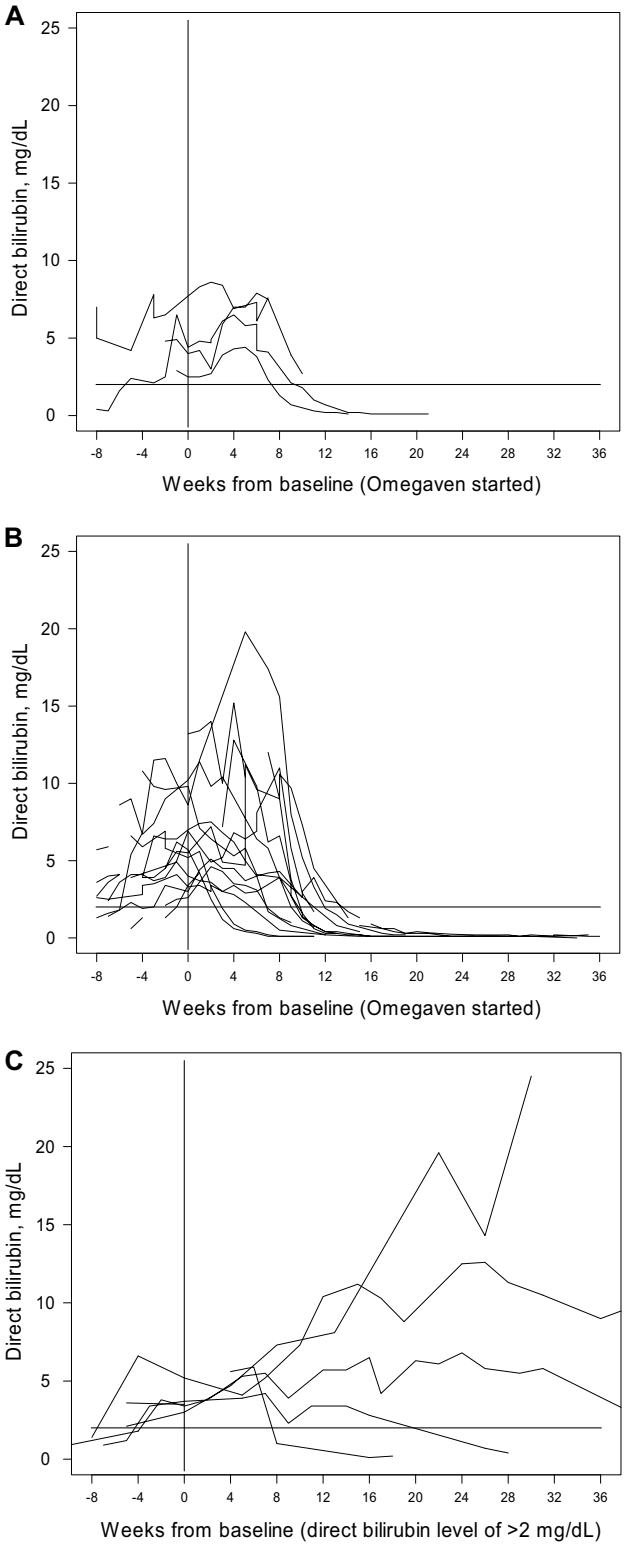

FIGURE 2
Individual direct bilirubin trajectories over time (weeks from baseline) for children who received higher (A) and lower (B) mean daily doses of fish oil during treatment, that is, below and above the cutoff of 0.87 g/kg per day (value corresponding with the 80th percentile). C, The trajectories of direct bilirubin levels are plotted for children in the soybean-emulsion group receiving an equivalent mean daily dose of lipid from baseline to the end of parenteral nutrition, that is, <0.98 (20th percentile of the soybean-emulsion group) and >0.67.

[CI]: 1.6–14.1) times larger rate of reversing cholestasis than those in the historical cohort (Table 3). After adjusting for the effect of baseline bilirubin level, gestational age, and necrotizing enterocolitis, the hazard ratio was 6.8 (95% CI: 1.7–27.8). When also including patients who died or underwent transplantation, the crude hazard ratio was 7.9 (95% CI: 2.6–24.0), and the adjusted hazard ratio was 15.9 (95% CI: 3.7–68.4). Other baseline patient characteristics were not included in the model, because they did not impact the coefficient of treatment or were not statistically significant predictors of the time to reverse cholestasis. These characteristics included PN and EN calories, daily soybean oil dose, PN dextrose and protein intake, birth weight, gender, and other diagnosis. The times for reversal of cholestasis in the 2 children who did not die and received a mean daily dose of fish oil during PN >0.87 mg/kg per day (maximum of 0.92 mg/kg per day) were 7.6 and 9.4 weeks. Both children reversed cholestasis. In the soybean oil group, 3 children did not die and received a mean daily dose of soybean oil from baseline to the end of PN ≤0.98 mg/kg per day (minimum: 0.67 mg/kg per day), and of those, 2 reversed cholestasis during the study period. The times for reversal of cholestasis of these 2 children in the soybean oil group were 10.3 and 44.1 weeks. Differences in the distribution of the times to reverse cholestasis between these 2 fish-oil and soybean oil subgroups were statistically significant (P of log-rank test = .04). The size of this subgroup of subjects was small. However, these results suggest that the beneficial effect of fish oil on recovery from cholestasis was not because of a difference in the lipid dose between these 2 groups.

The occurrence of undesirable safety outcomes in children receiving fish-oil–based emulsions was comparable before and after treatment started (Table 4), except for a single subject who briefly displayed biochemical evidence of essential fatty acid deficiency (triene/tetraene: >0.2). No patients developed hypertriglyceridemia (triglyceride level: >400 mg/dL) or coagulopathy (international normalized ratio: >2) during therapy. Mean platelet counts were statistically significantly higher during the fish-oil course (P = .03). Central venous catheter infection, new infection rates, and weight-for-age z scores were comparable across periods.

Reversal of cholestasis after PN cessation occurred in 5 subjects in the fish-oil cohort within 8 weeks; the median time from PN cessation to reversal of cholestasis was 3 weeks. In the soybean group, reversal of cholestasis after PN cessation did not occur during the follow-up period. In the fish-oil cohort, the 5 subjects who reversed cholestasis after cessation of PN were comparable with the 11 subjects who reversed cholestasis during PN with respect to all of the baseline clinical and demographic characteristics (including age, gestational age, birth weight, and diagnosis), with the exception of direct bilirubin levels. The median direct bilirubin level of subjects who reversed after cessation of PN was 7.0 mg/dL (IQR: 4.0–10.2) and of subjects who reversed during PN was 4.4 (IQR: 3.0–5.7), and differences were borderline statistically significant (P of Wilcoxon test = .07). Hazard ratios, when analyzing reversal of cholestasis ≤8 weeks

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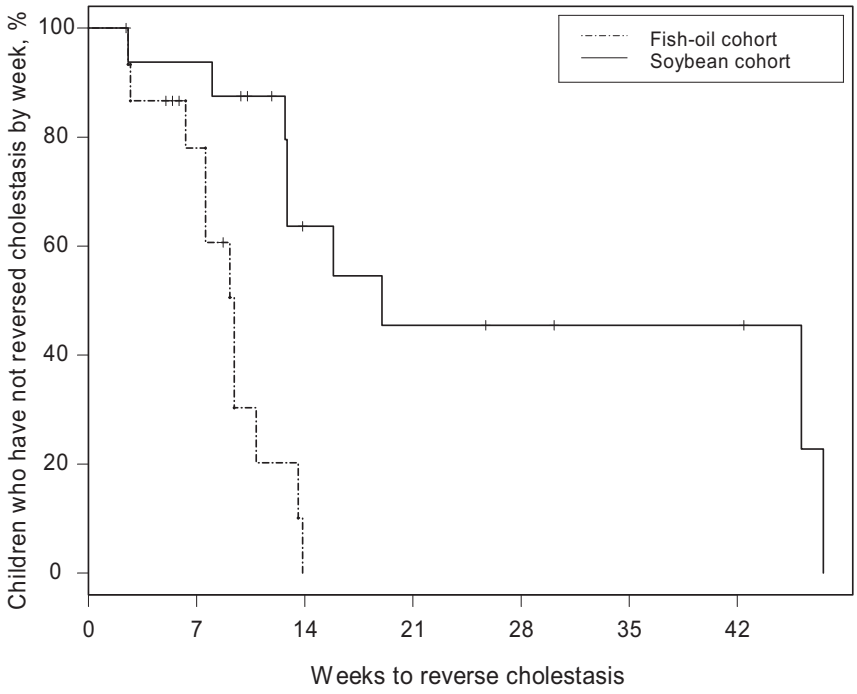

FIGURE 3
Kaplan-Meier curves with the proportion of subjects who reversed cholestasis according to different days in the fish-oil and historical cohorts.

after PN, were larger than those estimated with the primary outcome.

DISCUSSION

Infants, particularly premature infants, may survive without a lifelong dependence on PN with as little as 11 cm of initial bowel length,[16] attributed in part to rapidly growing bowel during this time. However, PNALD frequently occurs before bowel adaptation and growth are complete and EN can be tolerated. Therefore, it is a matter of what occurs first: bowel development or end-stage liver disease. The sequelae are significant considering that PNALD has a mortality rate as high as 100% in those children unable to be weaned off PN within a year of diagnosis.[17] Our results suggest that fish-oil–based emulsions may reverse PNALD when used in place of standard soybean emulsions. The experimental group reversed cholestasis, as measured by a decrease in direct bilirubin level, more frequently and more rapidly than the comparison group. The median time to reverse cholestasis was 9 weeks in the fish-oil and 44 weeks in the historical cohort, effectively halting the progression of PNALD and providing a chance to develop enteral tolerance and PN independence. Previous experience suggests that, in most cases, a reversal of cholestasis occurs after PN is discontinued and EN has been established,[8] but this experimental group consistently deviated from these norms. In comparison with the control group, these patients at baseline had lower enteral intake. However, once the serum bilirubin levels decreased (~4 weeks after the start of treatment), enteral intake increased, which may be attributed, in part, to enhanced bile excretion and improved nutrient absorption. In addition, fewer deaths and transplantations were observed in the fish-oil group.

More intriguing is the finding that 3 patients in the experimental arm experienced normalization of their serum bilirubin levels with <10% enteral intake, whereas no patient in the control group was able to achieve similar outcomes. We are not aware of this ever occurring at our institution until the use of this therapy. In addition, our results suggest that reversal of cholestasis in the fish-oil group occurred regardless of the lipid dose. A similar decay of trajectories of direct bilirubin in the higher and lower mean doses of fish oil was observed for nearly all of the subjects, particularly those who did not die. Moreover, subjects in the fish-oil group experi-

TABLE 3 **Increase in the Rate (as Measured by the Hazard Ratio) of Reversing Cholestasis (3 Consecutive Direct Bilirubin Levels of ≤2 mg/dL) While Receiving PN in the Fish-Oil Cohort Compared With the Historical Cohort**

Variables	Analyzing Survivors Only ($N = 30$)		Analyzing All Subjects ($N = 39$)	
	Hazard Ratio (95% CI)	P[a]	Hazard Ratio (95% CI)	P[a]
Crude estimate				
Fish oil (vs soybean)	4.8 (1.6–14.1)	.005	7.9 (2.6–24.0)	.0002
Adjusted estimates				
Fish oil (vs soybean)	6.8 (1.7–27.8)	.007	15.9 (3.7–68.4)	.0002
Gestational age, wk	0.82 (0.63–1.07)	.14	0.92 (0.74–1.14)	.46
Bilirubin at baseline, mg/dL	0.78 (0.58–1.06)	.12	0.85 (0.65–1.12)	.24
Diagnosis of necrotizing enterocolitis (vs other)	0.06 (0.004–0.770)	.03	0.15 (0.02–1.29)	.08

All of the estimates were obtained in proportional hazards models. The time to reverse cholestasis was based on the first of 3 consecutive bilirubin levels ≤2 mg/dL.
[a] The P values were estimated through likelihood ratio tests.

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TABLE 4 **Comparison of Safety Markers for the Period Before Fish Oil, From 30 Days After Starting Fish Oil Until the End of Follow-up (Primary Comparison) and From the Date in Which Fish Oil Was Started Until Day 30 of Treatment**

Variable	Before Fish Oil ($N = 17$)[a]	After Fish Oil	
		30 d to End ($N = 17$)[a]	0–30 d ($N = 18$)
Essential fatty acid deficiency			
Triene/tetraene ratio >0.2, *n* (%)	—	1 (5.9)	1 (5.6)
Maximum triene/tetraene, median (minimum, maximum)	—	0.05 (0.016, 0.28)	0.04 (0.02, 0.33)
Hypertriglyceridemia (triglycerides > 400 mg/dL), *n* (%)	1 (5.9)	0 (0.0)	0 (0.0)
Maximum triglycerides, median (minimum, maximum)	196 (89, 441)	162 (51, 336)	182 (107, 383)
Bleeding and coagulopathies			
INR >2, *n* (%)	1 (5.9)	0 (0.0)	0 (0.0)
Mean INR, median (minimum, maximum)	1.1 (0.99, 1.71)	1.1 (0.96, 1.33)	1.1 (0.92, 1.39)
Mean level of platelets, median (minimum, maximum)	175 (78, 390)	292 (103, 462)	220 (88, 380)
Minimum level of platelets, median (minimum, maximum)	104 (30, 298)	179 (26, 331)	145 (41, 268)
Infections			
No. of new infections per week, median (minimum, maximum)	0.24 (0, 1.29)	0.25 (0.06, 1.56)	0.25 (0, 1.0)
No. of central line infections per week, median (minimum, maximum)	0.13 (0, 0.57)	0.18 (0, 1.0)	0 (0, 0.67)
No. of Gram-positive infections per week, median (minimum, maximum)	0 (0, 0.29)	0 (0, 0.35)	0 (0, 0.25)
No. of Gram-negative infections per week, median (minimum, maximum)	0 (0, 0.26)	0.01 (0, 1.0)	0 (0, 0.33)
Growth			
Weight-for-age *z* score, mean ± SD	−4.2 ± 1.6	−4.5 ± 2.0	−4.4 ± 1.7

— indicates that the triene/tetraene ratio was not available before treatment; INR, international normalized ratio. Variables represent summaries over time for the specified period. Summary statistics were estimated including all of the subjects with information before ($n = 17$) and after ($n = 18$) administration of fish-oil emulsion.

[a] The *P* values were estimated for the primary comparison (ie, before fish oil versus for the period starting 30 days after the beginning of therapy and finishing at the end of the follow-up time). *P* values were obtained by using Wilcoxon signed-rank test (when reporting medians) for continuous variables and McNemar test for binary variables and included only the 17 patients with information before and 30 days to the end of follow-up after fish-oil emulsion started. All of the *P* values were >.25, except for mean level of platelets ($P = .03$), minimum level of platelets ($P = .13$), and weight-for-age *z* score ($P = .19$).

ence reversal of their cholestasis faster than subjects in the soybean-emulsion group, when comparisons were done only among subjects in the fish-oil and soybean oil cohorts who received equivalent mean doses of fat emulsion from baseline to the end of the study, These results, however, should be interpreted with caution, because the sample size of this subgroup was small.

The administration of fish-oil–based emulsions seems safe. After 4 weeks of therapy, only 1 patient displayed biochemical, not clinical, evidence of essential fatty acid deficiency (triene/tetraene ratio: >0.2). In this case, the patient's fish-oil emulsion had been discontinued, whereas EN was increased, in the presence of fat malabsorption; biochemical essential fatty acid deficiency occurred only after the patient had not received fish-oil emulsions for 3 weeks. The fish-oil emulsion was actually resumed to correct the observed deficiency. There was no increase in bleeding events, no change in growth, and no increase in infection rate. Two children receiving fish-oil–based emulsions died from causes unrelated to treatment. One died from aspiration pneumonia and sepsis. Another died when care was withdrawn because of irreparable cardiopulmonary disease.

The etiology of PNALD may be because of the use of soybean-based emulsions, secondary to proinflammatory metabolites of ω-6 fatty acids,[18] and decreased hepatic clearance of the parenteral lipid.[19] Soybean-derived lipids contain phytosterols (eg, stigmasterol, b-sitosterol, and campesterol) that are linked with impairment of biliary secretion.[20] Past and very recent studies have suggested that phytosterols may be the "hepatoxic" or "cholestatic" component of soybean-derived lipid emulsions, with recent molecular mechanisms of phytosterols being suggested.[21,22] It has also been suggested that ω-6 fatty acids may contribute to impaired immunologic function.[23] This multitude of factors results in a cholestatic, steatotic liver that is especially susceptible to inflammatory insults (eg, bloodstream infections, surgery, and hepatotoxic medication).[24] In turn, repeated liver injury results in fibrosis, cirrhosis, and end-stage liver disease. Fish-oil–based emulsions address these problems on several fronts. ω-3 fatty acid metabolites are less involved in the inflammatory response,[18] and animal models have shown that parenteral fish oil does not impair biliary secretion and may prevent steatosis.[12,25,26] Hence, the liver is not predisposed to inflammatory insult, and liver injury can be prevented.

Comparisons based on a historical cohort could result in bias. Because previous medical charts were less complete, more follow-up data were missing in this group. A delay in the time to reverse cholestasis could result from missing bilirubin measurements and result in overesti-

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mating the effect of fish-oil–based emulsions. To minimize these biases, bilirubin levels were imputed for certain data points. It is unlikely that worse outcomes in the historical cohort were because of poorer management of care, resultant from historical trends. For example, mortality in the historical cohort, uniformly recorded over time, did not increase. Furthermore, the rate of increase of bilirubin level in both groups was similar. Underestimation of fish-oil–induced reversal of cholestasis could result from enrollment under a compassionate protocol, because the patients were more severely ill and were higher risk because of several prognostic factors, including gestational age. The impact of these biases should be reduced when estimating adjusted effect in multiple regressions.

CONCLUSIONS

We have demonstrated that the use of fish-oil–based emulsion in infants who depend on PN as a life-sustaining measure may reverse cholestasis and fatal liver disease. More importantly, we have not observed any deleterious adverse effects of treatment. These benefits may be because of the absence of soybean oil or because of the pharmacologic effects of fish oil; however, this hypothesis is difficult to test because of the need to provide essential fatty acids in parenterally fed patients. Ideally, a prospective randomized, controlled trial comparing fish-oil emulsions with soybean emulsions in the treatment of established PNALD should be conducted, but this type of study would be difficult to conduct, because some may consider it unethical to perform a study where children with preexisting PN liver injury could potentially be randomly assigned to a treatment group in which they would continue to receive a soybean oil–based parenteral lipid emulsion. A prospective, randomized trial is underway at our institution to assess the efficacy of fish-oil–based emulsions in the prevention of cholestasis in which in infants who have never been exposed to either type of lipid emulsion are randomly assigned to either conventional soybean oil emulsion or fish-oil emulsion at the start of their PN course.

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