

Revisiting Parenteral Nutrition-Associated Liver Disease (PNALD)

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Abstract

Parenteral Nutrition-Associated Liver Disease (PNALD) causes progressive cholangitis which can lead to liver failure and cirrhosis especially in infants. Developments in the understanding of its pathogenesis are revisited in the context of the need for guidelines for its safe management and support

Keywords

parenteral nutrition, cholestasis, liver failure, liver fibrosis, jaundice

Introduction: PNALD: the perfect storm

Parenteral Nutrition associated liver disease (PNALD) is characterised by progressive cholestasis associated with a serum conjugated bilirubin above 34 $\mu\text{mol/l}$ during two consecutive measurements in patients dependent on parenteral nutrition (PN). Fifteen to 40% of adults on long term home PN may also develop PNALD, often in association with intestinal failure. However PNALD has been studied much more intensively in the paediatric age group.

PNALD is commonest and most severe in premature neonates, often in association with the short bowel syndrome (SBS). The incidence of SBS in neonates has been estimated at 24.5 per 100,000 live births. Two thirds of these patients will develop PNALD with a case fatality rate of 37.5%¹. Histologic evidence of cholestasis is seen within 2 weeks and varying degrees of liver fibrosis within 6 weeks of starting PN.² PNALD occurs in up to 70% of such patients during 8-14 weeks of PN³ and may progress to cirrhosis and fatal liver failure. PNALD is the leading indication for liver/ intestine transplantation in infants.⁴ Failure to wean infants onto enteral feeding increases the need for liver and intestinal transplantation. Immature liver enzyme systems, gut mucosal atrophy with loss of hormonal output from the unfed gut, intestinal bacterial translocation, PN component toxicity, and PN-mediated exaggerated inflammatory response creates a “perfect storm” for progression to liver failure. This article revisits the multiple proposed aetiologies and pathogenetic mechanisms which inform proposed therapeutic strategies in PNALD and revisits the evidence-base on how they may impact on the progress of this disease.

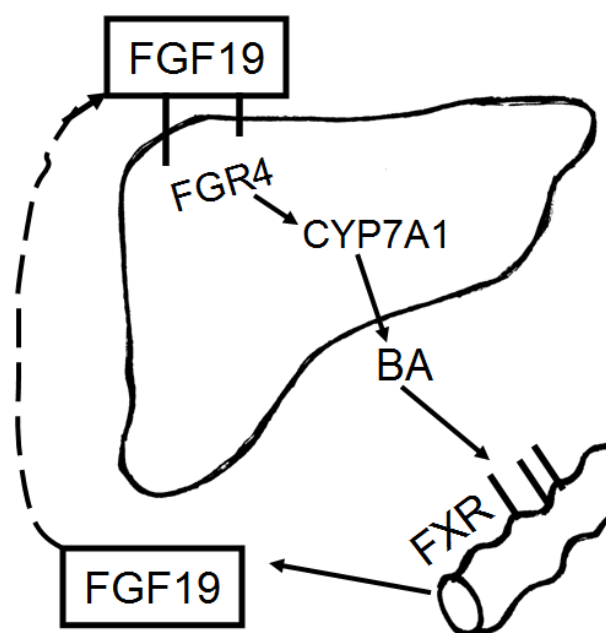
The cause of PNALD? : A Crime by omission?

In the search for the aetiology of PNALD, failure or inability to feed the gut is slowly replacing PN-related toxicities on centre-stage as the prime crime, with bile acids (BA) emerging as the main culprit. Healthy subjects economise on

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intestinal BA by their ileal reabsorption and their subsequent enterohepatic circulation. Bile acids complete the round trip from ileum to liver and back about twice during every meal. Luminal BA, particularly chenodeoxycholic acid (CDCA) act as ligands to, and activate, the nuclear farnesoid X receptor (FXR) in the intestinal epithelium which then releases fibroblast growth factor 19 (FGF 19).⁵ FGF 19 reaches the liver via the portal vein, and after binding to fibroblast growth factor 4 receptor (FGFR4) it represses the activity of cholesterol 7 alpha hydroxylase, a rate-limiting enzyme in hepatic BA synthesis (Figure 1). Disruption of the enterohepatic BA circulation by isolation of significant lengths of small intestine from exposure to food and bile leads to unrepressed hepatic BA synthesis which can be corrected by enteral administration of chenodeoxycholic (CDCA) but not Ursodeoxycholic acid (UDCA).⁶ Resumption of enteral feeding may also help to restore enterohepatic BA recirculation.

Figure 1: Bile acids (BA) bind to farnesoid X receptor (FXR) receptor in terminal ileum causing release of fibroblast growth factor 19 (FGF19) which travels through the portal circulation to the liver where it binds to its receptor FGFR4 leading to suppression of cholesterol 7 alpha hydroxylase (CYP7A1), a rate limiting enzyme in bile acid synthesis.



Food and bile acids exert both trophic and incretin effects on the intestinal epithelium through the bile salt-activated G protein-coupled receptor

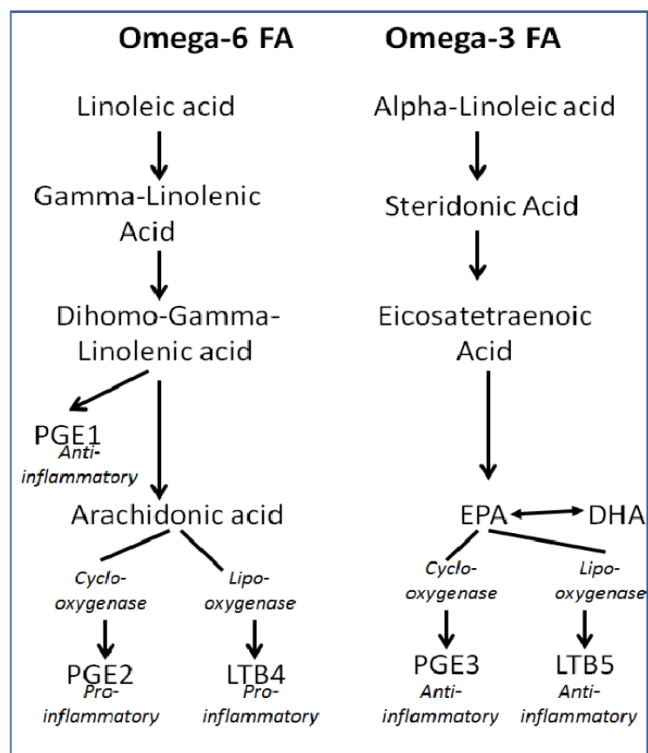
TGR5 which regulates secretion of glucagon-like peptides (GLP) 1 and 2 by intestinal endocrine cells.⁷ GLP2 reverses intestinal mucosal atrophy while GLP1 restores the insulin response to food⁸ and its deficiency may be partly responsible for glucose intolerance, dyslipidaemia and fatty liver seen in some patients on long term PN.

Gut rest, systemic antibiotics, and mucosal atrophy with low IGA are all potential causes of intestinal dysbiosis. Overgrowth of gram-negative species reminiscent of the dysbiosis observed in the gut of cirrhotic patients⁹ is often present in intestinal failure patients. Small intestinal bacterial overgrowth increases gut permeability, allowing translocation of bacteria to the liver where endotoxin disrupts bile acid transport.¹⁰ Here FXR and TGR5 have again been implicated.⁷ FXR modulates intestinal epithelial carbonic anhydrase¹¹ which regulates luminal pH and therefore bacterial levels while TGR5 reduces the expression of cytokines like TNF which mediate liver injury.¹²

Preventing irreversible liver damage: A fishy business?

Infant PN regimens typically infuse 2- 3g/ kg /day of soyabean oil as an intravenous fat emulsion (soya-IVFE) e.g. Intralipid (Fresenius kabi®) containing a high ratio (7:1) of omega-6 to omega-3 polyunsaturated fatty acids (PUFA), both of which compete for the cyclooxygenase and lipoxygenase pathways (see figure 2). Omega 6 PUFAs, predominantly Linoleic acid, are metabolized to 2-series prostaglandins e.g. PGE 2-series, and 4-series leucotrienes e.g. LTB4 via the cyclooxygenase and the lipoxygenase pathways respectively. Both LTB4 and PGE2 are proinflammatory and predominate on the antiinflammatory effects of PGE1 produced upstream in the same pathway. In contrast omega-3 PUFAs, mainly alpha-linolenic acid, docosahexanoic acid (DHA) and eicosapentaenoic acid (EPA) are precursors of anti-inflammatory mediators such as PGE3 and LTB5 which reduce inflammation. Arachidonic acid exerts some of its proinflammatory effect by activating the nuclear factor kappa B (NF-κB).¹³ In contrast omega-3 fatty acids inhibit NF-κB activation via a peroxisome proliferator-activated receptor (PPAR alpha) -dependent pathway. Metabolites of omega-3 PUFA that resolve inflammation such as resolvins have been shown to be hepatoprotective in mice.¹⁴

Figure 2: Omega-6 fatty acids are predominantly metabolized to proinflammatory mediators PGE2 and LTB4 while omega-3 fatty acids are predominantly metabolized to anti-inflammatory mediators PGE3 and LTB5



The above contrast between different PUFAs has led to trials of intravenous fish oil-based emulsions (fish-IVFEs) such as Omegaven (Fresenius Kabi®) which has a very low ratio of omega-6 to omega-3 PUFA (1:8) mainly containing EPA and DHA (see figure 2) which modulate PPAR alpha.⁷ Fish oils induce more beta-oxidation of fats and reduce lipogenesis thus reducing fat deposition in the liver. Compared to plant oils they do not contain phytosterols and they have a higher content of tocopherol (see below). They still await FDA approval and are only used in the USA on a compassionate basis. At lower doses of IVFA fish-IVFE monotherapy may not meet the infant's requirement for essential fatty acids. This has prompted the manufacture of mixed lipid emulsions such as SMOFlipid (Fresenius Kabi®) (30% soyabean oil, 30% MCT, 25% olive oil, 15% fish oil).

Soya and other plant-based emulsions also contain phytosterols which are potentially hepatotoxic. PN abolishes the protective limitation on the absorption of these plant sterols normally afforded by the enteral route.¹⁵ El kasmī et al, in a model of PNALD in parenterally fed mice with

intestinal injury, showed that parenteral fish oils prevented liver damage and hepatic macrophage activation by soya-IVFEs, and that stigmaterol promotes cholestasis and Kupffer cell activation by suppressing canalicular bile transport expression through antagonism of FXR nuclear receptors.

Soya IVFEs are also relatively deficient in alpha-tocopherol which is a crucial antioxidant in the PNALD scenario. The dyslipidaemia and hepatic steatosis which characterise both PNALD and non-alcoholic fatty liver are associated with the accumulation of long chain fatty acids (LCFA) in hepatocytes. Disposal of LCFA by hepatic mitochondrial B-oxidation is overwhelmed and rescue pathways such as peroxisomal beta-oxidation and microsomal omega-oxidation are activated.¹⁶ All this extra oxidative stress produces excess reactive oxygen species (ROS) such as superoxide which risks overwhelming the enzymes such as catalase, superoxide dismutase and the antioxidants such as tocopherol and glutathione which scavenge free radicals, which would otherwise damage hepatocytes through lipid peroxidation of their membranes. The burden on tocopherol is further exaggerated by oxidative stress secondary to bile duct damage. During PNALD vitamin E comes to the rescue, not only by means of its classical antioxidant function, but also specifically by activating xenobiotic receptors such as the pregnane X receptor (PXR)¹⁷ that wake up auxiliary scavenging by conjugation, sulphation and by cytochrome P450.

Coping with PNALD: too many verdicts awaited?

Treatment of PNALD aims to halt the hepatic fibroinflammatory reaction before it becomes irreversible. Early and repeated attempts to introduce enteral feeding are of utmost importance as many of the above aetiologies will be reversed. Enteral feeding should be carefully advanced and PN reduced.¹⁸ Even non-advanced minimal enteral feeding will help to reverse gut atrophy and restore gut hormonal responses (see Table 1). Non-nutritive sucking will help to prepare the infant for later feeding. Measures that maximize the length and function of residual viable bowel should be put in place including serial transverse enteroplasty (STEP) procedures if appropriate. When enteral feeding is contraindicated e.g. early phase of necrotizing enterocolitis the following alternative

strategies have been studied in the literature:

IVFE restriction: Restriction of soya IVFE to 1g/kg/day from the start of PN has been associated with significant reduction in PNALD but data on detailed nutritional parameters and essential fatty acid status have not been studied.

Cyclic PN: The use of cyclic PN throughout the intravenous feeding period has not been adequately studied in the literature¹⁸ and may be poorly tolerated in acutely ill neonates

Fish IVFE: Following the first use of fish-IVFE in a teenager with soy-allergy in 2005¹⁹ fish-IVFE administration has been reported in infants with PNALD to reverse cholestasis, reduce PN duration, reduce mortality, reduce transplant rates and to stabilize but not to prevent the early onset of liver fibrosis.¹⁸ Most of these studies used historical PNALD cohorts treated with soy-IVFE. A metanalysis in 2012 by Seida et al²⁰ of five randomised controlled (RCT) trials and 3 prospective cohort studies reported insufficiency of high quality data to support the use of fish-IVFEs in children. The study was heterogeneous and fish-IVFEs were not always the sole source of lipid in the study group. A more recent metanalysis²¹ of two RCTs (Jadad score of 5) and five non-randomized studies of fish-IVFE as the sole or partial source of intravenous lipids concluded that IVFEs are effective in reversing but not in preventing PNAC in neonates requiring prolonged nutritional support.

Mixed IVFEs: The low content of essential fatty acids (EFAs) in fish-IVFEs has caused at least a theoretical concern that infants with fat malabsorption receiving low volumes of PN may develop EFA deficiency. This has prompted the combined use of soy and fish-based IVFEs (mixedIVFEs) such as SMOF (Fresenius Kabi®: soya, olive and fish oils, medium chain triglycerides) which have been reported to be of benefit in PNALD but not adequately compared to fish-IVFEs.¹⁶ Research in this area is limited by the fact that fish oil-based IVFEs are still awaiting approval by the FDA in the USA. The ASPEN 2014 guidelines failed to recommend the use of fish/mixed IVFEs pending further trials.²²

Prevention of sepsis: Judicious use of antibiotics and other measures that prevent peritoneal, catheter-related and other sources of sepsis are expected to improve PNALD outcome. The ASPEN 2014 guidelines on PNALD failed to recommend ethanol locks of central PN catheters due to unresolved concerns regarding risks of thrombosis and disrupted catheter integrity.²²

UDCA: A retrospective cohort study²³ reported that cholestasis took longer to resolve in 64 neonates treated with UDCA than in controls. This is not surprising since UDCA has minimum affinity to FXR as compared with the natural bile acid CDCA. The ASPEN 2014 guidelines were unable to recommend UDCA treatment due to insufficient evidence in four analysed reports.

Table 1: Strategies that may limit liver failure in PNALD

Strategy	Comment
Start minimal enteral feeding early	Reverses gut atrophy, may be poorly tolerated
Advance enteral feeding	Restores bile acid homeostasis
Restrict calories administered as IVFE	Other calorie sources need high infusion volumes
Administer cyclic TPN	Faster volume infusion may be poorly tolerated
Use fish based IVFE	Theoretical risk of essential fatty acid deficiency
Use mixed IVFEs	Not adequately compared to fish-IVFEs
Treat with ursodeoxycholic acid	Poor efficacy as only weak FXR agonist
Treat with potent FXR agonist (Obeticholic acid)	Favourable reports from phase 2 clinical trial

FXR agonists: The above-mentioned ability of CDCA, a natural human BA to bind to the farnesoid X receptor (FXR) has prompted the synthesis of a 6 α -ethyl derivative of CDCA called Obeticholic acid (OCA), a first-in-class drug that binds to FXR with 100 times more potency than CDCA.²⁴ Phase II human clinical trials have shown that OCA is safe, and effective in reducing liver inflammation and fibrosis in type 2 diabetes and non-alcoholic fatty liver (NAFLD), and in reducing serum alkaline phosphatase, a surrogate marker of risk of fibrotic progression in primary biliary cirrhosis. In addition to its ability to modulate liver regeneration after liver injury, FRX is expressed in human stellate cells in which it reduces expression of extracellular matrix proteins with a potential to reverse hepatic fibrosis which has already been demonstrated in rats.²⁵ FRX agonist was more effective than CDCA in a piglet model of PNALD.²⁶

Conclusion

Available management strategies improve cholangitis in some PNALD scenarios but do not prevent fibrosis which appears to start very early in the course of the condition and which is often self-perpetuating through stellate cell activation. Of the two outcomes, prevention of fibrosis is likely to be more challenging than reversal of cholangitis. Perhaps the most effective strategy is to start enteral feeding early and to advance feeds while tapering PN. However, the patients with the highest mortality (typically premature / low birthweight, septic, and with acute enteric pathology such as necrotizing enterocolitis) will not tolerate this approach. The emerging challenge is to discover agents which will restore enteric hormonal, cytokine, and metabolic homeostatic responses during the early phase of intestinal failure. These could be bile acids,²⁷ gut hormones or agonists to the various gut receptors such as FRX. Intense research in the field of NAFLD- cirrhosis is likely to discover new FRX agonist in other drug classes e.g. the nonsteroidal FXR agonist GW4064.²⁸ Comprehensive guidelines to manage and prevent PNALD should be based on RCTs that control for gestational age, birthweight, type and dose of IVFE, and length of residual functional small intestine with clearly defined endpoints, such as the prevention or reversal of liver fibrosis and the reversal of cholangitis.

References

1. Wales PW, de Silva N, Kim JH, Lecce L, Sandhu A, Moore AM. Neonatal short bowel syndrome: a cohort study. *J Pediatr Surg.* 2005;40(5):755-62.
2. Zambrano E, El-Hennawy M, Ehrenkranz RA, Zelterman D, Reyes- Múgica M. Total parenteral nutrition induced liver pathology: an autopsy series of 24 newborn cases. *Pediatr. Dev. Pathol.* 2004;7:425–32.
3. Christensen RD, Henry E, Wiedmeier SE, Burnett J, Lambert DK. Identifying patients, on the first day of life, at high-risk of developing parenteral nutrition-associated liver disease. *J Perinatol.* 2007; 27:284.
4. Duro D, Kamin D, Duggan C. Overview of pediatric short bowel syndrome. *J Pediatr. Gastroenterol. Nutr.* 2008; 47(Suppl 1):S33–S36.
5. Jain AK, Teckman JH. Newly Identified Mechanisms of Total Parenteral Nutrition Related Liver Injury *Advances in Hepatology, Hindawi, Volume 2014, Article ID 621380, 7 pages.*
6. Lundasen T, Galman C, Angelin B, and Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man, *Journal of Internal Medicine* 2006; vol. 260 (6): 530–6.
7. Kumar JA, Teckman JH. Controversies in the Mechanism of Total Parenteral Nutrition Induced Pathology *Children* 2015, 2, 358-370; doi:10.3390/children2030358, www.mdpi.com/journal/children/
8. Drucker, D.J. The role of gut hormones in glucose homeostasis. *J. Clin. Investig.* 2007; 117: 24–32.
9. Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature.* 2014 Sep 4;513(7516):59-64.
10. Bolder, Ton-Nu HT, Schteingart CD. Frick E, Hofmann AF. Hepatocyte transport of bile acids and organic anions in endotoxemic rats: Impaired uptake and secretion *Gastroenterology* 1997; 112 (1): 214-225.
11. Lee FY, Lee H, Hubbert ML, Edwards PA, Yanqiao Zhang Y. FXR, a multipurpose nuclear receptor. *Trends in Biochemical Sciences* 2006; Vol 31 (10) 572–80.
12. Keitel V, Donner M, Winandy S, Kubitz R, Häussinger D. Expression and function of the bile acid receptor TGR5 in Kupffer cells *Biochemical and Biophysical Research Communications* 2008; 372: 78–84
13. Camandola S, Leonarduzzi G, Musso T, Varesio L, Carini R, Scavazza A, et al. Nuclear factor kB is activated by arachidonic acid but not by eicosapentaenoic acid. *Biochem Biophys Res Commun.* 1996 13; 229(2):643-7.
14. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G et al. Resolvins: a family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. *J Exp Med.* 2002;196:1025–37.

15. El Kasmi KC1, Anderson AL, Devereaux MW, Vue PM, Zhang W, Setchell KD et al. Phytosterols promote liver injury and Kupffer cell activation in parenteral nutrition-associated liver disease. *Sci Transl Med.* 2013 Oct 9; 5(206): 206 ra137. doi: 10.1126/scitranslmed.3006898.
16. Nandivada P, Carlson SJ, Melissa I. Chang MI, Cowan E, Gura KM, Puder M. et al, Treatment of Parenteral Nutrition-Associated Liver Disease: The Role of Lipid Emulsions *Adv. Nutr* 2013 ; 4: 711–7.
17. Stedman CA, Liddle C, Coulter SA, Sonoda J, Alvarez JG, Moore DD et al M. Nuclear receptors constitutive androstane receptor and pregnane X receptor ameliorate cholestatic liver injury. *Proc Natl Acad Sci USA.* 2005;102:2063–8.
18. Tillman EM. Review and Clinical Update on Parenteral Nutrition–Associated Liver Disease *Nutrition in Clinical Practice* 2013 28(1); 30-9.
19. Gura KM, Parsons SK, Bechard LJ, Henderson T, Dorsey M, Phipatanakul W et al. Use of a fish oil-based lipid emulsion to treat essential fatty acid deficiency in a soy allergic patient receiving parenteral nutrition. *Clin Nutr.* 2005 Oct;24(5):839-47.
20. Seida JC, Mager DR, Hartling L, Vandermeer B, Turner JM. Parenteral ω -3 fatty acid lipid emulsions for children with intestinal failure and other conditions: a systematic review. *J Parenter Enteral Nutr.* 2013 Jan;37(1):44-55.
21. Park HW, Lee NM, Kim JH, Kim KS, Kim S-N. Parenteral Fish Oil–Containing Lipid Emulsions May Reverse Parenteral Nutrition–Associated Cholestasis in Neonates: A Systematic Review and Meta-Analysis *J Nutr.* 2015 Feb;145(2):277-83.
22. Wales PW, Allen N, Worthington P, George D, Compher C, American Society for parenteral and enteral nutrition. A.S.P.E.N. Clinical Guidelines: Support of Pediatric Patients with Intestinal Failure at Risk of Parenteral Nutrition–Associated Liver Disease *Journal of Parenteral and Enteral Nutrition* 2014; 38(5) 538– 57.
23. Thibault T, McMahon J, Faubert G, Charbonneau J, Malo J, Ferreira E et al. Mohamed I. Parenteral Nutrition-Associated Liver Disease: a Retrospective Study of Ursodeoxycholic Acid Use in Neonates *J Pediatr Pharmacol Ther* 2014;19(1):42–8.
24. AH, Carey EJ, Lindor KD. Recent advances in the development of farnesoid X receptor agonists *Ann Transl Med.* 2015 Jan; 3(1): 5.
25. Fiorucci S, Rizzo G, Antonelli E, Renga B, Mencarelli A, Riccardi L et al. A farnesoid x receptor-small heterodimer partner regulatory cascade modulates tissue metalloproteinase inhibitor-1 and matrix metalloprotease expression in hepatic stellate cells and promotes resolution of liver fibrosis. *J Pharmacol Exp Ther* 2005;314:584-95.
26. <https://www.ars.usda.gov/research/publications/publication/?seqNo115=317851>.
27. Jain AK, Stoll B, Burrin DG, Holst JJ, Moore DD. Enteral bile acid treatment improves parenteral nutrition-related liver disease and intestinal mucosal atrophy in neonatal pigs. *Am J Physiol Gastrointest Liver Physiol.* 2012 Jan 15;302(2):G218-24.
28. Maloney PR, Parks DJ, Haffner CD, Fivush AM, Chandra G, Plunket KD et al. Identification of a chemical tool for the orphan nuclear receptor FXR. *J Med Chem* 2000; 43:2971-4.