TPN Cholestasis: Prevention and Treatment

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Objectives

- Review the history and clinical relevance of Parenteral Nutrition Associated Cholestasis and Liver Disease: PNAC
- Discuss the physiology of bile formation and flow
- Understand the causes of cholestasis, unique risk to the neonate and the role of septic mediators
- Outline the role of phytosterols and alternate lipid sources
- Explore new models
Liver: The Integrator of Metabolism & Inflammation
Key Events Over Past 40 years

- **1968**: TPN use in infants Wilmore & Dudrick *JAMA*
- **1971**: 1st report of TPNAC Peden et al *J Peds*
- **1980’s**: Modification of TPN constituents
- **1980’s-90’s**: Animals on TPN → cholestasis
- **1990’s → present**:
  - Hepatobiliary transporter genes
  - Genetic causes of cholestasis
- **2006**: Fish oil based lipid
intrahepatic cholestasis
cholate stasis
Bile duct proliferation
Kupffer cell hyperplasia
PNAC- Typical Clinical Findings

- Persistent Jaundice
- Hepatomegaly (occurs within 2-4 weeks)
- Portal Hypertension:
  - Splenomegaly
  - Ascites
  - Caput medusa
  - GI Bleeding
- Ostomies
- Poor enteral advancement
PNAC- A multi-factorial disease

TPN (IV a.a., CHO, lipids)

- Sepsis/Inflammation
- Lack of enteral feeding
- Prematurity
- Surgical resections

Cholestasis PNAC

End Stage Liver Disease PNALD
Contributors to PNAC

- Hepatic Immaturity
- TPN Toxin
- Sepsis & Inflammation
- Lack of enteral feeds
Predictors of Cholestasis in Preterm Infants

![Prevalence of Cholestasis](chart)

**Table 4** Logistic regression analysis, deriving odds ratios for developing PNALD, using the birth weight categories and surgical conditions as independent variables.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Odds ratio estimate</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight 1000–1499 g</td>
<td>2.8</td>
<td>2.1–3.7</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth weight 750–999 g</td>
<td>8.2</td>
<td>6.0–11.2</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth weight 500–749 g</td>
<td>13.1</td>
<td>9.4–18.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Birth weight &lt;500 g</td>
<td>30.7</td>
<td>9.5–56.2</td>
<td>0.000</td>
</tr>
<tr>
<td>NEC with laparotomy</td>
<td>11.7</td>
<td>10.0–13.6</td>
<td>0.001</td>
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<tr>
<td>NEC with drain</td>
<td>23.6</td>
<td>15–105.0</td>
<td>0.000</td>
</tr>
<tr>
<td>Gastroschisis</td>
<td>20.3</td>
<td>4.9–83.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Omphalocele</td>
<td>3.1</td>
<td>1.9–4.9</td>
<td>0.043</td>
</tr>
<tr>
<td>Diaphragmatic hernia</td>
<td>4.0</td>
<td>1–15.0</td>
<td>0.002</td>
</tr>
<tr>
<td>Jejunal atresia</td>
<td>24.0</td>
<td>9.0–64.1</td>
<td>0.000</td>
</tr>
<tr>
<td>ECMO</td>
<td>4.3</td>
<td>1.5–12.5</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Abbreviations: ECMO, extracorporeal membrane oxygenation; NEC, necrotizing enterocolitis; PNALD, parenteral nutrition-associated liver disease.

Risk of Cholestasis Increased in Prematurity and SGA

Kleinman & Sandler in *Pedi GI Disease* Walker eds, 1996
SGA Confers Greater Risk of Cholestasis

Patient characteristics and nutrition history

<table>
<thead>
<tr>
<th></th>
<th>Cholestasis (n = 79)</th>
<th>No cholestasis (n =152)</th>
<th>P value</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td>28 ± 6</td>
<td>30 ± 6</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>963 ± 465</td>
<td>1090 ± 463</td>
<td>.049</td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>27 ± 2</td>
<td>27 ± 2</td>
<td>.362</td>
<td></td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>49/30</td>
<td>80/72</td>
<td>.173</td>
<td></td>
</tr>
<tr>
<td>Race (W/H/B/other)</td>
<td>37/28/10/4</td>
<td>84/41/20/7</td>
<td>.453</td>
<td></td>
</tr>
<tr>
<td><strong>SGA</strong></td>
<td>23 (29)†</td>
<td>17 (11)</td>
<td>&lt;.01</td>
<td>3.3 (1.6-6.6)</td>
</tr>
<tr>
<td>Late-onset sepsis</td>
<td>63 (80)</td>
<td>52 (34)</td>
<td>&lt;.01</td>
<td>2.7 (1.2-6.0)</td>
</tr>
<tr>
<td>NEC</td>
<td>40 (51)</td>
<td>11 (7)</td>
<td>&lt;.01</td>
<td>7.5 (3.0-18.9)</td>
</tr>
<tr>
<td><strong>Required surgery</strong></td>
<td>6</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPD</td>
<td>51 (65)</td>
<td>38 (25)</td>
<td>&lt;.01</td>
<td>2.8 (1.4-5.9)</td>
</tr>
<tr>
<td>LOS (days)</td>
<td>106 ± 40</td>
<td>72 ± 40</td>
<td>&lt;.01</td>
<td></td>
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<tr>
<td><strong>Nutrition history</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPO before fed (days)</td>
<td>10 ± 9</td>
<td>6 ± 4</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>DOL FEN achieved</td>
<td>43 ± 25</td>
<td>23 ± 11</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Total days PN</td>
<td>63 ± 34</td>
<td>24 ± 14</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Days NPO before FEN</td>
<td>11 ± 12</td>
<td>3 ± 5</td>
<td>&lt;.01</td>
<td></td>
</tr>
<tr>
<td>Days NPO after FEN</td>
<td>12 ± 17</td>
<td>2 ± 4</td>
<td>&lt;.01</td>
<td></td>
</tr>
</tbody>
</table>

W/H/B/other, White/Hispanic/black/other; LOS, length of stay; DOL, day of life.
Mean ± SD.
†n (%).

J Peds 2008.152:(1), 59–62
70% Incidence of Liver Disease with Surgical NEC

Diagnosed with NEC
N=464

Surgery
N=180

<14 days of PN at liver disease diagnosis, end of study, or death
N=40

Abnormal liver function prior to surgery
N=6

Insufficient data:
Direct bilirubin and ALT both unknown
N=7

Analysis Dataset
N=127

Liver disease
N=89 (70%)

No liver disease
N=38 (30%)

Odds ratio (95% CI)  P

Weeks of PN* 2.37 (1.56–3.60)  <0.0001
Small-bowel resection or jejunostomy 4.96 (1.97–12.51)  0.0007

CI = confidence interval; PN = parenteral nutrition; PNALD = parenteral nutrition–associated liver disease.
*Cumulative exposure from birth through 4 weeks after surgery.

SBS mortality Correlates with Increased Bilirubin Levels

Teitelbaum *Curr Opin Ped* 1997
Probability of Survival in SBS

Early Persistent Cholestatic Jaundice

Primary Anastamosis

Mortality Increased in Patients with Maximum Conjugated Bilirubin >10 mg/dL.

Willis T C et al. JPEN 2010;34:32-37
Time to Recovery Correlated with Peak Bilirubin Level

Willis T C et al. JPEN 2010;34:32-37
It’s All About the Bilirubin- or is it??

Characteristics (n=31)

- Male sex, n (%) 18 (60)
- Gestational age (wk), mean ± SD 31.3 ± 5.1
- Residual small bowel length (cm), mean ± SD 86.8 ± 54.5
- Presence of ileocecal valve, n (%) 16 (59)
- Duration of PN (days), median (IQR) 124 (93-232)
- Peak ALT before cessation of PN (U/L), median (IQR) 157 (116-239)
- Peak direct bilirubin before cessation of PN (mg/dL) 5.3 (3.4-11.2)
PNAC-putative mechanisms

• The TPN solution is toxic
  • aluminum and manganese (Fell, 1996) not Cu (McMillan 2008)
  • amino acids, specifically tryptophan (Merritt, 1984)
  • lipid peroxides (Helbock, 1993), phytosterols (Clayton, 1998)

• The TPN solution is deficient
  • taurine (Cook, 1984)
  • micronutrients (Se, Mb, etc.) (Poley, 1981)
  • ω3 lipids (Chen 2003)

• The gut is the problem
  • abnormal flora (Pierro, 1998) & bacterial translocation (Alverdy, 1988)
  • bacterial product (LPS) translocation (Lichtman, 1991)
  • enteral fasting leads to disordered GI hormones (Lucas, 1983)

• The immature liver is the problem
  • enterohepatic circulation of bile acids (Balistreri, 1983)
  • enrichment with monohydroxylated bile acids (Fouin-Fortunet, 1982)
Contributors to PNALD

- Hepatic Immaturity
- TPN Toxin
- Sepsis & Inflammation
- Lack of enteral feeds
**Importers**

- NTCP
- OATP’s

**Exporters**

- OSTα/β
- MRP3 & MRP4

**Sinusoidal Membrane**

- Bile acids
- Organic anions
- Na⁺

**Canalicular Membrane**

- Bile acids
- Various conjugates
- Conjugated Bilirubin & other conjugates

**Unknown**

- Bile acids
- Various conjugates
- Conjugated Bilirubin & other conjugates

**Transporters**

- FIC1
- BSEP
- ABCG5/G8
- MDR3
- MRP2
Canalicular Transporters Determine Biliary Components

Solute composition of human bile

- Bile acids: 41% (30 mM)
- Conjugated Bilirubin & other conjugates: 17%
- Phospholipids: 17%
- Electrolytes: 31%
- Cholesterol: 3%
- Proteins: 7%
Developmental Expression of BSEP RNA

Adapted from Chen *J Hep* 2005

BSEP MRP2 MDR3 NTCP

Fetus Adult

Human

Rat

Bsep/Actin, % Adult level

Birth

19 DF 21 DF 1 DAY 1 WK 2 WK 4 WK ADULT

Adapted from Tomer *Ped Res* 2003
Polymorphic variants in the human bile salt export pump (BSEP; ABCB11): functional characterization and interindividual variability

Richard H. Ho\textsuperscript{a,c}, Brenda F. Leake\textsuperscript{b}, Dawn M. Kilkenny\textsuperscript{c}, Henriette E. Meyer zu Schwabedissen\textsuperscript{e}, Hartmut Glaeser\textsuperscript{f}, Deanna L. Kroetz\textsuperscript{d} and Richard B. Kim\textsuperscript{e}

180-fold range in NTCP mRNA
19-fold range in BSEP mRNA
31-fold range in BSEP protein expression
Decreased Bile Acid Pool

- Neonates have a smaller bile acid pool
- More of the toxic bile acid species
- Poor enterohepatic recirculation
- Bile acid loss with ostomies and loss of ileum
Contributors to PNAC

- Hepatic Immaturity
- Sepsis & Inflammation
- TPN Toxin
- Lack of enteral feeds
TPNAC: role for septic mediators

Serum bile acids (μM)

- Enteral (18) 13.1
- TPN (14) 13.7
- TPN + Sepsis (6) 66.1

Rising serum BA levels on TPN before sepsis

Manginello & Javitt, J Peds, 1979
PNALD: role for LPS & Cytokines

- Kupffer cell activation
- cholestasis
- GB sludge/stasis
- luminal stasis/strictures
- endotoxin
- ↑ mucosal permeability
- bacterial overgrowth
- ↑ loss of bile acids
LPS Decreases BSEP RNA levels

Ghose Nuc Receptor 2004

Pirovino et al, Gastro, 1989
Sepsis/LPS → ↓ BA Transporters

Importers
- NTCP
  - Na+
- OATP's
- Organic anions
- Bile acids

Exporters
- OSTα/β
  - Bile acids
- MRP3 & MRP4
  - Bile acids
  - Various conjugates
- ABCG5/G8
- BSEP
- MDR3
- MRP2

Unknown
- Conjugated Bilirubin
- & other conjugates
- Sterols
- Phospholipids
- Various conjugates
- Bile acids

LPS
Bile acid accumulation $\rightarrow$ hepatocyte damage

- $\uparrow$ apoptosis
- $\Delta$ membrane fluidity
- $\Delta$ cell signaling
  - Fas, PKC, Ca$^{2+}$, ...
- ? role in hepatocyte transformation
- $\uparrow$ pro-inflammatory cytokine expression

The hepatocyte must maintain safe intracellular BA levels

Guicciardi & Gores, *Dig Liver Dis* 2002
Paumgartner & Beuers, *Hepatology* 2002
Contributors to PNAC

- Hepatic Immaturity
- TPN Toxin
- Sepsis & Inflammation
- Lack of enteral feeds
Lack of enteral feeding and Cholestasis
The TPN Solution

- Absent element (ω3 lipids)
- Pro-inflammatory compound
- Lipid peroxides
- Phytosterols
Phytosterolemia in Children With Parenteral Nutrition–Associated Cholestatic Liver Disease

PETER T. CLAYTON,∗,‡ ANN BOWRON,∗ KEVIN A. MILLS,∗ AHMED MASSOUD,† MINNE CASTEELS,∗ and PETER J. MILLA∗,§

∗Metabolic Disease Unit and 3Gastroenterology Unit, Institute of Child Health, London; and †Hospital for Sick Children, London, England

**Phytosterols**

Cholic acid

10% Intralipid

Cholesterol 304 mg/L
Total Plant Sterols 370
  − Campesterol  84
  − Stigmasterol  76
  − β-Sitosterol 210

Carter, BA et al Ped Res 2007
Stigmasterol: an FXR antagonist in vitro

9cRA CDCA

RXR FXR

Bsep mRNA

Relative BSEP RNA

Carter, BA et al Ped Res 2007
Reversal of Parenteral Nutrition–Associated Liver Disease in Two Infants With Short Bowel Syndrome Using Parenteral Fish Oil: Implications for Future Management

Kathleen M. Gura, PharmD, a,b, Christopher P. Duggan, MD, MPH, b Sharon B. Collier, MS, RD, b, Russell W. Jennings, MD, c Judah Folkman, MD, c Bruce R. Bistrian, MD, c, Mark Puder, MD, PhD
Soy vs Fish

- Soybean extract
- Reversed ratio of phytosterols:cholesterol compared to normal human diet
- Rich in ω6 fatty acids: pro-inflammatory
- Slow bile flow in animal models
- Correlated with cholestasis

- Refined fish, EHA, DHA and α-tocopherol
- Rich in ω3 fatty acids: anti-inflammatory
- Contains no phytosterols
- Deficient in some essential fatty acids
- Can reverse many of the signs/symptoms of PNAC
- Compassionate use only
Fish vs. Soy

Le, Sem Ped Surg, 2010
Overlapping Timelines

Stopping Soy Lipid & Starting Fish Lipid


Colomb, *JPEN*, 2000
Safety and Efficacy of a Fish-Oil–Based Fat Emulsion in the Treatment of Parenteral Nutrition–Associated Liver Disease

Kathleen M. Gura, PharmD, Sang Lee, MD, Clarissa Valim, MD, ScD, Jing Zhou, MS, Sendia Kim, MD, Biren P. Modi, MD, Danielle A. Arsenault, BS, Robbert A. M. Strijbosch, BS, Suzanne Lopes, RN, Christopher Duggan, MD, MPH, Mark Puder, MD, PhD

Departments of *Pharmacy and Division of Gastroenterology and Nutrition, †Surgery, ‡Clinical Research Program, and •Department of Nursing, Children's Hospital Boston, Harvard Medical School, Boston, Massachusetts

**Pediatrics 2008**

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

<table>
<thead>
<tr>
<th>Variables</th>
<th>Fish Oil (N = 18)</th>
<th>Soybean (N = 21)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Demographic</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male), ( n ) (%)</td>
<td>12 (67)</td>
<td>10 (48)</td>
<td>.23</td>
</tr>
<tr>
<td>Age, mean ± SD, wk</td>
<td>14 ± 7</td>
<td>14 ± 20</td>
<td>.85</td>
</tr>
<tr>
<td>Birth weight, mean ± SD, kg</td>
<td>2.03 ± 1.85</td>
<td>2.23 ± 1.00</td>
<td>.69</td>
</tr>
<tr>
<td>Gestational age, mean ± SD, wk</td>
<td>30 ± 4</td>
<td>34 ± 5</td>
<td>.03</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intralipid</th>
<th>Liposyn II</th>
<th>Omegaven</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oil</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Soybean</td>
<td>10</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Safflower</td>
<td>—</td>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>Fish</td>
<td>—</td>
<td>—</td>
<td>10</td>
</tr>
<tr>
<td><strong>Fats, %</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic</td>
<td>50</td>
<td>65</td>
<td>0.1–0.7</td>
</tr>
<tr>
<td>( \alpha )-Linolenic</td>
<td>9</td>
<td>4</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>EPA</td>
<td>—</td>
<td>—</td>
<td>1.3–2.8</td>
</tr>
<tr>
<td>DHA</td>
<td>—</td>
<td>—</td>
<td>1.4–3.1</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>—</td>
<td>—</td>
<td>0.1–0.4</td>
</tr>
<tr>
<td>Glycerol</td>
<td>2.3</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Epa phospholipid</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>Phytosterols, mg/L¹⁰</td>
<td>348 ± 33</td>
<td>383</td>
<td>0</td>
</tr>
</tbody>
</table>

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

Is it the delivery of $\omega 3$ lipids, or

the discontinuation of soy lipids?
Clinical experience

- “Toxin” in iv soy lipid?
  - TPNAC resolves when enteral feeding replaces parenteral feeding. Regardless of enteral formula composition (ω-3 containing or not).
  - Oral soy-based formulas do not contribute to TPNAC → intestinal exclusion of a soy component (ABCG5/G8).
  - These infants start life without cholestasis (i.e., Direct Bili level is normal at first, then rises with time on iv soy lipid).

- ω-3 deficient?
  - No evidence that normal infants, enterally-fed, become cholestatic on non-ω-3 containing formula.
  - No head-to-head trial of preemies on soy vs fish oil.
Persistence of fibrosis on ω3 lipids

- 66 infants
- Bx at 6 m (on PN x 4.7 m)
- 70% on ω3 lipids

- 55% of biopsies with fibrosis
- 3/8 with cirrhosis

• No biochemical evidence of cholestasis

Failure of Resolution of Portal Fibrosis during Omega-3 Fatty Acid Lipid Emulsion Therapy in Two Patients with Irreversible Intestinal Failure

Jason S. Soden, MD, Mark A. Lovell, Kristin Brown, David A. Partrick, and Ronald J. Sokol

MVID
Switch from soy to ω3 lipids at 9 months

Fitzgibbons, J Ped Surg, 2010

J Peds, 2010
Lipid Limitation

- By decreasing the dose of soy-intralipid emulsion IL (S-ILE) the liver may be exposed to less inflammatory/hepatotoxic compounds
  - Total lipid load
  - Omega-6 fatty acids
  - Phytosterols

- Accumulation of fat in Kupffer cells
  - Leads to phagocytic dysfunction
  - Impaired clearance of endotoxin
  - Overload of reticuloendothelial system
  - Lipid peroxidation and hepatic inflammation
Intravenous Fat Emulsions Reduction for Patients with PNALD

The effect of lipid restriction on the prevention of PNAC in surgical infants

Effect of decreased parenteral S-IILE on hepatic function in infants at risk for PNALD: a pilot study
Novel Lipid Formulations

- Next generation blended lipid solutions
- “SMOF Lipid”- Soy (30%), Medium Chain (30%), Olive Oil (25%), Fish (15%)
- SMOF lipid in infants with SBS: safe, well-tolerated, decreased bilirubin and improved lipid profile

  Goulet et al. JPEN 2010;34:p.485-495

- Approved in Europe and in Phase I/II in Canada
SMOF Lipid Trials

- Can SMOFLipid®, A Composite Parenteral Nutrition Lipid Emulsion, Prevent Progression Of Parenteral Nutrition Associated Liver Disease In Infants?
  - The Hospital for Sick Children, Toronto; Randomized, Open-Label; Ongoing

- Comparison of liver function with two new/mixed intravenous lipid emulsions in children with intestinal failure: SMOF lipid vs Lipofundin
  - Great Ormond Street Hospital for Children, London
  - Addition of MCT to soybean ILE was associated with improved liver function. Even greater improvement when olive and fish oils were also added, with higher incidence of resolution of abnormal liver function tests and reduced inflammation.
SMOF Lipid Trials

• Resolution of parenteral nutrition-associated jaundice on changing from a soybean oil emulsion to a complex mixed-lipid emulsion.
  • SMOF lipid vs. Intralipid; retrospective cohort; Birmingham Children’s Hospital
  • After 6 months, 5 of 8 children in the SMOFlipid and 2 of 9 children in the Intralipid group had total resolution of jaundice.

• Fish Oil (SMOFlipid) and olive oil lipid (Clinoleic) in very preterm neonates.
  • Randomized Controlled, Blinded Trial; Preterm neonates (23-30 weeks); King Edward Memorial Hospital Western Australia
  • SMOFlipid was safe, well tolerated, and showed beneficial effect in terms of reduction of oxidative stress by reducing lipid peroxidation levels in high-risk preterm neonates.
Summary

- PNALD is an *acquired* disease of infants, due to a combination of:
  - immaturity
  - inflammation
  - lack of enteral feeds
  - putative TPN toxins

- New insights from:
  - Molecular mechanisms of bile formation
    - *focus on adaptation to cholestasis & NR regulation*
  - Sepsis-associated mediators
  - Phytosterols
  - ? Alteration in outcome by changing lipid solution