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Pediatric Intestinal Failure Associated Liver Disease is Revered with Six Months of Intravenous Fish Oil

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Pediatric Intestinal Failure Associated Liver Disease is Reversed with Six Months of Intravenous Fish Oil

A thesis submitted in partial satisfaction of the requirement for the degree Masters of Science in Clinical Research

By

Kara Lynne Calkins

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ABSTRACT OF THESIS

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Masters of Science in Clinical Research

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Professor Robert M. Elashoff, Chair

ABSTRACT OF THE THESIS

Pediatric intestinal failure associated liver disease (IFALD) is associated with a high morbidity and mortality. Studies have suggested that when intravenous (IV) soybean oil (SO) is replaced with fish oil (FO), direct hyperbilirubinemia is more likely to resolve. The necessary duration of FO has not been established. This study seeks to determine if 24 weeks of FO is an effective and safe therapy for IFALD.

The thesis of Kara Lynne Calkins is approved.

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Table of Contents

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Introduction

Parenteral nutrition (PN) is a major contributing factor to the long-term survival of children with intestinal failure (IF)[1]. IF results in prolonged PN dependence secondary to intestinal malabsorption. With an incidence of 3-5 cases per 100,000 births, IF in children is commonly caused by shortened and/or dysfunctional bowel due to necrotizing enterocolitis and congenital gastrointestinal disorders such as gastroschisis, intestinal atresias and volvulus[1-4]. For children with IF, PN serves as a bridge to bowel adaptation, a process by which the intestine recovers and improves its absorptive capacity. During this time, PN provides necessary fluids and micro- and macronutrients to prevent dehydration and promote growth and neurodevelopment.

The process of bowel adaptation can take weeks to years and can be complicated by intestinal failure associated liver disease (IFALD) which in turn is associated with a high morbidity and mortality[1,3,4-7]. Pediatric IFALD is hallmarked by cholestasis exhibited biochemically by direct hyperbilirubinemia, elevated serum liver transaminases, and histological findings on liver biopsy including cholestasis, steatosis, lipidosis and eventually fibrosis and cirrhosis. As IFALD progresses, patients can develop end stage liver disease exhibited by ascites, coagulopathy, portal hypertension, and hepatic encephalopathy. While an isolated intestine or combined liver-intestine transplantation may be life-saving for children with IF, these may not be feasible options for some children because of their small size, co-morbidities, and lack of timely organ availability[8]. The wait-list mortality rate for children awaiting a combined liver-intestine transplantation has historically been much higher than those awaiting transplantation of other solid organs[8]. For those who receive a transplant, the five-year survival is 50-70% and can be complicated by graft rejection, infection, and malignancy[1,8].

The safest and most effective treatment for IFALD remains successful rehabilitation to full enteral nutrition. However, enteral autonomy in children with IF may take time, or may not be feasible at all due to a host of factors. Moreover, IFALD has been shown to develop as early as a few weeks to months after PN initiation, particularly in low birth weight infants[5,9-11].

Intravenous (IV) fatty acid emulsions are prescribed with PN to provide additional calories, prevent essential fatty acid deficiencies, promote growth and neurodevelopment, and improve metabolic efficiency. Available fatty acid emulsions differ in composition and recommended dose, which complicate direct comparisons of efficacy. While the US Food and Drug and Administration (FDA) approved soybean oil-based emulsions (SO) have been traditionally prescribed at a maximum dose of 3-4 g/kg/d, a non-FDA approved fish oil-based emulsion (FO) used in Europe and Asia is prescribed at 1 g/kg/d and is available in the US under compassionate use and research protocols.

The development of IFALD in children has been associated with the composition and dose of fatty acid emulsions[9-15]. Animal studies, case reports and cohort studies have provided encouraging evidence that when 1g/kg/d of exclusive FO is substituted for SO, biochemical IFALD is more likely to resolve[9-13,16-19]. However, the required duration of FO monotherapy is unknown because prior studies usually prescribe FO until PN discontinuation[9-11]. Despite these reports and increased experience with FO at several US centers, there remain significant barriers for administering FO to IF patients due to a much higher unit cost, lack of FDA approval, and uncertainty of long-term safety and efficacy.

From the available studies in the literature, biochemical reversal of IFALD with FO monotherapy has been shown to occur between 35 days and 24 weeks[9-24]. We sought to determine whether a finite period of FO monotherapy followed by resumption of SO and

continued PN was safe and efficacious in children with IF. This study reports on the preliminary results of a prospective clinical trial in which 24 weeks of FO was substituted for SO in 10 subjects with IFALD. Subjects were compared to 20 historical controls who received SO. A FO treatment course of 24 weeks was selected based off available evidence at the design of this study, our experience with FO under compassionate use, and to minimize FO cost and potential and unforeseen adverse events, specifically effects on growth and neurodevelopment[9,12,20,21].

Methods

Patient Population

Ten interventional subjects were recruited from the University of California, Los Angeles (UCLA) Mattel Children's Hospital. Eligibility criteria included clinical evidence of IFALD, a direct bilirubin ≥ 2 mg/dL on two consecutive measurements separated by at least 1 week, expected PN course > 30 days, an acquired or congenital gastrointestinal disorder, > two weeks of age but < 18 years of age, greater than 60% of total calories from PN, and failure of standard therapies used to treat IFALD. Subjects with a primary liver disease, inborn error of metabolism, seafood, egg or FO allergy, hemorrhagic disorder, hemodynamic instability or shock, comatose state, stroke, pulmonary embolism, myocardial infarction, diabetes, or fatal chromosomal disorder or on extracorporeal membrane oxygenation were excluded.

Twenty historical controls were selected from an institutional review board (IRB) approved home PN and neonatal intensive care database from 2004 to 2009. Controls satisfied applicable inclusion and exclusion criteria.

IF was defined as PN dependence for > 60 days. PN dependence was defined as receiving > 60% of one's calories from PN. The number of gastrointestinal surgeries, not including gastrostomy tube placements or rectal biopsies, and bloodstream infections (a positive blood culture, signs and symptoms suggestive of sepsis, and need for at least five days of IV antibiotics) were compared between the two groups.

Written informed consent was obtained from interventional subjects and the study was approved by the UCLA IRB and FDA (IND 105,326) beginning in August 2009. A waiver of consent was approved by the IRB for historical controls. The study is registered at www.clinicaltrials.gov (NCT00969332).

Study Procedures and Methods

Once enrolled, each of the 10 interventional subjects received 0.5 g/kg/d IV for the first two days, then 1 g/kg/d IV of FO (Omegaven®, Fresenius Kabi, Bad Homberg Germany) over 8-24 hours in the inpatient or outpatient setting for an intended period of 24 weeks. FO was to be discontinued prior to 24 weeks if the subject no longer required PN, underwent liver and/or intestinal transplantation, or developed serious adverse complications from FO. In order to observe for tolerance, the first two FO doses were administered in the inpatient setting or UCLA's Clinical and Translational Science Institute. If a subject demonstrated persistent or recurrent cholestasis after 24 weeks of FO and continued to satisfy inclusion and exclusion criteria, he or she could receive two more courses of FO monotherapy for a maximum of 48 additional weeks. The 20 historical controls received SO (Intralipid®, Fresenius Kabi, Bad Homberg, Germany) at doses selected by their primary caregivers. Data collection for all 30 subjects began at the time the subject enrolled in the trial or the control subject satisfied inclusion

and exclusion criteria. Data collection then continued for 6 months or until death, transplant or PN discontinuation, whichever came first. FO subjects were followed post intervention for another 1.5 years.

Study Outcomes

Outcomes were measured after 24 weeks of FO or SO treatment or death, transplant, or PN discontinuation, whichever came first. The primary outcome was time to resolution of cholestasis (defined as a direct bilirubin < 2 mg/dL on two consecutive measurements separated by one week). Secondary outcomes included death, transplant, and full enteral feeds. Tertiary outcomes included number of hospital readmissions, number of inpatient days, and normalization of serum liver transaminases on two consecutive measurements separated one week. Measures of safety included growth (weight, length, and head circumference) and the development of an essential fatty acid deficiency (triene:tetraene ratio ≥ 0.5) in the FO group, and platelet counts and International Normalized Ratios (INR) in both groups.

Statistical Methods

Continuous variables were compared between groups using Wilcoxon rank sum test. Categorical variables were compared between the groups using Fisher's exact test. Laboratory values were transformed using a log base 10 scale since these values are normally distributed on the log scale. Median profiles were compared over time using a repeated measure analysis of variance model. Since not all laboratory values were measured exactly at four, eight, 12, 16 and 24 weeks for a given subject, some of the subjects' values were aligned to these times using local linear interpolation. Median platelet, albumin, triglyceride and INR

values were compared at baseline, eight, 12, 16, and 24 weeks. There was no extrapolation beyond times where values were not observed.

The probabilities of experiencing cholestasis resolution across time were estimated using the Kaplan-Meier method. The Kaplan-Meier curves were compared between the groups using the log rank test.

Growth for FO subjects was assessed by Z-scores. All anthropometric measurements were corrected for prematurity if the subject was less than two years of age. Mean and standard deviations for weight, length and head circumference were obtained for the Center for Disease Control and Prevention (CDC).

A p-value < 0.05 was considered statistically significant.

Results

Baseline characteristics between the FO and SO groups were similar (Table 1). While not statistically significant, the FO group was older at the start and end of the study. The study population was premature with a median gestational age of 34 weeks in both groups. The primary gastrointestinal diagnoses for the two groups were similar. FO and SO's median number of gastrointestinal surgeries and mean small bowel length, if measured and recorded by the surgeon, were comparable. The majority of the subjects had surgical short bowel syndrome. The median small bowel length (range) was 23 cm (0-51 cm) and 27 cm (0-55 cm) in the FO and SO group, respectively. As defined, IF was present in 80% of the FO group and 55% of the SO group.

PN at the beginning and end of the study was comparable between the two groups with respect to duration, total calories, glucose delivery rate, and amino acid dose (Table 2). However, at the start and end of the study, the mean intravenous fat dose (±SD) was less in the FO group in comparison to the SO group (1.5±0.9 g/kg/d vs. 2.6±0.7, $p < 0.006$ and 0.9±0.3 g/kg/d vs. 1.7±1.1, $p=0.02$). While not statistically significant, at the beginning of the study, the FO group received more enteral calories than the SO group with a median intake of 23 kcal/kg/d (range 0-43 kcal/kg/d) compared 4 kcal/kg/d (range 0-39 kcal/kg/d) ($p=0.05$). During the study, the number of bloodstream infections did not differ between the FO and SO groups. The FO group had a median of 1.5 infections (range 0-4) and the SO group had a median of 0.5 infections (range 0-3) ($p=0.14$). There was no difference in the use of ursodeoxycholic acid between the FO and SO group, respectively (30 vs. 25%, $p=0.99$). The SO group, however, had more gastrointestinal surgeries during the study period when compared to the FO group with a median of 1 (range 0-2) vs. 0 (range 0-1) ($p=0.02$).

At baseline, the FO and SO group's median (±SEM) total and direct bilirubin were comparable (10.2±1.1 vs. 7±0.5 and 6.1±0.8 vs. 4.1±0.4 mg/dL, $p = 0.2$ and 0.3, respectively). Baseline median aspartate aminotransferase (AST), alanine transferase (ALT), gamma-glutamyl transpeptidase (GGT) (data not shown), and triglyceride concentrations were similar between the two groups (Figure 1). The FO group's baseline median albumin was higher than the SO group's albumin (3.6±0.09 vs. 3.1±0.05 mg/dL, $p = 0.04$).

Compared to the SO group, the FO group's direct bilirubin decreased significantly at 8 (2.9 ± 0.4 vs. 6.6 ± 0.6 mg/dL, $p = 0.03$), 12 (1.3 ± 0.2 vs. 6.6 ± 0.5 mg/dL, $p < 0.0001$), 16 (0.7 ± 0.1 vs. 6.3 ± 0.6 mg/dL, $p <0.0001$), 20 (0.3 ± 0.04 vs. 7 ± 0.7 mg/dL, $p <0.0001$) and 24 weeks (0.2 ± 0.03 vs. 7.4 ± 1.1 mg/dL, $p < 0.0001$). Total bilirubin followed a similar trend.

Compared to the SO group, the FO group's AST significantly decreased specifically at weeks 12, 16, 20 and 24, and ALT decreased later beginning at week 16. At week 16 only, albumin was increased significantly in the FO group when compared to the SO group (3.6±0.1 vs. 3.0±0.1, p=0.02). At weeks 16 and 24, the FO group's triglycerides were markedly less than that of the SO group (Figure 1).

Primary Outcome

Median follow-up time for the primary endpoint for the time to resolution of cholestasis was 11.5 weeks (range 2.4 – 18 weeks) and 24 weeks (range 5.4 – 24 weeks) for the FO and SO group, respectively. 70% (7 subjects) of the FO group experienced resolution of cholestasis, while 5% (1 subject) of the SO group experienced resolution of cholestasis. The Kaplan-Meier method estimates (±SEM) that 75% (±16%) in the FO group will experience resolution of cholestasis by 17 weeks vs. 6% (±6%) in the SO group will experience resolution of cholestasis by 17 weeks ($p < 0.0001$) (Figure 2). After 11.5 weeks of FO monotherapy, 50% of subjects will biochemically reverse their IFALD. The hazard risk ratio for resolution of cholestasis was 21.5 (95% confidence interval (3.8, 404)) for the FO group while it was 0.05 (95% confidence interval (0.0002, 0.26)) for the SO group (p=0.0002).

Secondary and Tertiary Outcomes

There was no difference in death, transplant, percentage of patients on full enteral feeds, number of readmissions, number of inpatients days, and percentage of subjects with normalization of liver function tests (Table 3). 20% of FO subjects expired, while 10% of SO subjects expired (two subjects vs. one subject). 10% of subjects in the FO and SO group

received a transplant (one subject vs. two subjects). Both FO subjects who died were diagnosed with septic shock and medical care was withdrawn because continuation of care was considered futile. While one FO subject who died demonstrated hepatic deterioration, the other subject's cholestasis had resolved. The two deaths in the SO group were attributed to liver failure.

Safety

There was no difference in platelet concentrations and INR at baseline and weeks 8, 16, and 24 between the two groups (Figure 3). Mean Z-scores for weight and head circumference at baseline and the end of the study were comparable for the FO group. FO's mean length Z-score (±SEM) at the end of the study increased when compared to baseline (-0.9±0.3 vs. -1.8±0.4, p= 0.03) (Figure 4). None of the FO subjects developed an essential fatty acid deficiency and the range for mean triene:tetraene ratios during the study was 0.01-0.03.

Follow-up

Eight subjects including the one subject who received a transplant were alive at the end of FO intervention. When FO was discontinued, six of the eight subjects (75%) remained PN dependent and were transitioned to SO with a mean dose of 0.9 g/kg/d (range 0.6-1 g/kg/d). One subject was not available for long-term follow-up. Subjects were followed for a median 1.9 years (range one-two years). During follow-up, one subject remained free of cholestasis and experienced intestinal adaptation after 12 additional months of PN and SO. Another subject received a multi-visceral transplant and re-developed cholestasis prior to transplant.

At the end of follow-up, three of the seven children (43%) continued to receive PN with a mean SO dose of 1 g/kg/d (range 0.5-1.3 g/kg/d) and PN kcal/kg/d of 63.5 kcal/kg/d (range 44-

75 kcal/kg/d). These subjects continue to have normal direct bilirubin concentrations. Median serum ALT and AST concentrations at the end of the follow-up period decreased when compared to measurements at FO termination (32 and 33 U/L vs. 67 and 81 U/L, respectively).

Discussion

The substitution of 1 g/kg/d of FO monotherapy for 24 weeks in 10 IFALD subjects was associated with biochemical reversal of cholestasis and improved liver function at the end of FO intervention when compared to 20 historical controls who received SO at variable doses. After a median of 1.9 years of follow-up, all surviving subjects are free of cholestasis including those who received a transplant or continue to receive PN with SO.

In this study, the FO and SO group were at high risk for complications from IF based on their prematurity, low volumes of enteral nutrition, unfavorable gastrointestinal anatomy, and baseline serum direct bilirubin concentrations and liver function tests. Neonates born premature and with congenital or acquired gastrointestinal disorders are at high risk for IFALD because they are exposed to long PN courses due to intestinal dysfunction[3,5,6]. Predisposing factors for these populations include an immature biliary system, small for gestational age, necrotizing enterocolitis, abdominal surgeries, intestinal resections, and sepsis[3,4,22,23]. All of these factors center around insufficient enteral intake. As gestational age decreases and baseline serum bilirubin concentrations increase, resolution of IFALD with FO appears to be less likely[11].

Children with congenital gastrointestinal disorders are predisposed to develop IFALD[5,14]. As serum bilirubin concentrations climb, so does mortality[7,11]. In comparison to babies who are not jaundiced, mortality in neonates with congenital gastrointestinal disorders with a conjugated

bilirubin > 2 mg/d increases from 2 to 17%[7]. In fact, 38% of patients with a conjugated bilirubin ≥ 10 mg/dL will die or require a transplant referral[7]. Premature neonates and those with ultra-short gut, intestinal discontinuity, without distal ileum and/or an ileocecal valve, and repeated episodes of sepsis are at highest risk[1,6,5,23].

PN-specific contributors to IFALD include PN duration and an excess, deficiency, or imbalance in PN macro- or micronutrients[5,24]. In this study, glucose delivery rate and amino acid dose were comparable, and baseline median PN duration was 137 and 72 days in the FO and SO group, respectively. Reflecting changes in medical practice, the FO group was receiving less SO (g/kg/d) at baseline than historical controls[14-15]. After FO termination, subjects were prescribed PN with a low dose of SO (mean 0.9 g/kg/d). During follow-up, lipid-sparing was continued, and only one subject re-developed IFALD requiring a transplant. Prior observations have demonstrated that SO dose reduction can result in resolution of cholestasis or a decreased incidence of cholestasis in children and adults[15,24-26]. When compared to a historical cohort who received the standard SO dose, surgical neonates with cholestasis who received 1 g/kg of SO twice a week had an increased incidence of IFALD resolution (42 vs. 10%)[15].

Our results lend further support to case reports and recent studies[9-13,16-20]. Puder *et al* and Premkumar *et al* published the results of the largest cohorts to date[10,11]. One study compared 42 IFALD subjects to 49 historical controls while the other study prospectively followed 57 subjects[9,10]. While our study's sample size is much smaller than these studies, our FO cohort would be predicted to be at high risk for IFALD complications including death. When compared to the Premkumar *et al* and Puder *et al* study, our median age is 4.5 vs. 7.5 vs. 3 months and baseline direct bilirubin is 6.1 vs. 8.1 vs. 5.5 mg/dL[9,10]. The median small bowel length in our study for the FO group was 23 cm resulting in a median enteral tolerance of 23 kcal/kg/d at the

start of the study and a meager 9 kcal/kg/d increase by the end of the study. After 6 months of FO therapy and a median follow-up of 1.9 years, 75% and 43% of the cohort remained PN-dependent.

Seventy percent of the FO cohort achieved biochemical reversal of their cholestasis during FO treatment. Our data predicts that approximately 50% of children with IFALD will reach this goal by study week 11.5. This rate of reversal is comparable to the cohort described by Premkumar et al where 83% of surviving subjects achieved reversal with a median time of 35 days[11]. Approximately 45 and 60% of the populations described by Puder et al achieved this goal with a median time of 9-12 weeks[9,10]. In addition to gestational age and severity of IFALD, co-morbidities, enteral nutrition at baseline, the intestine's ability to adapt, and number of septic episodes and abdominal surgeries during treatment may serve as predictors of FO non-responders and slow responders[11,27].

A major confounding factor of all FO studies, including our study, has been the dose of the lipid product[9-14]. It remains unclear if dose alone, composition, or dose and composition are factors that aid in the biochemical resolution of IFALD. Interestingly, in the study by Diamond et al, four of the nine subjects experienced resolution of their cholestasis with a combination of FO and SO, each dosed at 1 g/kg/d, while five subjects were switched to FO monotherapy prior to reaching their endpoint[12]. Moreover, in this study, only one of the five PN-dependent subjects re-developed cholestasis while receiving SO after FO treatment. This brings into question if an emulsion composed entirely of FO at 1 g/kg/d is required for the treatment of IFALD. Randomized controlled trials have demonstrated that emulsions composed of various types of oils can prevent and reverse IFALD[28-34]. SMOF® (Fresenius Kabi, Bad Homberg, Germany), an emulsion comprised of some FO and SO, along with medium chain triglycerides in the form

coconut oil and monounsaturated fatty acids in the form of olive oil has been associated with improved liver function and can be dosed ≥ 1 g/kg/d[28-32]. A mixed emulsion may be more physiological, better satisfy fatty acid requirements, and preserve or improve growth and neurodevelopment in a nutritionally vulnerable population.

FO's mechanism is likely multifactorial. While SO is made up of the essential polyunsaturated fatty acids, linoleic and ∝-linolenic acid, it predominantly contains omega-6 fatty acids. FO, on the other hand, is mainly comprised of the omega-3 fatty acids, eicosapentaenoic and docosahexaenoic acid, which are absent in SO, but can be synthesized from ∝-linolenic acid. Linoleic acid is converted to arachidonic acid, which generates inflammatory prostaglandins, leukotrienes, and thromboxanes. ∝-linolenic acid is metabolized to eicosapentaenoic and docosahexaenoic acid, which are known for their anti-inflammatory properties and role in visual and cognitive development. By the "traditional" definition, essential fatty acids must be consumed in the diet and cannot be synthesized *de novo*. However, in the premature and SBS population, specifically those with cholestasis, provision and absorption of endogenous fatty acids may be limited[35,36,37]. At the same time, production of downstream products may be insufficient because of an increased demand or deficiencies of desaturase enzymes. Placental transfer of polyunsaturated fatty acids mainly occurs in the third trimester, thereby increasing the risk for deficiencies in preterm babies[37]. As a result, PN dependent neonates whose lipid source is SO have increased omega-6:omega-3 fatty acid ratios, thereby promoting inflammation and hepatic injury[38].

Polyunsaturated fatty acids promote lipid peroxidation. In order to minimize oxidative stress, PN is protected from light and vitamin E is added. The amount of vitamin E in SO may be inadequate and is in the form, ϒ-tocopherol, which is not as effective in preventing free

radical generation when compared to α-tocopherol, which FO contains and is provided in a much larger concentration. Moreover, preterm and SBS children are deficient in vitamin E because they lack exogenous sources and have decreased adiposity[39].

While SO contains a high amount of phytosterols, FO lacks phytosterols. Phytosterols reduce biliary flow by antagonizing the farnesoid X receptor, a bile acid nuclear receptor that regulates the multi-drug resistant genes 1 and 2 (*mdr* 1 and 2). These genes encode P-glycoproteins that are responsible for promoting bile acid secretion [40-43]. Animal studies have demonstrated that FO increases biliary flow, while SO impairs flow[44]. As a result, it is not surprising that bilirubin profiles improve prior to other hepatocellular indices as described in this study and other studies[9-12].

There are concerns that FO may cause an essential fatty acid deficiency. This study supports previous findings, which suggests that FO contains sufficient omega-6 fatty acids to prevent the development of an essential fatty acid deficiency as measured by a triene-tetraene ratio. While we used a liberal cut-off, a triene:tetraene ratio ≥ 0.5, the mean triene:tetraene ratio was between 0.01-0.02[9]. Triene:tetraene ratios, however, should be used with caution for detecting omega-6 and -3 fatty acid deficiencies. When absolute concentrations of specific polyunsaturated fatty acids are measured, there could be fatty acid deficiencies and toxicities with FO and SO. In order to develop an essential fatty acid by means of a triene:tetraene ratio, there must be an increase in mead acid, and decrease in arachidonic acid. Because desaturase enzymes have a higher affinity for omega-3 fatty acids, mead acid production many not increase with FO, which has adequate provisions of ∝-linolenic acid and high concentrations of eicosapentaenoic and docosahexaenoic acid.

One concern with lipid minimization is the decreased caloric intake from fat and possible adverse effects on growth and neurodevelopment. Premature and SBS children have high rates of growth failure and developmental delays[3,45-48]. This study, along with other SO lipid minimization and FO studies, have not reported a change in short-term growth[9,15]. While there was improvement in the Z-score for length, overall growth in our study population was suboptimal. Long-term studies are lacking and needed. In order to make-up for decreased fat calories, clinicians may increase glucose delivery rates, which may promote lipid deposition in the liver and peripheral tissues, IFALD, hyperinsulinemia, and carbon dioxide retention[24].

Lastly, because FO is antithrombotic, there is a theoretical concern that FO can increase the risk of bleeding in a population whose baseline risk is already increased. Similar to previous studies, there was no increase in bleeding, and INR and platelet counts remained unchanged or improved[9-11].

While FO appears to be associated with a biochemical reversal of IFALD, it is unclear if transplant-free survival is altered. Liver and intestine transplants are life-saving and mortality rates continue to improve with modern medicine and surgery. In our study, transplant represented an important modality for two subjects to achieve a PN-free state without cholestasis. One subject achieved this goal during FO intervention, and the other subject later satisfied this goal during follow-up.

Data on FO's effect on liver histology is lacking, and while cholestasis may reverse, it is unclear if portal hypertension or fibrosis can be reversed[49]. In the setting of IFALD, animal and human studies have demonstrated that biochemical parameters may be imprecise[49-50]. Moreover, while some studies have associated parenteral omega-3 fatty acids with hepatic collagen deposition, others have demonstrated improved histology on liver biopsy[51-52]. Despite 24 weeks

of FO and biochemical IFALD resolution, one of our subjects continued to demonstrate advanced fibrosis on liver biopsy requiring a transplant. Some patients regardless of a positive FO response may ultimately require an intestinal transplant with or without a liver because of prolonged IF, repeated episodes of sepsis and catheter replacements, severe malnutrition, or unchanged or progressive liver fibrosis. Others may opt for a transplant for quality of life issues and the desire to be PN-free.

Limitations of this analysis include the use of historical controls, missing data in the historical cohort, interpolation for laboratory data. In attempt to overcome problems associated with historical controls, the sample size for the control cohort was doubled. While subjects were not matched on specific confounding variables, they were relatively similar with regards to most baseline characteristics except for enteral nutrition, entry SO dose, lipid dose at the end of the study, and gastrointestinal surgeries. These factors, except for gastrointestinal surgeries, would positively favor the primary outcome, and cannot be ignored. Moreover, in the past 10 years, a multi-disciplinary approach to IF has been utilized at our institution, which has been associated with an improved prognosis for IF patients[54,55].

In addition, considering the number of comparisons made between the two cohorts, there is an increased risk of a type I error. A priori, we opted not to adjust for multiple comparisons. Our goal was to clearly define the cohorts' risk factors for IFALD and associated complications.

While the study remains open and data continues to be collected, follow-up post-24 weeks of FO will be essential to determine if patients who continue on PN and restart SO remain free of cholestasis and if transplant-free survival is decreased.

Conclusion

The goal for IF patients is to safely bridge them to enteral autonomy or transplant if needed. A contemporary, multi-disciplinary approach to IFALD, with attention to nutrition, infection control, gastrointestinal surgeries, and the psychosocial dynamics of the family unit, cannot be underestimated. While FO monotherapy may play an important role in the treatment of this disease, attention must still be given to IFALD prevention and other factors that drive the development and progression of this disease. Long-term follow-up on growth and neurodevelopment are lacking. This report provides support for the safety and efficacy of FO for a finite period in the pediatric IFALD population.

Variable	FO (n=10)	SO (n=20)	p value
Age at Start of Study, days	136 (33-334)	76 (27-866)	0.29
Age at End of Study, days	259 (81-456)	241 (96-1035)	0.66
Sex – Male % (n)	30 (3)	65 (13)	0.12
Gestational Age (weeks)	34 (25-39)	34 (25-39)	0.89
Birth Weight (kg)	2.3 (0.9-3.1)[†]	2.3 (0.9-3.3)[‡]	0.85
Primary Gastrointestinal Diagnosis			
-gastroschisis % (n)	40 (4)	40 (8)	0.37
-intestinal atresia % (n)	20 (2)	20 (4)	
-necrotizing enterocolitis % (n)	20 (2)	25 (5)	
-volvulus % (n)	0 (0)	15 (3)	
-malabsorption syndrome % (n)	10 (1)	0 (0)	
-MMIHS % (n)	10 (1)	0 (0)	
No. of Prior Gastrointestinal Surgeries	1 (0-5)	2 (1-8)	0.65
Intestinal Failure - Yes % (n)	80 (8)	55 (11)	0.25
Small Bowel Length (cm)	23(0-51)[§]	27(0-55)[¶]	0.78
Presence of an Ileocecal Valve – Yes % (n)	60 (6)	55 (11)	0.13
100% of Colon Present – Yes % (n)	70 (7)	55 (11)	0.69
Small Bowel Connected to Colon – Yes % (n)	70 (7)	55 (11)	0.69
Ursodeoxycholic acid – Yes % (n)	30 (3)	25 (5)	0.99

Table 1. Characteristics of subjects in the fish oil (FO) and soybean oil (SO) group at baseline. Results are depicted either as a median with the corresponding range or percentage (n). [†]Data was only available for 8, [‡]19, [§]7, and [¶]13 subjects. MMIHS, Megacystis Microcolon Intestinal Hypoperistalsis Syndrome.

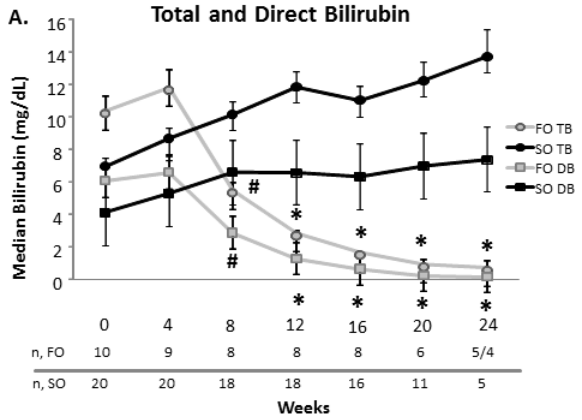
Variable	FO (n=10)	SO (n=20)	p-value
At the Beginning of the Study			
PN duration (days)	137 (33-334)	72 (27-710)	0.09
GDR (g/kg/min)*	16.0±4.4	15.2±3.8[†]	0.96
Amino Acids (g/kg/d)*	2.8±0.8	2.7±0.7[‡]	0.94
Soybean Oil (g/kg/d)*	1.5±0.9	2.6±0.7[§]	0.006
PN kcal/kg/d*	98±17	95±20[§]	0.83
Enteral kcal/kg/d	23 (0-43)	4 (0-39)[§]	0.05
Solid Foods – Yes % (n)	0 (0)	5 (1)	0.99
At the End of the Study			
GDR (g/kg/min)*	17.0±6	15.0±4[¶]	0.66
Amino Acids (g/kg/d)*	1.9±0.5	1.8±0.8[§§]	0.68
Fish or Soybean Oil (g/kg/d)*	0.9±0.3	1.7±1.1[††]	0.02
PN kcal/kg/d*	74±20	80±24[§§]	0.85
Enteral kcal/kg/d	32 (0-109)	0.6 (0-70)[*]	0.21
Solid Foods – Yes % (n)	40 (4)	25(5)	0.43

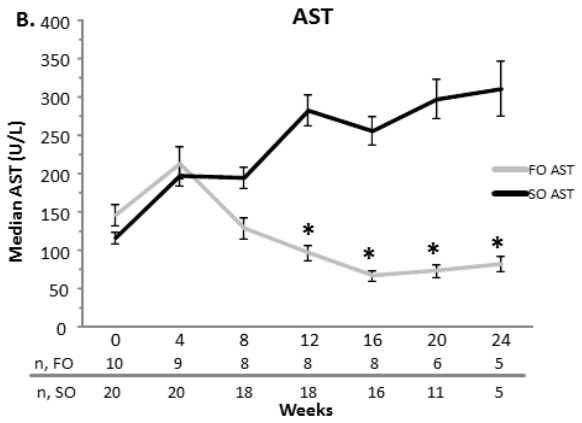
Table 2. Nutritional characteristics of the fish oil (FO) and soybean oil (SO) group. Results are depicted either as a median with the corresponding range, mean ± standard deviation (*), or percentage (n). PN, parenteral nutrition. [†]Data was only available for 15, [‡]17, [§]18, [¶]13, [§§]14, [††]12, and *16 subjects.

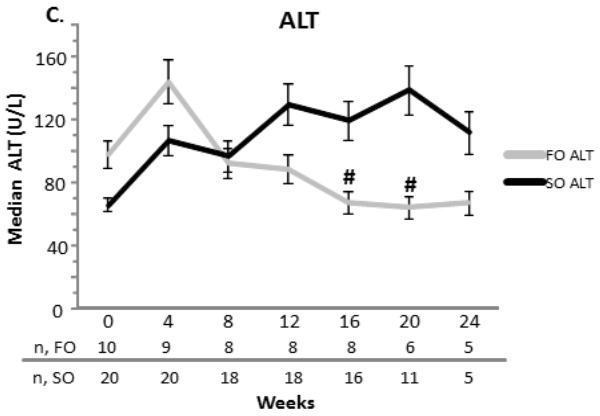
Outcome	FO (n=10)	SO (n=20)	p-value
Death – Yes % (n)	20 (2)	10 (2)	0.58
Transplant – Yes % (n)	10 (1)	10 (2)	0.99
Full Enteral Feeds – Yes % (n)	10 (1)	15 (3)	0.99
Number of Readmits	2 (0-4)	1 (0-4)	0.38
Number of Inpatient Days	39 (17-59)	87 (16-170)	0.08

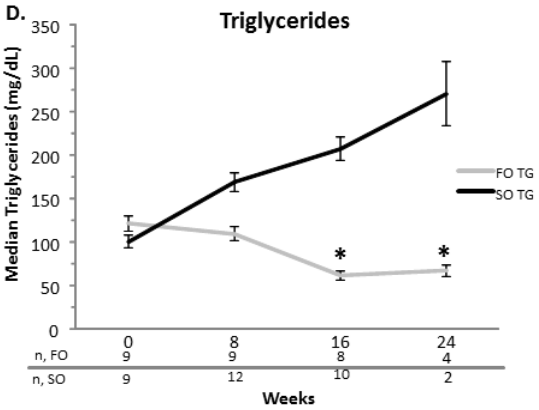
Table 3. Secondary and tertiary outcomes for the fish oil (FO) and soybean oil (SO) group.

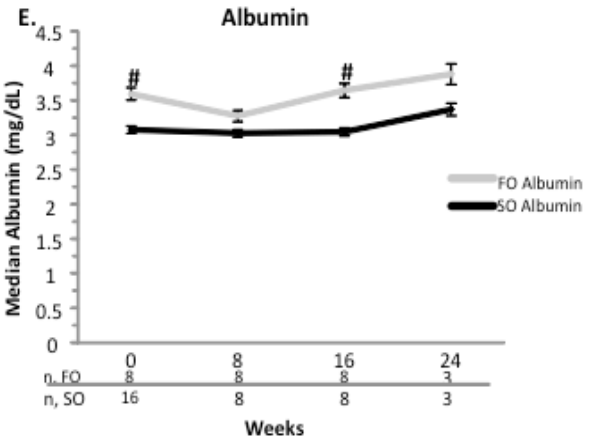
Results are either as medians with the corresponding range, or a percentage (n).


A.
Total and Direct Bilirubin
Median Bilirubin (mg/dL)
FO TB
SO TB
FO DB
SO DB
n, FO
n, SO
Weeks


B.
AST
Median AST (U/L)
FO AST
SO AST
n, FO
n, SO
Weeks


C.
ALT
Median ALT (U/L)
FO ALT
SO ALT
n, FO
n, SO
Weeks





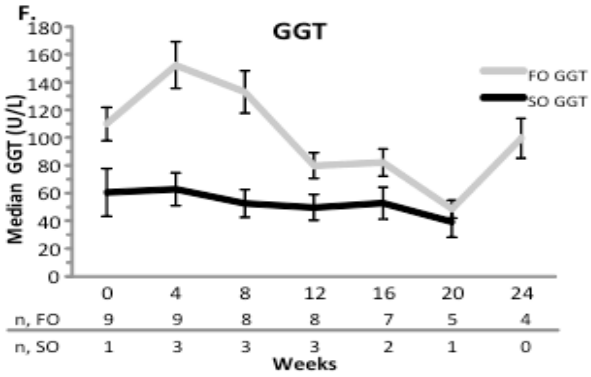


Figure 1A-F. Median serum total bilirubin (TB) and direct bilirubin (DB), aspartate aminotransferase (AST), alanine transferase (ALT), triglyceride, albumin and gamma-glutamyl transferase concentrations with the standard error of the mean for the fish oil (FO) and soybean

oil (SO) group. Sample sizes (n) are depicted for FO and SO by week beneath the graph. * $p < 0.001$. # $p < 0.05$.

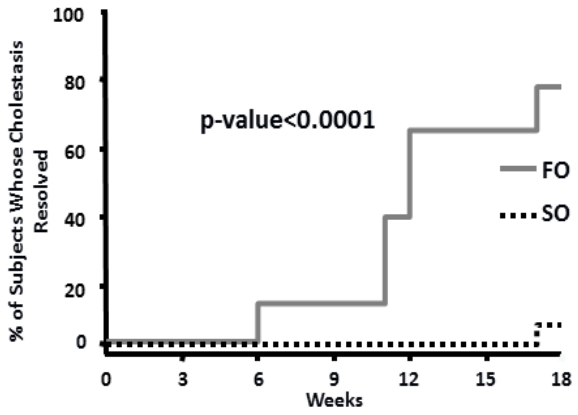
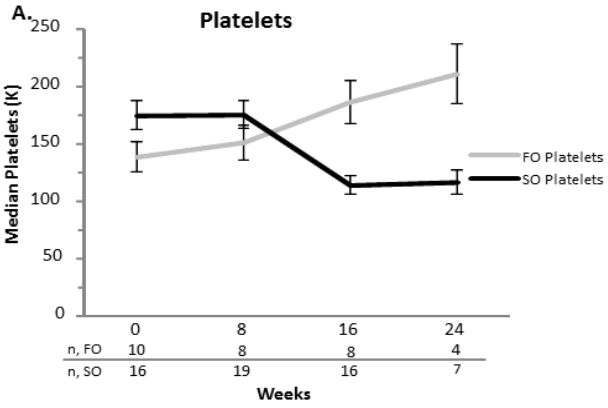


Figure 2. Kaplan-Meier curves for resolution of cholestasis.



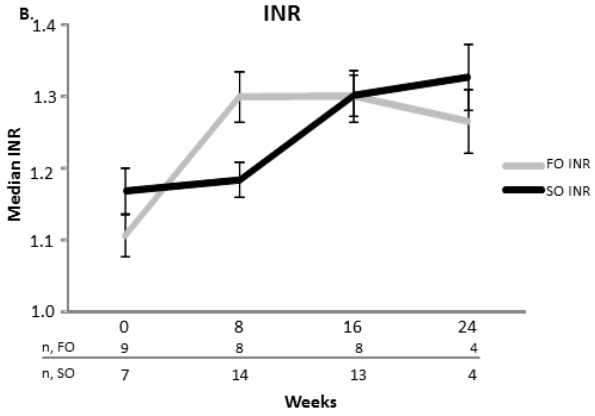


Figure 3A and B. Median platelets and INR values with the standard error of the mean for the fish oil (FO) and soybean oil (SO) group. Sample sizes (n) are depicted for FO and SO by week beneath the graph.

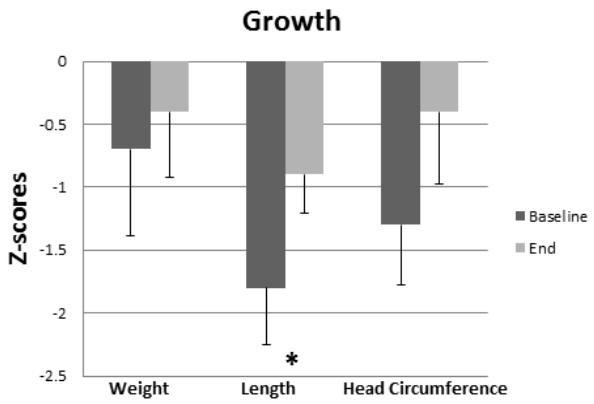


Figure 4. Median Z-scores for weight, length, and head circumference with the corresponding standard error of the mean for the fish oil (FO) group at baseline and the end of the study. *p < 0.05.

Appendix

Limitation of this study, as previously mentioned, include: 1. A small sample size, 2. use of historical controls, 3. interpolation. The subjects in this study had advanced IFALD, which is considered a rare pediatric disease. As a result, the number of subjects available for recruitment at a single institution is limited. This study remains open to enrollment. As of October 1, 2013, a total of 28 subjects have been enrolled in the study. Collaborations with outside institutions have been attempted, but have been unsuccessful for various reasons.

To date, there has yet to be a masked randomized controlled trial comparing FO to SO in children with advanced IFALD. Considering this report, along with previously published data, such a trial would most likely be considered unethical and not feasible due to the demand for FO and high mortality associated with advanced IFALD[9-12]. At time this study was designed, there was a lack of equipoise and unwillingness among investigators, clinicians, and parents/legal guardians to randomize a potentially eligible subject with advanced IFALD to either FO or SO even with a cross-over design. As a result, it was decided, a priori, to use historical controls as a comparison group. In order to overcome some of the limitations associated with historical controls, the control sample size was doubled and controls satisfied applicable inclusion and exclusion criteria. Matching, based off of gender, gestational age, and/or primary gastrointestinal diagnosis, was attempted but not possible due to the limited number of historical controls.

The historical controls were similar to controls except for two variables: lipid dose and enteral feeds. At the time of enrollment, the FO group was receiving less SO and more enteral feeds when compared to SO—two factors that would positively favor the primary outcome. Another pitfall associated with historical controls is that prevention strategies and treatments for

IF and IFALD have drastically changed since the initiation of this study, all of which are associated with improved outcomes[54].

All laboratories performed to assess the efficacy and safety of FO were performed as part of standard of care. It would not have been feasible to mandate laboratory visits for solely for research purposes at specific time intervals (ie exactly at week 4, 8, etc) for a variety of reasons. In general, children with IFALD undergo laboratory assessment for the progression or regression of IFALD on a bi-weekly to monthly basis depending on illness severity. Moreover, this population is at high risk for contamination of their central venous catheters and anemia. Accessing a subject's catheter for research purposes would increase infection risk. In addition, additional blood draws outside of standard, routine medical care, would potentially worsen a subject's anemia, putting him/her at risk for needing a red blood cell transfusion or iron infusion. Transfusion exposure is generally limited in this population because of the possible need for a multi-visceral transplant. Moreover, when red blood cells lyse, direct bilirubin is released, worsening a subject's cholestasis. As a result, labs evaluating efficacy and safety were limited to routine, standard care, which was dictated by the medical team. Moreover, routine laboratory assessment included all of the necessary assessments for this study.

Laboratory values were collected at baseline and then by weekly intervals for 24 weeks for each subject. If a subject had multiple values available for a specific time interval, the first value was taken. An alternative method would be to calculate the average if multiple value were available for a specific time interval. For the analysis, for reasons of simplicity and clinical interpretation, baseline and study weeks 4, 8, 12, 16, 20 and 24 were analyzed. As a result, there was a risk for missing data at specific time points. For example, a subject may have had a laboratory assessment for a specific biomarker at baseline and weeks 3, 5, 7, 10, 12, 16, 18, and

24. Data would then be missing at study weeks 4, 8, and 20. In order to account for this data, interpolation was used. The slope was calculated for the biomarker between two time points (ie study weeks 3 and 5), and this value was added to study week 3 in order to interpolate for week 4. In general, serum direct bilirubin and liver function tests follow an upward or downward trend and do not change drastically from one day to the next in the absence of culture proven sepsis. Other options to account for missing data would include: last observation carried forward, taking the mean of a specific time interval and using this mean for the missing time interval (ie study weeks 1, 2, and 3 for study week 4), or bootstrapping. Overall, the method used to account for missing data, appears to be the most biologically plausible and appropriate for this study. In order to determine if this method was accurate, the analysis for serum direct bilirubin was performed without interpolation. In general, when these results are compared to the results with interpolation (Figure 1A), they appear similar (Appendix, Figure 1). As anticipated, there were less data points available for analysis without interpolation (Appendix, Table 1).

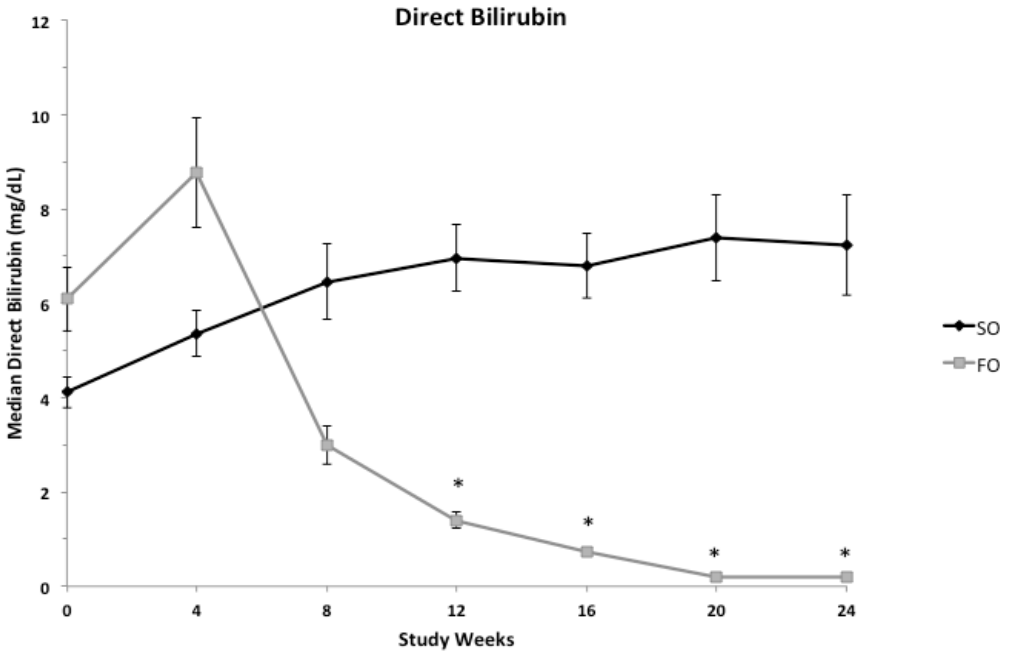
Another reason for missing data is death or enteral autonomy (discontinuation of PN). Approximately 10-20% of the population expired during the study. In cases of enteral autonomy, laboratory assessments may not be performed as frequently while receiving PN because direct hyperbilirubinemia would be anticipated to resolve over time. Ten to 15% of the study population achieved enteral autonomy. In both these situations when laboratory values were not available beyond a certain time point, extrapolation was not used.

In conclusion, this report provides support that FO biochemically reverses IFALD. In order to determine if long-term transplant-free survival, long-term follow-up (4.5 years post-intervention) for this cohort is in progress. Moreover, there is a need to determine the cost-

effectiveness of FO considering the cost of FO and transplant. FO can cost approximately 50 to several hundred US dollars per day, while SO costs only a couple of dollars[56]. A transplant costs approximately 1.5-1.9 million dollars in the first year. Lastly, there is a paucity of human data attempting to understand how FO biochemically reverses IFALD. In order to better understand FO's mechanism, a biorepository of human blood samples has been established to investigate the link between phytosterols, erythrocyte polyunsaturated fatty acids, inflammation, and bile acid transport.

	Sample Size, FO		Sample Size, SO	
	With Interpolation	Without Interpolation	With Interpolation	Without Interpolation
Baseline	10	10	20	20
Week 4	9	6	20	13
Week 8	8	6	18	6
Week 12	8	7	18	10
Week 16	8	7	16	10
Week 20	6	2	11	6
Week 24	5	5	5	4

Appendix Table 1. Sample sizes for the fish oil (FO) and soybean oil (SO) group for Figure 1A and Appendix Figure 1.



Appendix Figure 1. Median serum direct bilirubin (±SEM) over time for the fish oil (FO) and soybean oil (SO) group.

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