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Original article

Parenteral lipids and partial enteral nutrition affect hepatic lipid composition but have limited short term effects on formula-induced necrotizing enterocolitis in preterm piglets

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SUMMARY

Background & aims: Rapid transition from total parenteral nutrition (TPN) to enteral feeding is a risk factor for necrotizing enterocolitis (NEC) in preterm infants. We hypothesized that partial enteral nutrition with colostrum, increased proportion of n-3 polyunsaturated fatty acids (PUFA), or exclusion of lipid in TPN would affect short term NEC sensitivity and liver function.
Methods: Preterm piglets were fed for three days after birth: 1) TPN with a standard lipid emulsion (Nutriflex Lipid Plus, TPN control group, $n = 19$), 2) PN plus bovine colostrum as partial enteral nutrition (PN/COL, $n = 18$), 3) TPN with fish oil (FO) lipids (Omegaven, TPN/FO, $n = 19$), or 4) TPN with no lipid (TPN/NL, $n = 22$). After TPN, piglets were fed formula for two days before tissue collection.
Results: None of the treatments had consistent effect on NEC incidence (~40–50% across all groups), intestinal morphology and function, relative to TPN. In the liver, there were no signs of steatosis but PN/COL decreased the n-6 PUFA levels, leading to higher n-3/n-6 ratio, GGT activity, and plasma cholesterol and albumin levels, relative to TPN (all $p < 0.05$). TPN/FO increased the hepatic n-3 levels and n-3/n-6 ratio. TPN/NL treatment led to decreased hepatic n-6 level, n-3/n-6 ratio and bilirubin, albumin and triglycerides, and lowered blood clotting strength (−30%, TPN/NL vs. TPN/COL, $p < 0.05$).
Conclusion: Partial enteral nutrition with colostrum, increased n-3 PUFAs in TPN, or removal of lipid from the TPN, all affect hepatic lipids and proteins in preterm neonates. These effects do not translate into improved hepatic function or NEC resistance, at least not short term.

1. Introduction

In neonatal intensive care units total parenteral nutrition (TPN) is used to compensate for the inability to tolerate enteral nutrition because of an immature gastrointestinal tract (GIT) in premature infants. Too rapid enteral food introduction predisposes to necrotizing enterocolitis (NEC), a severe GIT inflammatory disorder.[1] NEC remains the primary underlying diagnosis (43%) leading to intestinal resection and the need for long term TPN in neonates.[2] TPN complications can be divided into hepatobiliary and intestinal complications,[3] but specifically in preterm neonates, these may also be connected. In a rodent model of NEC, an intimate relationship between the liver and the gastrointestinal tract has been demonstrated and alterations in the secretion and reabsorption of bile acids has been associated with NEC.[4] These liver and gut functions interact closely with enteral feeding and TPN may reduce bile acid secretion, create dys-regulated gut microbiota and immunity and interfere with intestinal morphology and function, even short term.[5–7]

The lipid composition of TPN may influence hepatic fat deposition and thereby hepatic function while effects on other organs such as the gut are poorly investigated. TPN-related hepatic steatosis and dysfunction are more common in preterm neonates than in adults and factors such as partial enteral nutrition, and amount and composition of TPN lipids, may help to preserve hepatic function.[8] The manifestation of parenteral nutrition associated liver disease (PNALD) covers the spectrum from mild fat infiltration,

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cholestasis, and steatohepatitis to extensive fibrosis and cirrhosis. However in neonates, the most common complication and sign of hepatic dysfunction is cholestasis and in pediatric patients the prevalence of PNALD has been reported to be 20–90%.[3] Even though most of these complications originate from prolonged use of TPN, complications can occur in children after only a few days of TPN.[9,10] Attempts have been made to set cut-off levels for bilirubin in order to predict later development of liver failure.[11]

TPN is typically given with a lipid emulsion from soybean oil to supply adequate amounts of non-protein calories as well as essential fatty acids for biological membranes.[2] However, the use of soybean oil-based emulsions, which are rich in n-6 poly-unsaturated fatty acids (PUFA), has been associated with cholestasis in children[12] while the use of n-3 long-chain PUFA (LCPUFA) may reverse PNALD .[13–15] Similar findings have been shown in piglet model of PNALD[16] and in piglets, it has been shown that the fatty acid composition of plasma and liver tissue are strongly correlated with the fatty acid profile of TPN.[17,18] Dietary fatty acids might play a role in NEC development, also when just given short term. Previous work on fatty acids and the relation to NEC has focused on exclusive enteral feeding without TPN. Enteral fatty acids and NEC has been investigated in experimental animal studies using mice[19] and rats[20] and also the limited clinical work in preterm infants.[21] An intriguing potential is that manipulation of TPN lipids might also be of importance and may produce short term anti-inflammatory effects on the immature gut to help protection against NEC.

Using a NEC model in preterm pigs[22] that allow TPN feeding and show high short term sensitivity to NEC after transition to formula feeding, our aim was to investigate the short term effects of TPN lipids on NEC development and hepatic function. Four groups of pigs were fed parenterally for three days, before transition to two days of enteral feeding with one of two formulas with different nutrient compositions. Our hypothesis was that short term use of n-3 LCPUFA, or partial enteral nutrition, would improve both the formula-induced NEC resistance and the hepatic structure and function.

2. Materials and methods

2.1. Animals and their treatment

Seventy-eight preterm piglets (Landrace × Large White × Duroc) were delivered by caesarean section from five sows at day 105 of gestation (92% gestation), as previously described.[23] Piglets were transferred to infant incubators, supplied with oxygen (0.5–2 L/min to achieve >92% arterial saturation), and were fitted with a vascular catheter and an orogastric feeding tube according to previously established protocol.[22,23] In contrast to humans, trans-placental transport of immunoglobulins does not occur in piglets, which makes neonatal piglets dependent on colostral immunoglobulins for passive immunization. Therefore, all piglets were immunized systemically following catherization with intra-arterial administration of plasma from their mother at 4, 12 and 20 h after delivery (doses were 4, 5 and 7 ml/kg respectively). The plasma was isolated aseptically from centrifuged maternal blood (4000 g, 4 °C, 10 min) collected from a maternal uterine artery at the end of caesarean section. All procedures were approved by the National Committee on Animal Experimentation in Denmark (protocol number 2004/561-910).

2.2. Nutritional regimens

The animals were stratified according to body weight into four different treatment groups: a control group receiving our standard TPN regimen (TPN, $n = 19$, Nutriflex Lipid Plus, Braun, Melsungen, Germany, lipid with a final n-6:n-3 PUFA-ratio of 7:1), TPN with fish oil (FO) based lipid (TPN/FO, $n = 19$, 1/3 of TPN lipid given as Omegaven, Fresenius Kabi, Bad Holmburg, Germany, final n-6:n-3 PUFA-ratio of 3:2.5), TPN with no lipid (TPN/NL, $n = 22$, TPN lipids replaced with isoenergetic levels of dextrose), and PN supplemented with enteral feeding of bovine colostrum (PN/COL, $n = 18$). For the latter group, bovine colostrum was collected from a dairy herd (Holstein Friesian, Gjordslev gods, Denmark) from the first milking after parturition and stored at −20 °C until use. The bovine colostrum was diluted 2:1 in tap water, before administration. The final lipid composition of the TPN is given in Table 1.

The TPN solution was infused continuously for 72 h via the arterial catheter by use of automatic syringe pumps (Infusomat Secura, Braun, Melsungen, Germany). The nutritional goal was to provide the piglets with sufficient energy and protein to allow for a slightly positive energy balance. The infusion rate for the three TPN groups was 4 mL/kg/h during the first 24 h postpartum, increasing to 6 and 7 mL/kg/h after 1 and 2 days, respectively, the macronutrient composition of the TPN solutions is given in Table 2. Hereby, the piglets received an average of 450 kJ/kg BW/d, 6.5 g amino acids per kg BW/d, and a fluid intake of 160 mL/kg BW/d during the TPN period. The piglets were given 4.3 g/kg BW/d of amino acids at day 1 and increasing to 7.6 g/kg BW/d at day 3. The lipid load was started at 3 g/kg BW/d and increased to 5 g/kg BW/d on day 3. The glucose infusion was started at 7 g/kg BW/d and increased to 12 g/kg BW/d at day 3. Specifically for the TPN/NL group, the glucose load was started at 13 g/kg BW/d and increased to 23 g/kg BW/d by day 3. The PN/COL group received 2 mL/kg BW/h during all 72 h and oral bovine colostrum was given starting at 2 mL/kg BW/h at day 1, increasing to 3.5 mL/kg BW/h and 4.5 mL/kg BW/h on day 2 and 3, respectively. Using the above protocols, all four groups were calculated to receive the same amount of energy during the first three days after birth (450 kJ/kg BW/d).

After 72 h, the piglets were taken off TPN and given formula milk, as part of our standard protocol for the NEC model[23] which was based on commercially available products used for feeding infants. We used two different formulas A and B which contained the following: A) 24 g/L Seravit, 75 g/L Liquigen-MCT, 60 g/L Pepdite (all from SHS International, Liverpool, UK), 45 g/L Calogen LCT, 50 g/L Protifar (both from Nutricia, Denmark) and B) 75 g/L Liquigen-MCT, 80 g/L Pepdite, 70 g/L Lacprodan (Arla, Århus, Denmark). The macro- and micronutrient composition of formula A was designed to match the macronutrient as well as micronutrient

Table 1
Composition of fatty acids in final TPN lipid emulsions (% of lipids).[a]

	TPN	TPN/FO
C10:0	2.4	2.2
C12:0 Lauric acid	0.1	0.1
C14:0 Myristic acid	0.1	1.9
C16:0 Palmitic acid	11.7	11.2
C18:0 Stearic acid	4.9	3.7
C18:1n-9 Oleic acid	22.5	16.4
C18:1n-7 Vaccenic acid	1.4	2.0
C18:2n-6 Linoleic acid	48.6	26.6
C20:4n-6 Arachidonic acid and C20:3n-3 Eicosatrienoic acid	0.4	1.1
C18:3n-3 α-linoleic acid	5.4	3.4
C20:5n-3 Eicosapentaenoic acid	0	9.6
C22:5n-3 Docosapentaenoic acid	0	1.1
C22:6n-3 Docosahesaenoic acid	0.2	9.5

TPN: TPN with a standard lipid emulsion (Nutriflex Lipid Plus), TPN/FO: TPN with fish oil (FO) lipids (Omegaven).

[a] % of total lipids in total parenteral solution determined by gas chromatography. Animals given partially enteral nutrition received the standard TPN as parenteral solution.