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Intestinal Microbiota, Lipids and the Pathogenesis of Intestinal Failure-Associated Liver Disease

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Intestinal failure (IF) is considered the end result of gastrointestinal disorders in which functional intestinal mass is inadequate to promote adequate growth, hydration, and electrolyte balance.¹ Today, a substantial number of infants and children with IF, caused by short bowel syndrome (SBS), necrotizing enterocolitis (NEC), gastroschisis, intestinal atresias, motility disorders and genetic enterocyte transport defects, depend upon long term parenteral nutrition (PN) for survival and the promotion of normal growth and development.

PN-associated cholestasis (PNAC), referring to the development of conjugated hyperbilirubinemia and impaired bile flow, implies that PN itself is the predominant factor responsible for liver injury.² However, recent understanding of the various factors causing liver injury in patients receiving PN has led to the broader descriptive term of IF-associated liver disease (IFALD),³ replacing the previous term PN-associated liver disease (PNALD). IFALD is defined as cholestasis and progressive biliary cirrhosis in the setting of PN in a patient with underlying intestinal disease, resection or dysfunction, if other specific causes of liver injury have been excluded.⁴ It is in these patients that the most severe, progressive, and sometimes fatal phenotypes of PNAC develop, hence IFALD has become the leading indication for intestinal and multi-visceral transplantation in children. In addition to

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progressive biliary cirrhosis and portal hypertension, hepatocellular carcinoma has been reported as a rare complication in children with liver cirrhosis secondary to long-term PN.⁵

Advanced IFALD is one of the most significant risk factors associated with mortality in infants on long-term PN.^{6, 7} There are several recent reviews on the pathogenic mechanisms predisposing to IFALD and various strategies to prevent or reverse established IFALD.^{2, 4, 8-10} Prematurity and small-for-gestational age, length of bowel remnant in those who had bowel resection, lack of enteral feeding, duration of PN, recurrent sepsis, protein under nutrition and excess of intravenous carbohydrate load have been considered as important factors for the development IFALD.³ Mechanisms receiving the most recent attention include the role of omega(ω)-6 polyunsaturated fatty acids (PUFA) and plant sterols found in the intravenous lipid emulsions (ILEs),⁸ bacterial overgrowth and microbiome dysbiosis of the small intestine,¹¹ the role of bacteremia and fungemia related to microbial translocation across the intestinal barrier and central line associated blood stream infections (CLABSI), and increased intestinal permeability leading to absorption of bacterial products from injured intestine inducing innate immune responses in the liver.^{12, 13} The purpose of the present review is to discuss the latest understanding of the role of various ILEs and intestinal microbial dysbiosis.^{14, 15}

Prevalence and Epidemiology of IFALD

The prevalence of IFALD varies with age and the underlying cause of IF. Criteria used to define IFALD generally include the presence of serum direct or conjugated bilirubin ≥ 2 mg/dL in an infant with duration of PN ≥ 14 days and no other cause for the cholestasis.^{16, 17} Others have included raised serum liver enzymes^{18, 19} and the presence or absence of end-stage liver disease^{20, 21} as other categories of IFALD. A recent systematic review of 23 studies reported the overall incidence of IFALD as 29.9%, with the incidence of 25.5% among extremely low birth weight and very LBW preterm neonates receiving PN, and incidence of 30.6% in term infants and children without IF.² The incidence was highest (49.8%) in pediatric patients with IF receiving PN.² The authors noted that there has been no obvious changes in the prevalence of IFALD over the last four decades (excluding very recent experience), although there is a lack of high-quality, prospective studies.² In support is a population-based, retrospective survey in infants with gestational age <30 weeks in Stockholm county, Sweden which reported the incidence of PNALD to be 14.8% in those born between 2006 to 2008, and 12.7% for those delivered between 2010 to 2011 ($p=0.52$).²² Thus the incidence of IFALD has been stable over the last few decades despite various prior efforts to prevent IFALD.

Pathogenesis of IFALD

Many factors, including host factors and nutrient factors, have been implicated in the development of IFALD. Nutrient factors include components of intravenous lipid emulsions (ILEs) and non-lipid nutritional considerations (Table I).⁴ Host factors, including prematurity, small for gestational age, abdominal surgery, and episodes of sepsis, have been reviewed elsewhere.^{4, 8, 9} However, none of the listed risk factors has been carefully studied in a prospective, controlled trial.^{4, 8, 9} In addition, no single risk factor has

been implicated as causative of liver injury in all patients on prolonged PN, thus, a multifactorial etiology seems likely.

Recent efforts to elucidate the mechanisms responsible for IFALD have focused on the role of soybean oil (SO)- and other plant oil-based lipid emulsions, the intestinal microbiome and the integrity and permeability of the intestinal wall, as well as the role of activation of the hepatic innate immune system, particularly Kupffer cells (Figure).^{12, 13} A better understanding of the pathological mechanisms of IFALD is required to identify potential therapeutic targets for prevention and treatment, which will require the use of appropriate animal models followed by translation into well designed clinical trials in affected children.

Intravenous lipid emulsions

Interest has been focused in recent years on the potential role of ILE in the pathogenesis of IFALD following reports of reversal of IFALD when ILE was switched from intravenous SO lipid emulsion to a fish oil (FO)-based emulsion.^{10, 23} Several mechanisms have been proposed.²³⁻²⁵ First is the potential role of SO- or plant-based lipid emulsions which are commonly used in the United States. SO-ILE are composed primarily of ω -6 PUFAs, including linoleic acid which is the precursor of arachidonic acid, the structural backbone of proinflammatory eicosanoids.²⁶ In contrast, the ω -3 PUFAs found in FO products but not in plant oils, such as α -linolenic acid, are converted into anti-inflammatory derivatives.²⁷ FO-ILE, approved for use in Europe but not in the United States, have a high ratio of ω -3 to ω -6 PUFA. It has been hypothesized that the potential benefit of FO-ILEs is due to downstream anti-inflammatory properties of ω -3 PUFA compared with the potential pro-inflammatory ω -6 PUFA forms. Although this is an attractive hypothesis, there are few data in children affected by IFALD to support it. Secondly, phytosterols, plant-based naturally occurring sterols found in SO-ILEs, have been shown to interrupt hepatocyte farnesoid X receptor (FXR) signaling and expression of downstream bile acid transporters, thus decreasing bile flow in animal studies.²⁸ Third, the cholestatic effect of ILE has been postulated to be related to the dose of lipid (and its constituents) itself. The dose of FO-ILE commonly administered in PN is only 30-40% that of SO-ILE. Finally, SO-ILE contain relatively low amounts of the antioxidant, alpha tocopherol, relative to the amount of PUFAs, potentially putting the infant at risk for oxidative stress and lipid peroxidation which have been demonstrated in the cholestatic liver.^{29, 30} FO-ILE contain far greater amounts of alpha tocopherol than SO-ILE.

To more clearly understand the role of lipid emulsions in IFALD, several animal models have been developed in recent years. By using an IFALD mouse model, which combines PN infusion with intestinal injury (which was accompanied by increased intestinal permeability) induced by dextran sodium sulfate (DSS), PN/DSS mice, we have demonstrated that SO-based PN solution caused hepatic injury, cholestasis and hepatic macrophage activation.¹² However neither intestinal injury nor PN alone led to these hepatic perturbations. In contrast, isocaloric PN solutions which were either FO-ILE based or were devoid of all lipids were not associated with these hepatic abnormalities.¹³ Importantly, adding stigmaterol, the putative cholestatic phytosterol, to the FO-ILE based PN solution recapitulated the hepatocyte injury, cholestasis and macrophage activation,¹³ which was

similar to that observed in the SO-PN group. Furthermore, *in vitro* studies demonstrated that stigmasterol itself, in addition to absorbed intestinal lipopolysaccharides (LPS), had the capacity to activate macrophages into a pro-inflammatory state. In addition, treatment of the PN/DSS mice with oral antibiotics or use of Toll Like Receptor (TLR) 4 mutant mice that fail to respond to LPS, prevented liver injury, cholestasis and macrophage activation, confirming the role of bacterial products absorbed through injured intestine.¹² On a molecular basis, the expression of the canalicular exporter for stigmasterol, Abcg5/g8, was downregulated in the mouse model, resulting in hepatic accumulation of stigmasterol.¹³ This was associated with inhibition of expression of the nuclear receptor Fxr, which in turn reduced the hepatocyte expression of Fxr-dependent genes, including the bile salt export pump (Bsep) responsible for driving bile flow.¹³ Thus, our studies in this novel mouse model provided experimental evidence that plant sterols in ILEs combined with increased absorption of LPS from the hyperpermeable injured intestine may play major roles in the pathogenesis of IFALD (Figure).

There are also human data supporting the role of plant sterols in IFALD. Clinical studies in neonates and children with IFALD receiving PN demonstrated that serum stigmasterol (and other phytosterols) was markedly elevated as compared with infants and children on PN who did not develop IFALD.^{31, 32} It is difficult in these clinical studies to determine if the elevated phytosterols were the cause or result of the cholestasis.

Other animal studies have not supported the contribution of phytosterols to PNALD.³³ Vlaardingerbroek et al, using premature piglets fed exclusively with PN, compared the effect of different ILEs (SO-ILE, FO-ILE and a mixture of SO, medium chain triglycerides, olive oil and FO-based ILE [SMOF, Fresenius-Kabi]) in inducing hepatic injury and cholestasis.³³ Although the plasma and hepatic phytosterol concentrations were highest in piglets fed with SO-based PN solution, there was only weak correlation between these phytosterol concentrations with the severity of hepatic injury and cholestasis. Moreover, the SMOF-based ILE, although containing phytosterols, was equally protective against PNALD as compared with FO-based ILE, which was devoid of phytosterols.³³ The authors suggested that other lipid components present in SMOF-based and FO-based ILE, such as vitamin E or ω -3 PUFA, may be protective against PNALD.^{33, 34} In subsequent experiments, the addition of α -tocopherol to SO-ILE prevented the increase in serum and hepatic markers of PNALD, as compared with PN solution containing SO-ILE alone.³⁴ Furthermore, addition of phytosterols to 100% FO-ILE did not reproduce evidence of PNALD.³⁴ It should be pointed out that there are important differences in this piglet model of PNALD and the mouse model of IFALD described above. In the piglet model, there was no intestinal injury or inflammation and by inference no major increase in intestinal permeability, components that are believed to be essential in the development of IFALD in infants.³³ In addition, all piglets received broad spectrum antibiotics throughout the course of the PN administration (which would alter intestinal microbiota and reduce absorbed intestinal LPS), the levels of circulating phytosterols achieved were only modestly elevated in the piglets supplemented with phytosterols, and macrophage activation was not investigated.³³ Thus, the applicability of this piglet model to IFALD, in which intestinal inflammation and altered permeability are intrinsic factors, is not clear. It is intriguing that addition of large amounts of α -tocopherol to

the PN solutions in the piglet provided protection against PNALD, inasmuch as cholestasis and toxic bile acid accumulation in the hepatocyte generate oxidative stress through mitochondrial pathways,³⁰ and α -tocopherol and other antioxidants have been shown to reduced bile acid-induced hepatic injury.²⁹

Intestinal Microbiome and Intestinal Permeability

It is well established in animal models that prolonged PN leads to a shift in the intestinal microbiota, favoring Bacteroidetes, such as *Clostridium difficile*, as compared with Firmicutes,^{35, 36} which was associated with activation of intestinal Paneth cells.^{36, 37} Moreover, the presence of bacterial overgrowth of the small intestine was demonstrated in a large series of infants and children with IFALD, and it was observed that this bacterial overgrowth was related to the severity of small intestine inflammation,³⁸ presumably impairing intestinal barrier function and promoting absorption of small molecules across the small bowel mucosa.³⁹ This relationship between bacterial overgrowth and inflammatory pathways was elegantly demonstrated in a PN-dependent mouse model in which a shift in the intestinal microbiota was associated with intestinal epithelial cell apoptosis, increased expression of mucosal proinflammatory cytokines and a loss of intestinal barrier function.³⁹

Altered intestinal microbial communities were demonstrated in the described mouse model combining intestinal injury with total PN.¹² A specific intestinal microbiome, identified by a metagenomic evaluation of colonic bacteria, was limited to those mice with PNAC. Specifically, overgrowth of *Erysipelotrichaceae* and S24-7 taxa of Bacteroidetes was observed in DSS/PN mice that developed cholestasis and liver injury,⁴⁰ which was associated with increased intestinal permeability and absorption of LPS.¹² In addition, this authors showed that treatment with a combination of four oral antibiotics, resulting in significant suppression of intestinal microbiota, prevented liver injury, cholestasis and activation of hepatic macrophages.¹²

Based on these observations, a restoration of intestinal integrity and barrier function would in theory prevent the occurrence of IFALD in patients receiving prolonged PN. Thus, the potential use of antibiotics, prebiotics or probiotics to modify the microbiome is an attractive strategy to prevent IFALD. An interesting study in preterm newborn pigs receiving PN showed that feeding with amniotic fluid before initiation of milk feeding led to a reduction in intestinal permeability and a change in intestinal microbiota, presumably related to growth or immunologic factors transferred in the amniotic fluid.⁴¹ However, whether such novel therapeutic approaches or other manipulations of the intestinal flora would be effective in preventing or treating human IFALD remains to be determined. Currently, there are no clinical data supporting the use of probiotics to prevent or treat IFALD in children or adults, and concerns remain about bacteremia from such agents in patients with central venous catheters.⁴² Surgical approaches to reduce bowel dilation, lengthen the intestine and improve motility appear to be the best current approaches for preventing bacteria overgrowth of the small intestine.⁴²

Bacteremia and innate immune response

Another important factor in the pathogenesis of IFALD resulting from increased intestinal permeability is the promotion of bacterial translocation, bacteremia or simply absorption of bacterial cell wall products capable of activating the innate immune system.¹⁵ Indeed the number and earlier timing of episodes of bacterial or fungal sepsis has been consistently associated with the development of IFALD.⁸ In the mouse IFALD model, interruption of TLR4 signaling was associated with prevention of liver injury and hepatic macrophage activation,¹² suggesting that LPS or other TLR4 agonists absorbed from the intestine were involved in IFALD pathogenesis. Meticulous care of central venous catheters including ethanol locks has been recommended to prevent CLABSI to potentially reduce the risk for innate immune cell activation and IFALD.^{4,8}

Preventive and therapeutic approaches

Various strategies have been proposed to prevent IFALD, and in patients who have established IFALD, to reverse the cholestasis and hepatic fibrosis (Table II). We will focus on those related to ILE and the intestinal microbiota.

Reduced soybean intravenous lipid dose

The prevailing view linking the development of cholestasis and the use of SO- and other plant oil-based ILEs stemmed from several clinical observations showing a temporal relationship between development of cholestasis and an increased dosage of SO-based ILE.⁴³ This was supported by a more recent study which showed that the development of advanced IFALD was related to the number of days of exposure to 2.5 mg/kg per day of parenteral ILE.⁴⁴ Subsequently, a prospective study comparing a cohort of surgical neonates receiving SO-ILE at a reduced dose (1 g/kg) with a historical standard dose cohort (2-3 g/kg) showed a reduction in the incidence of IFALD (22% vs. 43%, $P < 0.002$).²⁶ However, another retrospective review failed to demonstrate a benefit of reduced SO-ILE dose (52% IFALD in 1 g/kg group vs. 44% in 2-3 g/kg group; $P = 0.61$).⁴⁵ More recently, a prospective randomized clinical trial in preterm neonates 29 weeks gestational age compared treatment with a low dose (1 g/kg per day) vs. a standard dose (3 g/kg) of SO-(or SO+safflower oil) ILE with the primary outcome of cholestasis at 28 days of life, defined as a serum direct bilirubin $>15\%$ of total bilirubin.⁴⁶ The study found no differences in the percent of infants who went on to develop cholestasis (69% vs. 63%, low vs. standard dose, $p = 0.45$).⁴⁶ However it should be pointed out that the cohorts were not enriched for those having surgical intestinal disorders (10% vs. 8% had abdominal surgery, low vs. standard dose), and most infants ($>70\%$) were weaned off of PN by the time of the primary endpoint in both groups. Growth did not differ between groups. Although this well conducted study demonstrates no benefit of low dose SO-ILE in reducing the incidence of PNAC in preterm infants, IFALD *per se* was not studied. The concerns of a chronic reduction in the SO-ILE dose in the preterm infant are the development of essential fatty acid deficiency (reported in one study)²⁶ and possible effects on normal brain growth and development. Nevertheless, there has been a gradual change in clinical practice with a reduction in amount of SO ILE administered to infants showing early signs of IFALD.

Alternative sources of intravenous lipid

Another recent approach has been the replacement of traditional SO-ILEs with alternative lipid sources. This follows initial retrospective reports of reversal of cholestasis with discontinuation of SO-ILE (2-3 g/kg per day) and initiation of FO-ILE (1 g/kg per day).^{44, 47, 48} In another open label study in infants with IFALD, more patients achieved reversal of cholestasis in the FO-ILE group (19 of 38) as compared with the SO group (2 of 36; $P=0.005$).⁴⁹ It should be pointed out that a lower dose of FO-ILE is routinely used compared with SO-ILE, and the FO-ILE contains significantly more alpha tocopherol, both of which may also alter the risk for development of IFALD. Thus, a prospective, randomized trial comparing low dose FO-ILE with low dose SO-ILE (1 g/kg/d of each) was attempted in surgical infants believed to be at risk for IFALD. Unfortunately, this relatively small study failed to show the development of IFALD in any of the infants, thus it was not possible to determine if there was any protective benefit of FO-ILE, if the low dose of both lipid emulsions was protective, or if the selection criteria for infants at high risk for IFALD were not optimal.⁵⁰ Thus, although a properly powered randomized controlled trial has not been conducted, current evidence suggests that the use of FO-ILE is effective in reversing the established cholestasis associated with IFALD, but there is insufficient evidence for a preventative effect in neonates who require prolonged PN support.^{10, 51} An alternative lipid emulsion containing a combination of oils, SMOF, was not associated with cholestasis in the premature piglet model, suggesting that it may also have protective effects in humans.³³ However, two infants with surgical bowel conditions who developed IFALD while receiving SMOF (2-3 g/kg per day) required switching to a FO-ILE (1 g/kg per day) in order to reverse the cholestasis.⁵²

Final considerations of lipid modification

Authorities agree that few high quality clinical trials have been conducted to support use of alternative approaches to lipid emulsions.^{4, 8, 24, 25} Most studies have been retrospective, used a historical comparison group, used different doses of lipid, and were conducted in patients with quite advanced IFALD. In addition, with the exception of improving biochemical measurements of cholestasis and delaying the need for intestinal transplant evaluation (two important outcomes), there is evidence that the use of ILE containing ω -3 PUFA does not improve other important long-term clinical outcomes, such as the severity of hepatic fibrosis.^{4, 8, 25} Moreover, the long-term safety of reduced ILE doses in small infants remains to be proven, because infant brain growth and cognitive development depend on an adequate supply of PUFA and lipid. Recently, lower brain weight and alterations of brain PUFA content were demonstrated in newborn piglets receiving total PN with reduced dose SO-ILE or FO-ILE compared with normal dose SO-ILE and control piglets,⁵³ supporting this concern. Thus, there is an urgent need for high quality, well conducted clinical trials with clearly defined clinical outcomes and measurements, including cognitive and brain development, and longer term follow-up. It is equally important to note that because FO-based ILE is not licensed for use in the United States, its use increases the complexity and cost of care as it is more expensive than SO-ILE and can only be administered under Food and Drug Administration compassionate use protocols requiring special monitoring and reporting.

Current recommendations for use of ILE

The causal link between the use of standard dose SO-ILE and IFALD has not been firmly established in prospective, controlled clinical trials, leading most experts to conclude that the level of evidence in human trials for the proposed pathophysiologic mechanisms (role of SO-based ω -6 rich PUFA, and high level of plant phytosterols) as weak.^{2, 4, 8, 9} The evidence supporting the recommendation for a reduced dose of SO-ILE or replacing it with other source of ILE, such FO-based, is graded as weak or probably effective.^{2, 4, 8, 9} Nevertheless, these practices have been widely adopted in the United States based on clinical experience and absence of alternative effective approaches.

Discussion

IFALD is the greatest contributor to morbidity and mortality in infants and children with IF. Its pathogenesis is complex and multifactorial. Recently, interest has focused on the potential role of SO- and plant oil-based, ω -6 rich ILEs, altered intestinal permeability with absorption of bacterial products and activation of the innate immune system, and episodes of sepsis in the pathogenesis of IFALD. Reduction in the dose of SO-ILEs or replacement with non-soy based ILEs (such as FO-ILE or mixed lipid ILE) appears to reverse the cholestasis (and abrogate the need for intestinal transplantation) but probably has little effect on hepatic fibrosis. Other biologic pathways under investigation, including activation of hepatic-based innate immunity mechanisms, may yield new therapeutic targets. There is an urgent need to conduct high-quality, prospective trials with clearly defined outcome measures and long-term follow-up to ascertain the effects of these new strategies. Meticulous care of central venous catheters to prevent bacterial and fungal sepsis may also reduce innate system activation. Both surgical and medical measures taken to enhance intestinal adaptation and advancement of enteral feedings, thus allowing for reduction of PN dependence, will ultimately reduce the need for PN in infants with IF. The effect of α -tocopherol on the hepatotoxic effect of toxic bile acids and phytosterols, oxidative stress and the pro-inflammatory effect of SO-ILE, as well as restoration of intestinal mucosal integrity may provide novel therapeutic approaches in the prevention of IFALD in the future.

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Abbreviations used

CLABSI	central line-associated blood stream infections
DSS	dextran sodium sulfate
FO	fish oil
FOE	fish oil emulsion
IF	intestinal failure
IFALD	intestinal failure-associated liver disease

ILE	intravenous lipid emulsion
LBW	low birth weight
LPS	lipopolysaccharide
NEC	necrotizing enterocolitis
PN	parenteral nutrition
PNAC	parenteral nutrition-associated cholestasis
PNALD	parenteral nutrition-associated liver disease
SMOF	soybean oil, medium chain triglycerides, olive oil and fish oil lipid emulsion
PUFA	polyunsaturated fatty acid
SBS	short bowel syndrome
SO	soybean oil

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Figure 1.

Proposed pathogenesis of intestinal failure associated liver disease (IFALD).

Lipopolysaccharides (LPS) and other intestinal bacterial products are absorbed through the inflamed intestinal mucosa into the portal circulation as result of small bowel bacterial overgrowth, intestinal dysbiosis and increased intestinal permeability, and may also circulate in high concentrations as result of bacteremia and sepsis. LPS, through toll like receptor 4 binding, leads to activation of hepatic macrophages promoting release of cytokines which stimulate hepatocyte pathways that lead to interruption of FXR and LXR signalling. Reduced FXR signalling leads to suppression of BSEP and MRP2 expression, resulting in reduced canalicular secretion of conjugated bile acids and bilirubin and cholestasis. Phytosterols (particularly stigmasterol) derived from IV soy oil lipid emulsions may also activate hepatic macrophages. Macrophage derived cytokines impair LXR signalling causing reduced expression of ABCG5/G8 resulting in hepatocyte retention of sterols, such as phytosterols. Phytosterols interrupt FXR signalling and further reduce BSEP and MRP2 expression, resulting in cholestasis. LPS may also potentially directly affect hepatocytes, which also express TLR4, and suppress FXR and LXR signalling.

Table 1
Risk Factors and Pathogenic Mechanisms for Developing Intestinal Failure-Associated Liver Disease

Risk Factor	Proposed pathophysiologic mechanisms	Level of evidence
Host factors		
Prematurity and small-for-gestational age	Immature enterohepatic circulation leading to accumulation of toxic bile acids precipitating secondary oxidant injury	Class III
Sepsis	Circulating endotoxin activates Kupffer cells within the liver, stimulating the release of pro-inflammatory cytokines, generating inflammatory cascade	Class III
Intestinal surgery	Absent of enteral feeding leads to decreased secretion of enteric hormones causing intestinal and gall bladder stasis, predisposing to small bowel bacterial overgrowth and dysbiosis; compensatory bowel dilatation in short bowel syndrome. Altered intestinal permeability predispose to increased bacterial translocation	Class III
Necrotizing enterocolitis	Multifactorial; prematurity; abdominal surgery and bowel resection often necessary; prolonged absent/limited enteral feeding; peritonitis; altered intestinal permeability	Class II & III
Parenteral nutrition		
Lipid emulsions: plant- or soybean-based lipid emulsions	Plant- or soybean based lipid emulsions contains higher ω -6 PUFA which are precursors of pro-inflammatory eicosanoids. ω -6 PUFA administration predispose to hepatic steatosis. Phytosterols interfere with FXR signaling and decrease bile acid transport. Relatively low alpha tocopherol levels relative to PUFA in soybean based lipid emulsions.	Class III
Longer duration of parenteral nutrition	Longer exposure to PN	Class III
Excessive energy load	Excessive delivery of parenteral energy may lead to hepatic steatosis	No evidence
Amino acid component	Mechanism unclear	No evidence

Classification of evidence:

- Class I: prospective, randomized, controlled clinical trial. Primary outcome and exclusion / inclusion criteria clearly defined.
- Class II: prospective matched group cohort study in a representative population with masked outcome assessment.
- Class III: all other controlled trials (including well-defined natural history controls or patients serving as own controls) in a representative population. Outcome assessment is independent of patient treatment.
- Class IV: evidence from uncontrolled studies, case series, case reports, or expert opinions.

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Table 2
Suggested strategies for the prevention of intestinal failure-associated liver disease^{1,2}

Strategies	American Society for Parenteral And Enteral Nutrition (ASPEN) ^A		American Pediatric Surgical Association (APSA) ^B	
	Evidence on Effectiveness	Recommendation grade	Level of evidence	Rate of recommendation
<i>Non-nutrient factors</i>				
Ethanol lock	Low or very low	Weak		
Management by multidisciplinary team	Very low	Weak		
Oral choleretic agent: ursodeoxycholic acid	Very low	Weak		
Cholecystokinin-octapeptide			Class I & III	Established as ineffective
Oral erythromycin as prokinetic agent (or antibiotic effects)			Class II	Possibly effective
Oral supplementation of bile acids			Class III	Possibly effective
Cycling of PN			Class III	Possibly effective
Antibiotic treatment to prevent bacterial translocation and overgrowth			Class III	Unproven
<i>Nutrient factors</i>				
<i>Lipid emulsions</i>				
Reduction of lipid emulsion (soy-based lipid emulsion) to 1 g/Kg/day	Very low	Weak	Class III	Probably effective
Use of fish oil emulsion	Further research needed	No recommendation	Class IV	Unproven
Fat emulsion with soy oil, medium chain triglyceride, olive oil & fish oil	Further research needed	No recommendation	Class III	Unproven
Fat emulsion containing a blend of olive and soy oil	Further research needed	No recommendation		
<i>Non-lipid nutritional considerations</i>				
Supplementation of conditional amino acids and choline			Class IV	Possibly effective
Removal or reduction of manganese and copper as prophylaxis			Class III / IV	Probably effective
Trophic feeding			Class II	Probably effective

References

Note:

The GRADE methodology is described in detail in the paper by Druyvan ME, *et al* (Druyan ME, Compher C, Boullata JI, et al. Clinical guidelines for the use of parenteral and enteral nutrition in adult and pediatric patients: applying the GRADE system to development of A.S.P.E.N. clinical guidelines. *JPEN J Parenter Enteral Nutr.* 2012;36:77-80.

Rating of recommendation: A: established as effective, ineffective or harmful; B: probably effective, ineffective, or harmful; C: possibly effective, ineffective or harmful; D: data inadequate or conflicting, given current knowledge, treatment is unproven.

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- ¹Wales PW, *et al.* ASPEN Clinical Guidelines: support of pediatric patients with intestinal failure at risk of parenteral nutrition-associated liver disease. *J Parenter Enteral Nutr* 2014;38:538-557.
- ²Rangel SJ, *et al.* Parenteral nutrition-associated cholestasis: an American Pediatric Surgical Association Outcomes and Clinical Trials Committee systematic review. *J Pediatr Surg* 2012;47:225-240.
- ^AThe ASPEN Clinical Guidelines (Wales PW, *et al*) uses the GRADE methodology to develop its recommendation; high: further research is unlikely to change the confidence in any recommendation made; moderate: further research is likely to have an important impact on the confidence of recommendation; low: further research is very likely to have an impact on our confidence in the estimate of effect; very low: any estimate of effect is very uncertain.
- ^BThe American Pediatric Surgical Association (Rangers SJ, *et al*) adopted the following rating of evidence or therapeutic evidence: see footnote for Table 2 for the Classification of evidence.