Section of Pediatric Surgery
Children's Hospital
Helsinki University Central Hospital
Faculty of Medicine
University of Helsinki
Helsinki, Finland

LIVER DISEASE IN PEDIATRIC ONSET INTESTINAL FAILURE

Annika Mutanen (née Kurvinen)

ACADEMIC DISSERTATION

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Supervisor

Docent Mikko P. Pakarinen

Section of Pediatric Surgery

Children's Hospital

Helsinki University Central Hospital

University of Helsinki

Reviewers

Docent Harri Niinikoski

Department of Pediatrics

Turku University Hospital

University of Turku

Docent Taina Sipponen

Department of Medicine

Division of Gastroenterology

Helsinki University Central Hospital

University of Helsinki

Opponent

Professor Daniel H. Teitelbaum

Department of Surgery

University of Michigan

Ann Arbor, Michigan

USA

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To intestinal failure patients

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Abstract

Background. Intestinal failure (IF) is characterized by the reduced capacity of the intestine to digest and absorb nutrients and fluids required for the maintenance of energy, fluid, electrolyte, and micronutrient balance, as well as normal growth and development in children. In these studies, IF was defined by over 50% resection of small bowel and/or duration of parenteral nutrition (PN) over 30 days. Patients with IF are at risk for multiple potentially life-threatening complications, including IF-associated liver disease (IFALD).

Aims. To study the incidence of long-term PN and associated liver disease, characterize liver histopathology, and evaluate risk factors of IFALD during and after weaning off PN in pediatric onset IF.

Methods. Long-term effects of IF and PN on serum non-cholesterol sterols, including plant sterols and cholesterol precursor sterols, and liver biochemistry were evaluated prospectively in a single-centre (patients n=11) and a nation-wide study (patients n=39). Liver histology, risk factors of IFALD, and serum fibroblast growth factor 21 (FGF21) were studied in two cross-sectional studies (patients n=38 and n=35).

Results. During PN, serum plant sterol levels were significantly increased in neonates and children with IF compared to healthy controls ($P<0.05$ for all). In children, total and individual serum plant sterol levels associated with percentage of parenteral calories ($r=0.70$-0.92, $P<0.05$ for all), and reflected their distribution in PN lipid emulsions. In neonates, total duration of PN associated with serum cholestanol, stigmasterol, avenasterol, ALT, and AST ($r=0.472$-0.636, $P<0.05$). In neonates with IFALD, serum plant sterols ratios to cholesterol, especially stigmasterol, were increased compared with healthy controls, neonates without IFALD and children on PN ($P<0.005$ for all). After weaning off PN, IFALD persisted in 25% of neonates with 4.2- and 2.2-times higher serum stigmasterol and cholestanol ratios to cholesterol compared with neonates without IFALD ($P<0.05$).

Abnormal liver histology was found in 94% of IF patients on PN and 77% of patients weaned off PN (P=0.370). During PN, liver histology was weighted with cholestasis (38% of patients on PN vs. 0% of patients weaned off PN, P=0.003) and portal inflammation (38% vs. 9%, P=0.05). After weaning off PN, cholestasis resolved, but significant fibrosis (88% vs. 64%, P=0.143) and steatosis (50% vs. 45%, P=1.000) persisted. Fibrosis stage was associated with remaining small bowel length (r=-0.486, P=0.002), duration of PN (r=0.387, P=0.016), and number of septic episodes (r=0.480, P=0.002). In a multivariate analysis, age-adjusted small bowel length (ß=-0.553, P=0.001), portal inflammation (ß=0.291, P=0.030), and absence of ileocaecal valve (ß=0.267, P=0.048) were predictive for fibrosis stage.

IF patients with steatosis had markedly higher serum FGF21 concentration (median 626 vs. 108 pg/mL, P=0.002) and more advanced liver fibrosis (Metavir stage median 1.6 vs. 0.7, P=0.020) compared to those without steatosis. Serum FGF21 correlated with steatosis grade (r=0.589, P=0.001). Hepatic steatosis and serum FGF21 associated with duration of PN (r=0.471-0.580) and remaining small bowel length (r=-0.368- -0.502, $p<0.05$ for all). Liver steatosis grade (ß=0.630, P=0.001) predicted serum FGF21 concentration in a multivariate regression model.

Conclusions. During PN, serum plant sterol levels are high and reflect their distribution in PN lipid emulsions. In neonates, IFALD is frequent and associates with markedly increased serum plant sterols compared to healthy neonatal controls and children on PN with more mature liver function. After weaning off PN, serum stigmasterol and cholestanol remain high in neonates with persistent IFALD.

Liver histology is characterized by cholestasis, portal inflammation, liver fibrosis and steatosis in IF patients on PN. After weaning off PN, portal inflammation diminishes and cholestasis resolves, but fibrosis and steatosis persists. In addition to duration of PN, extensive small intestinal resection and loss of ileocaecal valve as well as septic episodes are major risk factors of histological liver fibrosis. Increased serum FGF21 levels reflect the presence and the degree of liver steatosis in patients with pediatric onset IF.

List of original publications

This thesis is based on the following publications:

I. Kurvinen A, Nissinen MJ, Gylling H, Miettinen TA, Lampela H, Koivusalo AI, Rintala RJ, Pakarinen MP. Effects of long-term parenteral nutrition on serum lipids, plant sterols, cholesterol metabolism, and liver histology in pediatric intestinal failure. J Pediatr Gastroenterol Nutr 2011;53:440-446.

II. Kurvinen A, Nissinen MJ, Andersson S, Korhonen P, Ruuska T, Taimisto M, Kalliomäki M, Lehtonen L, Sankilampi U, Arikoski P, Saarela T, Miettinen TA, Gylling H, Pakarinen MP. Parenteral plant sterols and intestinal failure-associated liver disease in neonates. J Pediatr Gatroenterol Nutr 2012;54:803-811.

III. Mutanen A, Lohi J, Heikkilä P, Koivusalo AI, Rintala RJ, Pakarinen MP. Persistent abnormal liver fibrosis after weaning off parenteral nutrition in pediatric intestinal failure. Hepatology 2013;58:729-738.

IV. Mutanen A, Heikkilä P, Lohi J, Raivio T, Jalanko H, Pakarinen MP. Serum FGF21 increases with hepatic fat accumulation in pediatric onset intestinal failure. J Hepatol 2014;60:183-190.

The publications are referred to in the text by their Roman numerals and are reprinted here with the permission of the publisher. In addition some unpublished data are presented.

Abbreviations

ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
APRI	AST-to-platelet ratio index
APTT	Activated partial tromboplastin time
AST	Aspartate aminotransferase
BMI	Body mass index
BSEP	Bile salt export pump
CIPO	Chronic intestinal pseudo-obstruction
CK7	Cytokeratin-7
DHA	Docosahexaenoic acid
EPA	Eicosapantaenoic acid
FGF21	Fibroblast growth factor 21
FXR	Farnesoid X receptor
GLP-1	Glucagon like peptide 1
GLP-2	Glucagon like peptide 2
GT	Glutamyl transferase
HbA1c	Glycosylated hemoglobin
HDL	High-density lipoprotein
H&E	Haematoxylin and eosin
HOMA-IR	Homeostasis model assessment for insulin resistance
HPEN	Home parenteral and enteral nutrition
HPN	Home parenteral nutrition
ICV	Ileocaecal valve
IF	Intestinal failure
IFALD	Intestinal failure associated liver disease
INR	International normalized ratio
ISO-BMI	Body mass index-for-age
LDL	Low-density lipoprotein
NASH	Nonalcoholic steatohepatitis
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit

PAS	Periodic acid-Schiff
PN	Parenteral nutrition
PNALD	Parenteral nutrition associated liver disease
PUFA	Polyunsaturated fatty acids
S-25-OHD	Serum 25-hydroxyvitamin-D
SBA	Small bowel atresia
SBS	Short bowel syndrome
SIBO	Small intestinal bacterial overgrowth
TPN	Total parenteral nutrition
TT	Tromboplastin time
VLBW	Very low birth weght

1 Introduction

Intestinal failure (IF) is characterized by the reduced capacity of the intestine to digest and absorb nutrients and fluids required for the maintenance of energy, fluid, electrolyte, and micronutrient balance as well as normal growth and development in children (Goulet O 2006). The most frequent causes of IF are short bowel syndrome (SBS) and intestinal motility disorders requiring long-term parenteral nutrition (PN) (Goulet O 2004). IF affects patients in all age groups from neonates to elderly with different etiologies in different ages. Congenital conditions, including necrotizing enterocolitis (NEC), gastroschisis, small bowel atresia (SBA), and midgut volvulus, cause neonatal IF requiring long-term medical and nutritional care (Finkel Y 2008). In children, IF most commonly results from midgut volvulus while mesenteric ischemia and Crohn's disease are among the leading causes in adults (Goulet O 2006, Pironi L 2006).

The development of PN in the 1960s led to significant improvements in the survival of IF patients (Wilmore DW 1968). More recent advances, including improved catheter care, awareness and aggressive treatment of septic episodes and small intestinal bacterial overgrowth (SIBO), new surgical approaches and improvements in enteral and parenteral nutrition, have significantly changed the treatment and improved outcomes of IF patients (Kelly DA 2006). Intestinal and multivisceral transplantation has become a treatment option to those with most severe and irreversible forms of IF (Fishbein TM 2006). Referral to multidisciplinary specialized IF treatment teams, including surgeons, gastroenterologists, nurses, and dieticians, has steadily shown to improve patient outcomes, including survival and quality of life (Sudan D 2005, Modi BP 2008).

Despite these advances in the management of IF, most patients suffer multiple complications during the course of the disease. Intestinal failure associated liver disease (IFALD) is a major complication and the leading cause of morbidity and mortality in pediatric and adult IF patients (Kelly DA 2006, Carter BA 2007, Pironi L 2012, D'Antiga L 2013). Etiology of IFALD is proposed as multifactorial, including duration and composition of PN, different components of PN, such as plant sterols, massive intestinal resection, SIBO, septic episodes, prematurity, low birth weight,

and lack of enteral nutrients (Beath SV 1996, Clayton PT 1998, Spencer AU 2005, Kelly DA 2010, D'Antiga L 2013). PN associated liver disease (PNALD), defined by liver biochemistry, occurs in up to 15 to 85% of neonates, children and adults on long-term PN (Kelly DA 2006, Pironi L 2012). After weaning off PN, liver biochemistry usually slowly normalize but liver histology may remain abnormal or the liver damage may even progress, as reported in few small series (Rodgers BM 1976, Dahms BB 1981, Vileisis RA 1982, Moss RL 1993, Hasegawa T 2002, Pichler J 2010, Yeop I 2012). Despite the various factors linked to development of IFALD, the exact mechanisms causing and maintaining the liver damage remain unknown. The aim of this thesis is to study the incidence of long-term PN and IFALD and to characterize liver function, risk factors of IFALD, and liver histopathology in neonates, children, and young adults with pediatric onset IF.

2 Review of the literature

2.1 Intestinal failure (IF)

2.1.1 History of parenteral nutrition and IF

As early as Egyptian times, liquid formulae, including wine, brandy, whey, and milk, were used to improve general health and to treat a range of ailments (Randall HT 1984). Until the delivery of food directly into the esophagus through a feeding tube was described in 1598, rectal infusion of nutrients was the only practical means to access the gastrointestinal tract of malnourished patients unable to maintain fluid and energy balance per orally (Harkness L 2002, Chernoff R 2006). The next major advancement occurred in the early seventeenth century when circulation was described by William Harvey and led to growing interest in intravenous administration of nutrients (Lagnas A 2008). In the late nineteenth century the first intravenous glucose and amino acid infusions were reported, but because of complications and limited caloric density, intravenous treatment was not safe (Wretlind A 1992). Until the late 1960s malnutrition secondary to IF was an irreversible and fatal condition. In 1968, Dudrick and Wilmore reported a successful long-term total PN treatment via superior vena cava catheter in a child with SBA and IF (Wilmore DW 1968, Dudrick SJ 1969). Soon after introduction of successful long-term PN, PN-associated complications affecting patient survival and outcome, including liver disease as a major complication, were reported (Peden VH 1971, Touloukian RJ 1973, Rager R 1975).

2.1.2 Definition of IF

IF is characterized by reduced capacity of the intestine to digest and absorb nutrients and fluids required for maintenance of energy, fluid, electrolyte, and micronutrient balance as well as normal growth and development in children (Goulet O 2006). This definition remains a matter of debate as various additional definitions have been suggested to describe the reduction of functional gut mass in patients with SBS after surgical bowel resection and in patients with disease-associated loss of absorption

due to intestinal motility disorders or enteropathies (O'Keefe SJ 2006). The definition of IF is usually combined with an estimate of absolute and/or percentage of remaining small bowel length and amount and/or duration of PN. In children, PN duration of over 30 days is often used as a sign of IF (Fitzgibbons SC 2010). The degree of IF may be defined according to the amount of PN required (Goulet O 2004). A massive resection leaving less than 40 cm or 30% of small bowel leads more likely to long-term dependence on PN in children (Beath SV 1996, Goulet O 2004, D'Antiga L 2013). Another approach is to estimate the remaining age-adjusted bowel length that takes into account the growth of the bowel (Struijs MC 2009). In most children, IF is considered reversible as adaptation may allow the discontinuation of PN, while preserving normal growth and development (Goulet O 2004). Some patients remain partially or totally dependent on PN and are thus considered to have irreversible IF (Goulet O 2004). Beside anatomical and nutritional criteria for defining IF, actual measurements of intestinal energy and wet weight absorption may be used to define IF in adults (Jeppesen PB 2000).

2.1.3 Incidence and prevalence of IF

Because of rarity of IF, varying definitions and multifactorial etiology the precise incidence and prevalence of IF in children and adults is unknown (Koffeman GI 2003, Youssef NN 2012). In adults, a retrospective European multicenter study reported estimates on IF incidence rates, expressed as patients/million inhabitants/years and defined by newly diagnosed cases of IF on home PN (HPN), ranging from 0.4 in Portugal to 3.0 in Netherlands (Bakker H 1999). The same data from European countries revealed the prevalence of IF in children over 16 years through adulthood to be 0.65 (patients/million inhabitants/year) in Spain, 1.1 in Portugal, 3.0 in Belgium, 3.6 in France, 3.7 in the United Kingdom and Netherlands, and 12.7 in Denmark (Bakker H 1999). Approximately 40 000 adult patients were dependent on HPN in 1994 in the United States (Howard L 1995). In a more recent study, 16 000 children were estimated to be on HPN in the United States (Spencer AU 2008).

In children, the most common type of IF in developed countries is SBS with an overall estimated incidence of from 3 up to 25 per 100 000 live births, being higher in

premature babies (Wallander J 1992, Guarino A 2003, Schalamon J 2003, Wales PW 2004, DeLegge M 2007, Casey L 2008, Wales PW 2010). In premature neonates with birth weight under 1500 g the average incidence of SBS was estimated to be 7/1000 (Cole CR 2008). These numbers are likely to be inaccurate because of ruling out IF patients weaned off PN and those with temporary need for PN, and including patients with diagnoses other than IF (O'Keefe SJ 2006). (Table 1)

Table 1. Incidence and prevalence of parenteral nutrition and short bowel syndrome in Europe and North America.

Reference	Country	Center(s)	Study design	Population	Scope	Incidence and prevalence
Howard L 1995	USA	HPEN registry	Retrospective	Adult	HPN	40 000 patients on HPN (year 1994)
van Gossum 1996	Europe	Multicenter	Retrospective	Adult	HPN	0.2-4.6/million inhabitants/year
Bakker H 1999	Europe	Multicenter	Retrospective	Adult	HPN	0.4-3/million inhabitants/year
Wales PW 2004	Canada	Population based, NICU	Retrospective	Neonates	SBS	22/1000 (2.2%) NICU admissions 25/100 000 live births 350/100 000 infants born <37 wk gestation
Colomb V 2007	France	Single center	Retrospective	Pediatric	HPN	20 new HPN patients/year
Cole CR 2008	USA	Multicenter	Prospective	Neonates	SBS in VLBW neonates	7/1000 (0.7%) VLBW infants
Salvia G 2008	Italy	Multicenter, NICU	Retrospective	Neonates	SBS	1/1000 (0.1%) live births 5/1000 (0.5%) NICU admissions

HPEN; home parenteral and enteral nutritio, HPN; home parenteral nutrition, NICU; neonatal intensive care unit, SBS; short bowel syndrome, VLBW; very low birth weight.

2.1.4 Causes of IF in children

The causes of pediatric IF vary according to the specific clinical setting surveyed. The etiology of IF can be divided into three categories: SBS, intestinal motility disorders, and congenital enteropathies (Fig 1 and Table 2). Moreover, the etiology of IF varies in different ages as NEC is the leading cause of IF in premature and/or low birth weight neonates while midgut volvulus, post traumatic resection, thromboembolic events, and inflammatory bowel disease may lead to IF later in childhood. In contrast, in adults SBS represents 75-80%, intestinal motility disorders 20%, and enteropathies 5% of IF and PN dependency (Bakonyi NA 2004, Pironi L 2006). In adults, the most common underlying diagnoses of IF are mesenteric ischemia and Crohn's disease. (Pironi L 2006, Goulet O 2006)

2.1.4.1 Short bowel syndrome

SBS results from massive resection of the small bowel and leads to malabsorption of nutrients, fluid, and electrolytes (Goulet O 2004). SBS is the most common condition causing IF (Goulet O 2006). In neonates, NEC is the most common cause of SBS (up to 55%), followed by intestinal atresia (10-25%), gastroschisis (13-20%), and midgut volvulus (6-14%) (Wales PW 2004, Casey L 2008, Salvia G 2008, Cowles RA 2010, Gutierrez IM 2011). In older children, SBS can result from midgut volvulus, post traumatic resection, thromboembolic events, and inflammatory bowel disease (Goulet O 2006). (Figure 2, Table 2)

Necrotizing enterocolitis

NEC is characterized by inflammatory bowel necrosis and is a major cause of mortality and morbidity of preterm infants. The proportion of neonates with NEC is significantly higher in preterm babies (born before 37 weeks of gestation) and those with low birth weight (under 1500 g) (Cole CR 2008, Navarro F 2009). NEC occurs in 90% of cases after starting of enteral feeds. Events leading to NEC are described as immature intestinal host defense and blood flow regulation, bacterial colonization, and inflammatory responses. The inflammatory changes of the bowel in NEC

primarily affect terminal ileum and proximal colon, but can also be widespread throughout the colon and small intestine. Treatment for NEC includes PN, nasogastric decompression, and broad-spectrum antibiotics. In case of perforation, persistent intestinal obstruction, deterioration despite maximal medical treatment, or development of intestinal stricture surgical treatment is indicated. In cases where major small bowel resection is required, infants with NEC will develop IF needing long-term PN. (Finkel Y 2008)

Gastroschisis

Gastroschisis is a congenital malformation characterized by evisceration of the bowel through an abdominal wall defect, generally to the right of intact umbilical cord, with no membrane covering the bowel. The prevalence of gastroschisis is estimated to be up to 4.0 per 10 000 births. Soon after birth, the reduction of the abdominal contents is done surgically through the abdominal wall defect and if not possible, a prosthetic silo is used. After surgical repair, feeding intolerance and need for long-term PN is highly prevalent in infants with gastroschisis caused by a combination of co-morbidities, including intestinal dysmotility, intestinal atresia (in 10-20% of infants with gastroschisis), perforation, and presence of necrotic bowel segments. Enteric nervous system damage seen in gastroschisis is suggested to relate on prenatal amniotic fluid exposure of the bowel. (Finkel Y 2008, Rodriguez L 2012)

Intestinal atresia

Intestinal atresia is a congenital malformation in which one or several bowel segments, classified according to localization as jejunoileal or colonic atresia, are narrow or absent. Multiple atresias may lead to SBS, IF, and need for long-term PN. Moreover, infants with intestinal atresia may experience intestinal dysmotility and feeding intolerance after surgical resection of the atretic segment and primary anastomosis. Delayed maturation of the myenteric plexus, alterations in intestinal pacemaker cells of Cajal and smooth muscle cells are suggested to result from fetal obstruction impairing development of the enteric nervous system. (Ozguner IF 2005, Finkel Y 2008, Dicken BJ 2011)

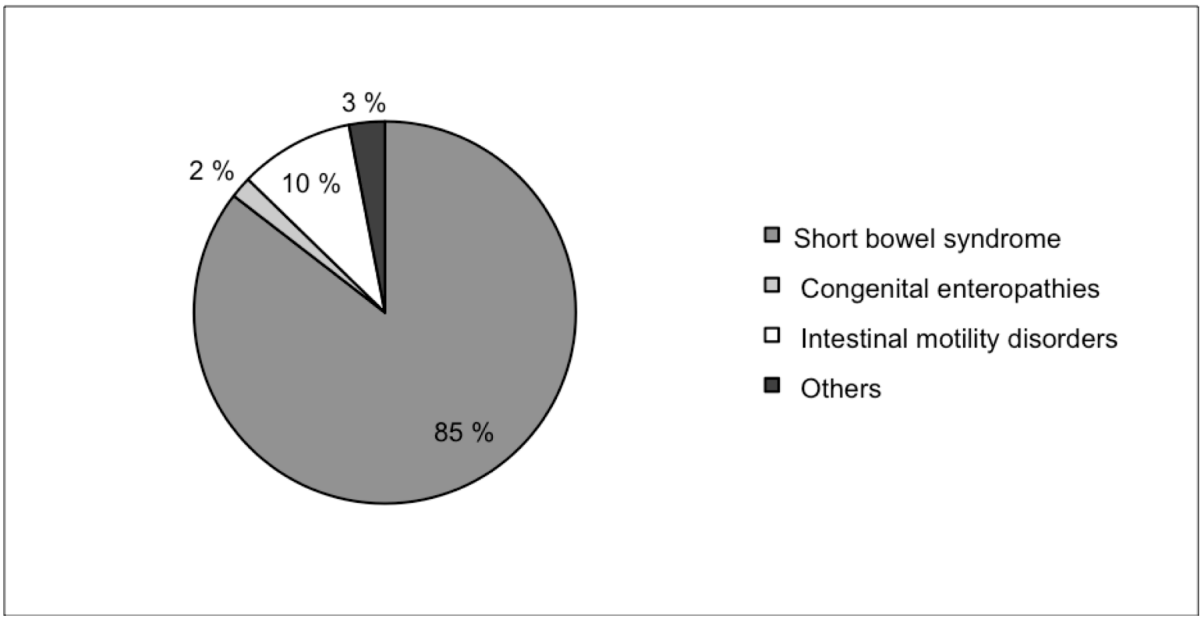

Figure 1. Distribution of diagnoses in patients with pediatric onset intestinal failure (n=165). Data adapted from multicentre study from Italy and single centre studies from North America (Casey L 2008, Salvia G 2008, Cowles RA 2010).

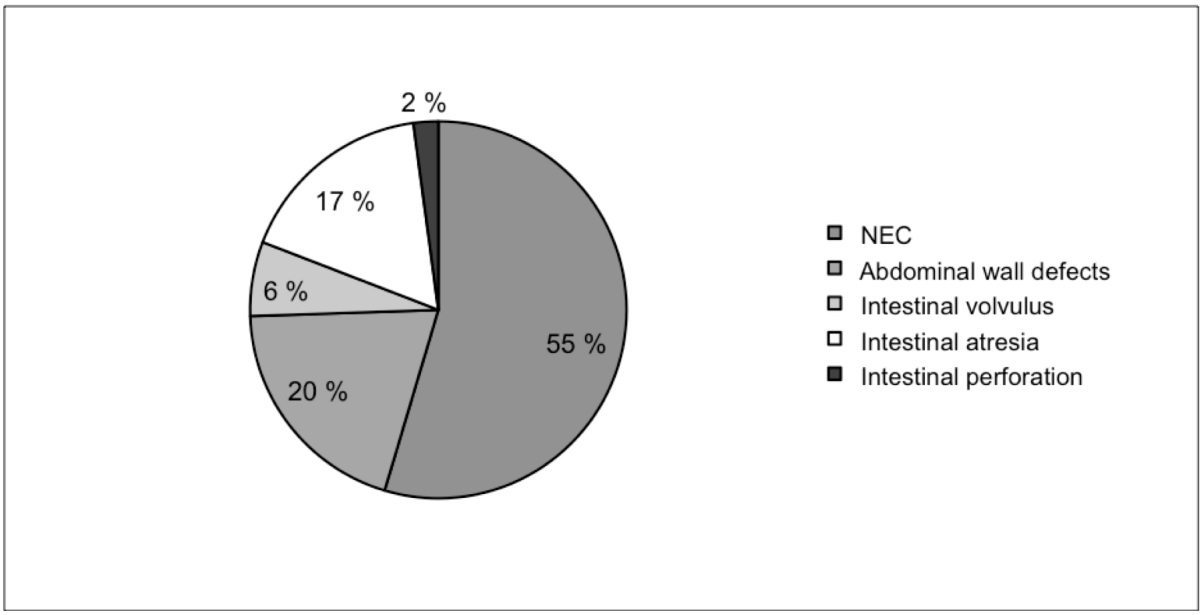

Figure 2. Distribution of diagnoses in children with short bowel syndrome (n=141). Data adapted from multicentre study in Italy and single centre studies in North America (Casey L 2008, Salvia G 2008, Cowles RA 2010). NEC; necrotizing enterocolitis.

Malrotation and midgut volvulus

Malrotation of the bowel occurs after incomplete rotation and fixation of the fetal bowel; typically the caecum does not reach the right ileal fossa. Furthermore, fixation of the gut may be aberrant, small bowel may be placed on the right side and colon on the left with band formation between the duodenum and right colon. Malrotation often causes bowel obstruction, and may lead to midgut volvulus and necrosis from midgut to proximal colon and eventually require major bowel resection leading to IF. (Finkel Y 2008)

2.1.4.2 Intestinal motility disorders

Intestinal motility disorders, characterized by bowel obstruction without luminal occlusion, may lead to IF and long-term PN even without any bowel resection (Connor FL 2006). Motility disorders account for up to 10% to 25% of pediatric patients with IF (Guarino A 2003, Pironi L 2006, Casey L 2008, Salvia G 2008, Cowles RA 2010). The main intestinal motility disorders include extensive Hirschsprung's disease and chronic intestinal pseudo-obstruction (CIPO). (Figure 1)

Extensive Hirschsprung's disease

Hirschsprung's disease is a congenital disorder affecting approximately 1 in 5000 newborns and is characterized by an interruption of the craniocaudal migration of neuroblasts and absence of ganglionic innervation of the affected bowel causing functional obstruction (Goulet O 2006). The length of the aganglionotic segment varies and in 75% of patients the aganglionosis is limited to the rectosigmoid (Chumpitazi B 2008). Rarely the aganglionosis extends to the proximal small intestine (extensive Hirschsprung's disease) leading to vomiting, bowel distension, malabsorption, and IF (Goulet O 2006). Extensive Hirschsprung's disease is the underlying diagnosis in 5% of pediatric patients with IF (Pironi L 2006, Mazariegos GV 2009, Gutierrez IM 2011). Moreover, even if the small bowel of a patient with Hirschsprung's disease has ganglion cells, it may have other neurologic abnormalities, for example involving Cajal cells with subsequent motility disorder, leading to IF (Vanderwinden JM 1996).

Chronic intestinal pseudo-obstruction (CIPO)

CIPO refers to a heterogeneous group of rare, severe and disabling disorders characterized by symptoms of bowel obstruction, including radiographic signs of dilated bowel with air-fluid levels, without lumen occluding-lesions (Di Lorenzo C 1999). In the United States, it is estimated that approximately 100 infants with congenital CIPO are born every year (Chitkara DK 2006). Symptoms associated to CIPO include abdominal distension (98%), vomiting (91%), constipation (77%), failure to thrive (62%), abdominal pain (58%), sepsis (34%), and diarrhea (31%) (Rudolph CD 1997). Intestinal dilatation and dysmotility incline the risk of SIBO and septic episodes and lead to malabsorption and malnutrition (Rudolph CD 1997). The diagnosis of CIPO is initially clinical, while radiographic studies are utilized to exclude mechanical obstruction (Chumpitazi B 2008).

CIPO may be congenital or acquired, primary or secondary (Rudolph CD 1997). In children, pseudo-obstruction is usually primary and congenital, with a small minority being hereditary familial processes, and symptoms present from birth or early infancy (Heneyke S 1999, Lehtonen HJ 2012). Based on histopathology and pattern of motility abnormalities, primary and secondary forms are classified as visceral myopathy and visceral neuropathy (Connor FL 2006). CIPO can also be secodary to mitochondrial disorders, diminished intestinal cells of Cajal, inflammatory condition or autoimmune disease (Chitkara DK 2006). No specific therapies for CIPO are available and special attention is placed on nutritional support and medical management (Chumpitazi B 2008). In selected CIPO patients, intestinal transplantation may be considered as the treatment of choice (Millar AJ 2009).

2.1.4.3 Congenital enteropathies

Congenital enteropathies are extremely rare disorders, including microvillus inclusion disease and intestinal epithelial dysplasia (tufting enteropathy), involving the development of intestinal mucosa and characterized by neonatal onset severe watery diarrhea leading to IF (Goulet O 2006). In autoimmune enteropathy, generally affecting neonates and children, although cases of adult onset have been reported,

autoimmune-mediated damage to the intestinal mucosa leads to villous atrophy of the intestine, intractable diarrhea, and IF (Montalto M 2009). Immunosuppressive therapies have been reported to be effective in pediatric and adult patients with autoimmune enteropathy (Gentile NM 2012). Most patients suffering from microvillus inclusion disease or intestinal epithelial dysplasia remain permanently dependent on PN and should be considered for intestinal transplantation (Goulet O 2006).

Table 2. Causes of intestinal failure at different ages.

Neonatal	Childhood	Adult
Short bowel syndrome	Short bowel syndrome	Short bowel syndrome
Necrotizing enterocolitis	Midgut volvulus	Ischemia
Gastroschisis	Trauma	Crohn's disease
Intestinal atresia	Mesenteric infarction	Volvulus
Midgut volvulus	Crohn's disease	Trauma
Vascular abnormalities/thrombosis	Vascular abnormalities/thrombosis	Tumor
	Tumor	
Motility disorders	Motility disorders	Motility disorders
Aganglionosis*	Aganglionosis*	CIPO
CIPO	CIPO	
Congenital enteropathies	Autoimmune enteropathy	Autoimmune enteropathy
Microvillus inclusion disease		
Intestinal epithelial dysplasia		
Autoimmune enteropathy		

Modified from Di Lorenzo C 1999, Goulet O 2004, Casey L 2008, Salvia G 2008, and Cowles RA 2010. *Extensive Hirschsprung's disease. CIPO; Chronic intestinal pseudo-obstruction.

2.1.5 Effects of bowel anatomy in IF

In all IF patients, the overall prognosis depends on the patient age at the time of resection, absolute and age-adjusted length of the remaining small bowel, but also the remaining anatomy and function of the intestine (DiBaise JK 2004, Spencer AU 2005, Goulet O 2006). In children with SBS, a short resection leaves more than 100-150 cm of small intestine, large resection between 40 to 100 cm, and a massive resection under 40 cm (Goulet O 2006). Goulet and co-workers reported that children with major small bowel resection and without ileocaecal valve (ICV) are more likely to remain dependent on PN; 40% of these children were on PN after 8 years (Goulet O 2005). In contrast, 80% of children with neonatal SBS and 40-80 cm of remaining small bowel and preserved ICV weaned off PN within one year (Goulet O 2005). In considering their prognosis, the age of the patients at the time of bowel resection should be considered as the growth potential of the bowel varies substantially according to patient age. For example preterm infants have great potential for bowel growth, as small bowel length doubles during the third trimester of gestation (Touloukian RJ 1983, Struijs MC 2009, Amin SC 2013). However, patients with intestinal motility disorder have a significant amount of intestine remaining and still may have severe and often irreversible IF (Di Lorenzo C 2010). Patients with intestinal motility disorders have demonstrated a lower probability of weaning off PN compared to patients with SBS (Goulet O 2006, Pakarinen MP 2009).

There are three major types of remaining bowel anatomy with different outcomes in IF patients: 1) end-jejunostomy, 2) jejunocolic anastomosis, and 3) jejunoileocolic anastomosis (DiBaise JK 2004). Patients with jejunoileocolic anastomosis usually have the most promising overall prognosis (DiBaise JK 2004). Anatomical factors associated with improved survival include intact ileocaecal valve and colon, takedown surgery after ostomy, and primary anastomosis (Quiros-Tejeira RE 2004). In contrast, ileal resection is poorly tolerated because of resulting malabsorption of bile acids (Youssef NN 2012). Resection of the ileocaecal valve facilitates the entry of colonic bacteria to populate the small intestine leading to SIBO and mucosal inflammation, thus impairing intestinal function, prolonging PN dependence, and promoting bacterial translocation and sepsis (Kaufman SS 1997, Yang H 2003, Goulet O 2004, Cole CR 2010). Enterectomy is associated with gastric

hypergastrinemia and hypersecretion (Williams NS 1985). In SBS, gastric hypersecretion can lead to fluid, electrolyte losses, peptic ulcers, and acidic environment in small bowel, resulting in bile acid precipitation with subsequent ineffective micelle formation, as well as inactivation of exocrine pancreatic enzymes (Kauffman SS 1997, Kocoshis SA 2010). Clinical features associated with different types of bowel resections are presented in Figure 3.

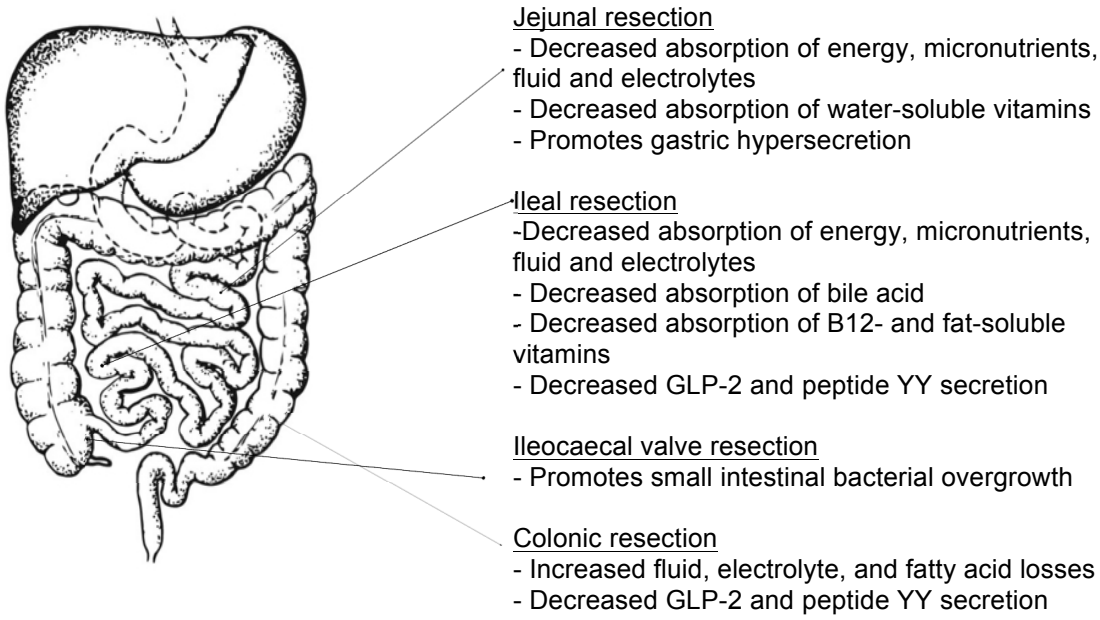

Figure 3. Clinical features associated to diffent types of intestinal resections. GLP-2; glucagon-like peptide 2.

2.1.6 Intestinal adaptation

Soon after major bowel resection, intestinal adaptation in the remaining bowel yields an increase in fluid and nutrient absorption to the level required to maintain energy and fluid balance (DiBaise JK 2004, Goulet O 2004). Based on animal and adult studies, changes in several interrelated systems, including morphological, functional, biochemical, and hormonal, appear to be involved in intestinal adaptation (Jeppesen PB 2002, Goulet O 2004). Adaptation includes bowel dilatation, muscular

hypertrophy, and mucosal hyperplasia (DiBaise JK 2004, Goulet O 2004). Mucosal hyperplasia is characterized by an increased number of enterocytes per unit, rate of enterocyte proliferation, villous height, and crypt depth (Goulet O 2004). Plasma citrulline, a non-essential amino acid mostly produced by enterocytes, is suggested as a marker of bowel function and adaptation in SBS (Bailly-Botuha C 2009, Stultz JS 2011).

The remaining small bowel can adapt and allow partial or total weaning off PN even when its remaining length below the ligament of Treitz is shorter than 15 cm (Goulet O 2004). The presence of colon has been shown to be beneficial in SBS patients; colon is able to absorb water, electrolytes and fatty acids, to slow intestinal transit, and to stimulate intestinal adaptation (DiBaise JK 2004). Moreover, the energy derived from bacterial fermentation of soluble fiber and carbohydrates to short-chain fatty acids in colon may be substantial (Nordgaard I 1996). The complex regulation of intestinal mucosal growth involves hormonal mediators such as glucagon like peptides 1 and 2 (GLP-1 and GLP-2), neurotensin, peptide YY, growth hormone, and insulin-like growth factor (Baksheev L 2000, Jeppesen PB 2002, Zhang W 2008, Zhu W 2009). Additionally, enteral feeding stimulates the release of enterotrophic hormones, including gastrin, cholecystokinin, and neurotensin, and may further promote intestinal adaptation (Goulet O 2004). Other factors potentially affecting adaptation include glutamine and epidermal growth factor, and short chain fatty acids produced by bacterial fermentation of dietary fiber in the colon (Noorgard I 1994, Briet F 1995, Wilmore DW 1999, Jeppesen PB 2002, Sham J 2002).

Various pharmacological agents, including growth hormone, peptide YY and GLP-2, have been studied to accelerate intestinal adaptation in SBS in animal models and in humans (Shulman DI 1992, Ulshen MH 1993, Benhamou PH 1997, Peretti N 2011, Jeppesen PB 2012, O'Keefe SJ 2013). In animal studies on bowel adaptation, growth hormone and peptide YY have shown promising results (Shulman DI 1992, Ulshen MH 1993, Benhamou PH 1997, Zhang W 2008, Zhu W 2009). However, in a prospective randomized study, growth hormone did not improve weaning off PN in children with SBS on long-term PN (Peretti N 2011). GLP-2 is a proglucagon-derived peptide produced by L-cells in the distal small bowel and colon in response to luminal nutrients (Goulet O 2004). In animal models, GLP-2 regulates gastric motility, gastric

acid secretion, intestinal hexose transport, and increases barrier function of the intestinal epithelium (Drucker DJ 2001, L'Heureux MC 2001). GLP-2 is shown to enhance the surface area of the mucosal epithelium by stimulation of the crypt cell proliferation, inhibition of the enterocyte apoptosis and crypt compartments (Drucker DJ 2001, L'Heureux MC 2001). In adult SBS patients with ileo-colic resection and impaired postprandial GLP-2 secretion, treatment with GLP-2 analogue, teduglutide, improved both weight gain and intestinal absorption of energy, and increased crypt depth and villus height (Jeppesen PB 2001). More recent results show that teduglutide is safe and reduces the number of days on PN in adult SBS patients (O'Keefe SJ 2013). These effects are explained by the decrease in GLP-2 levels due to distal small bowel and colonic resection and further reduction of adaptation (Scolapio JS 2001, Jeppesen PB 2002). The poor prognosis and relative lack of jejunal hypertrophy following ileal resection may be partly due to the resection of GLP-2 producing L-cells (Drucker DJ 2001, Topstad D 2001).

As intestinal adaptation progresses, the amount of PN required decreases and eventually intestinal autonomy is achieved and PN may be discontinued. Most children who survive SBS achieve independence from PN within the first five years, althought some continue to remain PN-dependent (Spencer AU 2008, Squires RH 2012). Children with IF may achieve intestinal autonomy even after many years on PN in contrast to adults who rarely wean off PN beyond two years on PN (Messing B 1999, Squires RH 2012). Long-term nutritional status and growth must be carefully monitored also after weaning off PN (Leonberg BL 1998, Goulet O 2005, Mutanen A 2013). Some children may experience malnutrition and growth failure after weaning off PN and require resumption of PN support, especially during puberty (Goulet O 2005, Miyasaka EA 2010).

2.1.7 Nutrition in IF

2.1.7.1 Parenteral nutrition (PN)

Patients with IF may receive all or some of the required nutrients and energy parenterally. The amount and composition of PN solution is adjusted based on patient age, weight gain, height-adjusted weight, head circumference, growth, enteral tolerance, hydration status, and liver function (Bines JE 2009, Pakarinen MP 2009). Short-term PN may be administered through a peripheral vein, but for long-term PN a tunneled central venous catheter is required. PN is started gradually, aiming at calculated energy requirements with avoidance of over feeding to prevent hyperinsulinemia, glucose intolerance, infectious problems, and IFALD (Cavicchi M 2000, Mirtallo J 2004). In metabolically stable patients, cyclic infusions with simultaneous enteral bolus feeds as tolerated are preferred to reduce risk of hyperinsulism and IFALD (Tillman EM 2013). Most parenteral energy is supplied as glucose together with an adequate amount of protein, including essential amino acids. Routine supplementation of fat- and water-soluble vitamins and trace elements as part of PN is recommended (Amin SC 2013). PN lipid emulsions, based on soy oil, olive oil, fish oil, or their different combinations, are rich sources of energy. Long-term use of PN lipids is linked to IFALD (discussed in more detail in chapter 2.2) (Carter BA 2007).

2.1.7.2 Enteral nutrition

Enteral nutrition is safer, cheaper, and easier to administer, but more importantly it has physiologic and metabolic advantages compared to PN (Bines JE 2009). Therefore, the management of IF includes early initiation and progressive advancement of enteral feeding as tolerated per orally or through percutaneous feeding tubes (DiBaise JK 2004, Pakarinen MP 2009). Enteral feeds should be tailored individually based on the remaining bowel anatomy and function (Bines JE 2009). Nutrients requiring little or no digestion to be absorbed from the intestine, such as hydrolysed proteins and medium-chain fatty acids, are prefered (Bines JE 2009).

Breast milk nutrition is advantageous in neonates with IF (Andorsky DJ 2001). To control fluid and electrolyte losses through high-volume intestinal excretions enteral sodium supplementation, aiming at urinary sodium levels above 30 mmol/L, is used (Pakarinen MP 2009). Patients with colon may benefit from a diet high in complex carbohydrates, possibly because of short chain fatty acid production by bacterial fermentation (Atia A 2011). Soluble dietary fibers, such as pectin or guar gum, are shown to impact on colon adaptation positively and increase stool consistency in animal models but lack of effects in humans (Kocoshis SA 2010, Jeppesen PB 2013). H_2-receptor blockers and proton pump inhibitors are used to suppress gastric acid secretion in SBS patients (Amin SC 2013). Anti-diarrhea agents, including loperamide, codeine, ursodeoxycholic acid, and cholestyramine, may be used to reduce gut motility and bile acid induced diarrhea in selected SBS patients (Dicken BJ 2011, Amin SC 2013, Jeppesen PB 2013).

2.1.8 Surgical treatment of IF

2.1.8.1 Autologous intestinal reconstruction

The goal of surgery is to correct the two major functional problems preventing enteral autonomy in IF: stasis in dilated bowel loops leading to SIBO and disordered motility (Jones BA 2010). The primary indication for autologous intestinal reconstructive surgery is to produce intestinal narrowing and lengthening in a patient who has reached a plateau in enteral tolerance despite maximal medical and nutritional management (Jones BA 2010). Many procedures aimed at enteral autonomy and weaning off PN have been developed, including reversed intestinal segments, intestinal valves, recirculating loops, longitudinal lengthening and tailoring (Bianchi), and serial transverse enteroplasty (STEP) (Waddell WR 1970, Persemedis D 1974, Bianchi A 1980, Stacchini A 1982, Panis Y 1997, Kim HB 2003). In the Bianchi procedure, the bowel and mesentery is divided longitudinally between mesenteric and antimesenteric border and finally anastomosed end to end, doubling the length of the bowel segment (Bianchi A 1980). The STEP procedure is based on alternative serial GIA-staplings on the mesenteric and antimesenteric border of the bowel creating a zig-zag pattern of longer and narrower bowel (Kim HB 2003). The Bianchi

and STEP procedures have been shown to improve long-term survival and enteral autonomy in selected children with severe SBS (Bianchi A 1980, Kim HB 2003, Jones BA 2010, Pakarinen MP 2013).

2.1.8.2 Intestinal transplantation

Intestinal transplantation has become a treatment option in patients with irreversible IF and associated life-threatening complications (Fishbein TM 2006). Referral to intestinal transplant assessment and consideration for transplant listing should be performed in case of children with irreversible IF, persistent hyperbilirubinemia and liver dysfuntion, thrombosis of 2 of the 4 upper body central veins (jugular and subclavian), or recurrent life-threatening septic episodes (Fishbein TM 2006, Beath S 2008, Avitzur Y 2010). Thus far, over 2000 intestinal transplantations have been performed worldwide; children account for more than 50% of the recipients (Avitzur Y 2010). The 1-, 3-, and 5-year survival of transplanted children is reported up to 80-95%, 65-84%, and 50-77%, being similar in patients with intestinal motility disorders and those with SBS, respectively (Avitzur Y 2010). Despite improvements in surgical technique, immunosuppressive medication, infection control, and understanding of intestinal transplant pathophysiology, the 10-year survival rate has stayed in 45 to 50% (Avitzur Y 2010). After intestinal transplantation, 80 to 100% of patients are able to wean off PN with improved quality of life (Sudan D 2004, Avitzur Y 2010, Ngo KD 2011, Pakarinen MP 2013).

2.1.9 Complications of IF

Children with IF and long-term PN are at risk for multiple potentially life-threatening complications such as liver disease, recurrent septic episodes, SIBO, metabolic derangement from gastrointestinal electrolyte losses, bone disease, and reduced axial growth affecting overall survival and quality of life of patients (Goulet O 2006). (Table 3)

Liver disease

IFALD, a major complication of IF, is further reviewed in chapter 2.2.

Recurrent septic episodes and loss of venous access

A central venous route for long-term PN is essential in the treatment of IF (Kelly DA 2006). Although catheter design and aseptic techniques have improved, central venous catheter related complications still have a significant impact on the outcome of IF patients (Andorsky DJ 2001, Pironi L 2003, Cober MP 2011). Squires and co-workers showed sepsis to be the second leading cause of death and reported 8.9 new catheter-related blood stream infections per 1000 catheter days among pediatric IF patients (Squires RH 2012). Poor catheter technique or insertion site and tunnel infections may lead to bacteremia and septicemia (Buchman AL 2003, Bines JE 2009, Cober MP 2011). The presence of a central venous catheter can lead to central venous thrombosis and even embolism and further loss of venous acces and inability to deliver PN (Buchman AL 2003, Cober MP 2011). Furthermore, IFALD has been shown to be more common in children with recurrent septic episodes related to either central line infections or bacterial translocation from the intestine due to SIBO (Candusso M 2002, Heine RG 2002, Kelly DA 2010).

Bacterial overgrowth

SIBO is a frequent complication of IF, which causes nutrient malabsorption, deconjugation of bile acids, impaired micellar solubilization, and fat malabsorption, as well as increased risk for bacterial translocation and sepsis (Goulet O 2006). In addition, SIBO may worsen the hepatotoxity of PN and provoke the development of IFALD (Wolf A 1989, Braxton C 1995, Kaufman SS 1997, Sondheimer JM 1998, Forchelli ML 2003, Kelly DA 2006). SIBO is likely to occur in IF patients without an ileocaecal valve leading to the entrance of colonic bacteria to the small bowel and those with poor motility and dilated bowel segments (Goulet O 2006). SIBO is associated with vomiting, diarrhea, bowel distension, metabolic acidosis, D-lactatic

acidosis, and failure to thrive (Goulet O 2006). In D-lactic acidosis intestinal bacteria produce D-lactate, which is not metabolized and can accumulate to toxic levels causing increased anion gap acidosis and sometimes neurological problems ranging from disorientation to coma (Amin SC 2013). To treat SIBO, rotating antibiotics, including metronidatzole, amoxicillin, ciprofloxacin, and fluconatzole, can be used empirically (Pakarinen MP 2009). In selected patients, performing an intestinal tapering and lenghthening procedures may be mandatory to help reduce SIBO (Goulet O 2006).

Impaired growth and weight gain

In children suffering from chronic diseases, progressive deteriorating axial growth, delayed pubertal maturation, and reduced pubertal growth spurt are often noted (Pozo J 2002, Simon D 2002). In a cross sectional study on patients with infantile-onset SBS at a mean age of 14.8 years, short stature and normal weight was reported (Olieman JF 2012). A retrospective study by Goulet and co-workers suggested that most neonates with SBS have normal growth pattern, experience normal pubertal development and achieve a normal adult height and weight (Goulet O 2005). As highlighted in figure 4, children with IF seem to be short and light weight for their age until puberty (height Z-scores from -1.4 to -0.8 in patients aged 1 to 12 years and weight Z-scores from -1.3 to -0.5 in patients aged 1 to 16 years, $P<0.05$), but most of them undergo normal pubertal development and attain normal adult height (Mutanen A 2013).

Metabolic bone disease

Low bone mineral contents have been described in children with SBS during and after weaning off PN (Dellert SF 1998, Olieman JF 2012). A majority of patients with pediatric onset IF at a median age of 9.9 years were shown to have decreased bone mineral density (Z-score ≤-1.0 in 70% of patients) with poor vitamin D status [serum 25-hydroxyvitamin-D (S-25-OHD) <50 nmol/l in 41% of patients] and secondary hyperparathyroidism (plasma parathyroid hormone >47 ng/l in 44% of patients)

equally during and after weaning off PN (Mutanen A 2013). Long-term PN, short remaining small bowel, increasing age, and insufficient calcium and vitamin D supplementation are risk factors for metabolic bone disease in pediatric onset IF (Mutanen A 2013).

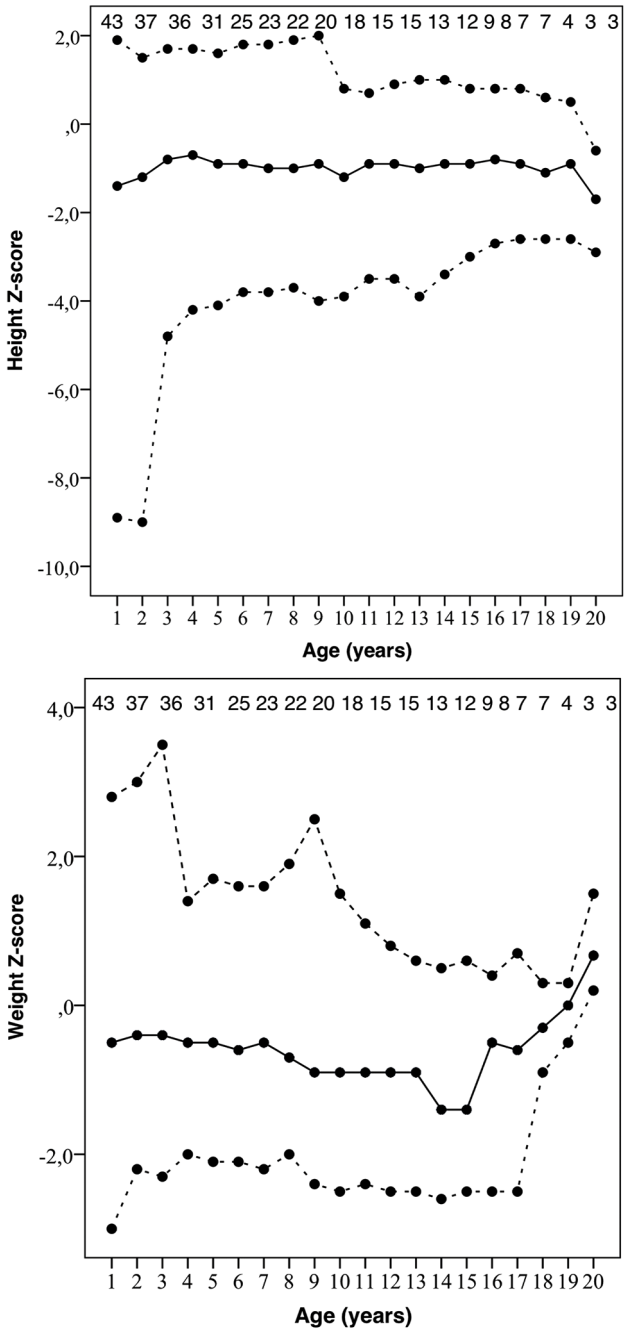

Figure 4. Height and weight (Z-scores) in intestinal failure patients from 1 to 20 years of age. The lines represent means and ranges. The number of patients at each measurement point is given above the curves. Adapted from Mutanen A et al. Horm Res Paediatr 2013;79:227-235 with permission of Karger.

Table 3. Complications associated to parenteral nutrition and intestinal failure.

Liver disease
Hyperbilirubinemia and abnormal liver enzymes
Histological cholestasis, steatosis and fibrosis
Cirrhosis
Liver failure
Cholelithiasis, biliary sludge, cholecystitis
Hepatocellular carcinoma
Recurrent sepsis
Central line associated
Bacterial translocation
Bacterial overgrowth
D-lactic acidosis
Loss of venous access
Thrombosis
Infection
Line dislodgement
Dehydration and electrolyte imbalance
Impaired growth in children
Metabolic bone disease
Renal dysfunction
Hyperoxaluria
Nephrolithiasis
Chronic renal insufficiency
Psychosocial

Modified from Goulet O 2006, Kelly DA 2006, Duro D 2008, and Bines JE 2009.

2.1.10 Patient outcomes in IF

Mortality associated with pediatric IF has decreased from 40% to under 10% in specialized centers and likely reflects the impact of multidisciplinary intestinal rehabilitation programs (Andorsky DJ 2001, Schalamon J 2003, Spencer AU 2005, Sudan D 2005, Thompson JS 2006, Diamond IR 2007, Torres C 2007, Cole CR 2008, Modi BP 2008, Hess RA 2011, Pakarinen MP 2013). Because mortality and patient outcomes are linked to the presence of liver disease, incidence of septic episodes, residual bowel anatomy, patient age, dependence on PN, and underlying disease, the reported mortality rates vary depending on the patient population

studied (Squires RH 2012). In adults with benign etiology of long-term home PN, the overall 5-year survival is estimated to be 75% depending on the underlying disease, age of patient, and remaining bowel anatomy (Staun M 2009). In mixed patient populations of children on HPN, the mean survival rate is reported to be generally better than in adults, with approximately 90% at 5 years (Pironi L 2012). In contrast, the survival rates of children listed for intestinal transplantation are as low as 35% at 3 years (Bueno J 1999, Gupte GL 2007). In a retrospective analysis of a multicentre cohort of 272 pediatric IF patients the cumulative incidences for enteral autonomy, death, and intestinal transplantation were 44%, 26%, and 23% by 36 months (Squires RH 2012).

Major negative prognostic factors in pediatric IF include presence of persistent liver disease, residual bowel length under 15 to 20 cm or under 10% of age-adjusted normal, presence of SIBO, prematurity and underlying disease (gastroschisis, mucosal inflammation) (Andorsky DJ 2001, Quiros-Tejeira RE 2004, Duro D 2008, Mian SI 2008, Goday PS 2009). In children with CIPO, onsets of symptoms before one year of age, involvement of urinary tract, midgut malrotation, or a myopathic histology are indicators of poor prognosis (Bond GJ 2004). IF children with residual bowel length over 40 cm and ileocaecal valve and/or colon preserved are more likely to have better outcomes (Andorsky DJ 2001, Quiros-Tejeira RE 2004, Duro D 2008, Mian SI 2008, Goday PS 2009).

2.2 Intestinal failure associated liver disease (IFALD)

2.2.1 Definition of IFALD

IFALD is defined as liver disease of clinical spectrum ranging from mild cholestasis, steatosis and fibrosis, to liver failure with cirrhosis, portal hypertension and coagulopathy (Kelly DA 2006, Tillman EM 2013). Clinical diagnosis of IFALD includes repeatedly abnormal liver biochemistry, such as conjugated bilirubin, alanine aminotransferase (ALT), and glutamyl transferase (GT), while ruling out other potential causes of liver disease, such as medication, viral etiology, mitochondrial disease, thyroid disease, hemolysis, cystic fibrosis, iron storage disease, α1-antitrypsin deficiency, and biliary tract abnormalities (Kelly DA 2010, Tillman EM 2013). The diagnostic limits for liver biochemistry values in IFALD vary, but >1.5 increase above the upper limit of normal for at least 2 of the 3 parameters, including conjugated bilirubin, ALT and GT, is widely used (Kumpf VJ 2006). Liver biopsy remains the gold standard for assessing histopathological changes in chronic and acute liver disease, including IF patients, although histopathological definitions of IFALD vary (Postuma R 1979, Benjamin DR 1981, Cohen C 1981 Wolfe BM 1988, Mullic FG 1994, Loff S 1999, Cavicchi M 2000, Peyret B 2011).

2.2.2 Risk of IFALD and IFALD related morbidity and mortality

IFALD is a major complication and the leading cause of morbidity and mortality in pediatric and adult IF patients (Kelly DA 2006, Carter BA 2007, Pironi L 2012, D'Antiga L 2013). The incidence of abnormal liver biochemistry, abnormal liver histology and liver failure varies between studies, probaply because of differencies in patient populations, underlying diseases, definition of IFALD and PNALD, duration of PN, and amount and composition of PN used. PNALD, defined by liver enzymes, is reported to occur in 15 to 60% of children and up to 85% of neonates (Kelly DA 2006, Pironi L 2012, Lauriti G 2014). In children with IF, the cumulative percentage of survival is significantly lower in patients with cholestasis compared to patients without cholestasis; 79% vs 95% at 1 year and 73% vs 88% at 3 years (Squires RH 2012). PNALD is seen more often and is associated with higher mortality in infants with

prematurity and low birth weight (Beath SV 1996, Christensen RD 2007, Pironi L 2012). Among children with IFALD, end stage liver disease is reported to occur in 3% up to 25% (Colomb V 2007, Nasr A 2007). End stage liver disease has a mortality rate approaching 100% within 1 year of diagnosis if weaning off PN is not achieved or liver and/or intestinal transplantation is not received (Chan S 1999, Wales PW 2005, Cowles RA 2010, Wada M 2013).

2.2.3 Etiology and pathophysiology of IFALD

The etiology of IFALD is multifactorial. In children, IFALD is related to duration, amount, and composition of PN, recurrent septic episodes, remaining bowel anatomy, SIBO, lack of enteral feeds, prematurity, and low birth weight. (Kelly DA 2006) (Table 4)

Duration, amount and composition of parenteral nutrition

The frequency of liver dysfunction, cholestasis and liver fibrosis is shown to increase with duration of PN (Beale EF 1979, Kelly DA 2006, Peyret B 2011). PN lipids and plant sterols have been implicated in the pathogenesis of IFALD (Clayton PT 1993, Clayton PT 1998, Btaiche IF 2002, Carter BA 2007, Llop M 2008). A lipid dose <1 g/kg/day is found to be beneficial in preventing IFALD (Cavicchi M 2000, Mirtallo J 2004). Furthermore, PN fat reduction is a clinically applied approach when signs of IFALD occur to prevent further liver damage (Cober MP 2010, Cober MP 2012).

PN lipid emulsions traditionally contain soy oil-based lipids with high concetrations of polyunsaturated fatty acids (PUFA), omega-6 fatty acids, and plant sterols, including stigmasterol, avenasterol, sitosterol and campesterol (Gabe SM 2013). Alternative lipid emulsions have been developed by partially or totally replacing soy oil-based lipid with olive oil- and/or fish oil-based emulsions. Olive oil-based lipid emulsions are rich in omega-6 fatty acids and oleic acid, a monounsaturated fatty acid that is less prone to lipid peroxidation than PUFA, and contain less plant sterols compared to soy oil-based lipid emulsion (Carter BA 2007, Gabe SM 2013). Fish oil-based emulsions

contain omega-3 fatty acids and long-chain PUFAs eicosapantaenoic acid (EPA) and docosahexaenoic acid (DHA) and are devoid of plant sterols (Carter BA 2007). The downstream products of omega-6 fatty acids are proinflammatory, including leukotriens, prostaglandins, and tromboxane 2 and may associate to PNALD (Gabe SM 2013). In contrast, EPA and DHA in fish oil are thougt to have anti-inflammatory and hepatoprotective potential via its production of anti-inflammatory leukotriens, prostaglandins, and thromboxane A3 (Gabe SM 2013).

Plant sterols are likely to be significant contributors to cholestasis in IF patients on PN (Gabe SM 2013). The serum levels of plant sterols have been shown to markedly increase in adults and children on soy oil-based PN with biochemical evidence of IFALD (Clayton PT 1993, Clayton PT 1998, Cavicchi M 2000, Hallikainen M 2008, Llop M 2008). Moreover, transition from soy oil-based PN to olive oil-based PN containing less plant sterols have been shown to decrease elevated liver enzymes, where as fish oil-based emulsions have been suggested to reverse cholestasis in neonates (Pálová S 2008, Puder M 2009). At the molecular level, plant sterols, especially stigmasterol, have been shown to antagonize nuclear farnesoid receptor X (FXR)-mediated bile acid homeostasis in hepatocytes and associate with hepatocyte damage (Wang H 1999, Carter BA 2007). Despite promising results on fish-oil based PN, devoid of plant sterols, it is not an universal solution for IFALD and an optimal parenteral lipid emulsion remains yet to be found (Tillman EM 2013).

Other components of PN have also been studied for significance in IFALD. Nutrient excess in the form of either parenteral glucose or amino acids have been associated with changes in liver histology and biochemistry, including elevated transaminases, steatosis, and cholestasis (Sheldon GF 1978, Carter BA 2007). Therefore, it is recommended that a balanced PN provide 70-85% of non-protein calories as carbohydrate and 15-30% as fat (Mirtallo J 2004). In neonates and children, specialized age-specific formulations of crystalline amino acids are routinely used to contain a balanced source of essential and nonessential amino acids (Spencer AU 2005).

Recurrent septic episodes

IFALD is more common in children with recurrent septic episodes, whether it is related to central line infections or bacterial translocation from the intestine (Braxton C 1995, Kaufman SS 1997, Sondheimer JM 1998, Forchelli ML 2003, Kelly DA 2006). A higher number of septic episodes has been shown to predict elevated bilirubin levels and cholestasis (Beath SV 1996). Intestinal stasis induced SIBO leads to reduction of bile flow, production of secondary bile salts, bacterial translocation, and sepsis, and therefore is suggested to affect development of IFALD (Kelly DA 2006).

Bowel anatomy and bacterial overgrowth

The absolute and percentage of age-adjusted small bowel length are predictive for survival, weaning off PN and cholestasis in children with IF (Spencer AU 2005). Peyret and co-workers reported in a retrospective study of 42 children on PN that histological liver fibrosis was associated with shorter length of small bowel (Peyret B 2011). Intestinal dysmotility and lack of ICV leading to SIBO may impair intestinal immunity, decrease intestinal immonoglobulin A levels, enchance production of hepatotoxic cytokines and increase intestinal permiability (Btaiche IF 2002). IFALD is shown to be more common in children with recurrent septic episodes related either to central line infections or bacterial translocation from the intestine due to SIBO (Candusson M 2002, Heine RG 2002, Kelly DA 2010). Furthermore, SIBO may worsen the hepatotoxity of PN and provoke development of IFALD (Wolf A 1989, Braxton C 1995, Kaufman SS 1997, Sondheimer JM 1998, Forchelli ML 2003, Kelly DA 2006).

Prematurity and low birth weight

Several case series have shown that prematurity and low birth weight are significant risk factors for PNALD and IFALD; The lower the gestation age, the higher the elevation of serum bilirubin concentrations and the more rapid and severe the

development of liver disease and jaundice (Pereira GR 1981, Beath SV 1996, Spencer AU 2005, Cristensen RD 2007, Robinson DT 2008). Premature infants have reduced bile salt pool, uptake and synthesis of bile salts, and entero-hepatic circulation compared to full term babies (Watkins JB 1975, Wessel JJ 2007). Moreover, the liver of premature babies is more susceptible to toxic damage (Watkins JB 1975, Wessel JJ 2007). Due to the physiologic immaturity of the neonatal liver and hepatic excretory system, premature neonates are likely to be at great risk for IFALD with increased mortality (Kelly DA 2010).

Lack of enteral feeding

IFALD is more frequent in children who are unable to tolerate enteral feeds compared to those who tolerate even partial enteral nutrition (Beath SV 1996, Kaufman SS 2002, Kelly DA 2006). In IF, the lack of enteral feeds may lead to reduced gastrointestinal hormone secretion, including GLP-2, peptide YY, gastrin, motilin, glucose-dependent insulinotrophic polypeptide, secretin, pancreatic polypeptide, cholecystokinin, and vasoactive intestinal peptide, decreased bile flow, formation of biliary sludge, intestinal stasis and SIBO which can further promote IFALD (Greenberg G 1981, Shulman RJ 2000). Interestingly, the use of cholecystokinin-octapeptide failed to reduce the incidence of PN associated cholestasis in a multicenter double-blind randomized controlled trial in neonates on PN (Teitelbaum DH 2005). Lack of enteral nutrition and use of total PN resulted in loss of villous height and decline in epithelial growth in intestine in animal models (Shou J 1994, Niinikoski H 2004). Furthermore, use of total PN leads to loss of epithelial barrier function in experimental models and in humans (Buchman AL 1995, Peterson CA 1997, Yang H 2003). This loss of intestinal barrier function may result in endotoxins and even bacterial translocation to the systemic circulation (Yang H 2009).

Table 4. Risk factors of intestinal failure associated liver disease.

Long-term parenteral nutrition
Components of parenteral nutrition
Excess lipids (>1 g/kg/day)
Plant sterols
Excess dextrose
Excess amino acids
Toxic components of PN (+/- peroxidation)
Lack of essential fatty acids
Lack of essential amino acids
Bacterial overgrowth
Septic episodes
Bacterial translocation
Central line associated
Bowel anatomy associated factors
Short remaining small bowel
Lack of ileocaecal valve
Intestinal inflammation
Prematurity and low birth weight
Lack of enteral feeding

Modified from Beath SV 1996, Kelly DA 2006, Goulet O 2006, and Carter BA 2007.

2.2.4 Clinical features of IFALD

Elevated liver biochemistry values, including ALT, AST (aspartate aminotransferase), GT, total and conjugated bilirubin, are the earliest sings of liver dysfunction in IF (Btaiche IF 2002). The time to onset of liver dysfunction after starting PN is difficult to predict and varies with the precence or absence of different risk factors of IFALD (Benjamin DR 1981). In infants on soy oil-based PN serum conjugated bilirubin, alkaline phosphatase (ALP), and AST concentrations were elevated after 2.2 ± 0.2, 4 ± 0.8, 4.6 ± 0.7 weeks on soy oil-based PN (Postuma R 1979). As liver dysfunction progresses, a fall in albumin and prolonged coagulation occurs (Kelly DA 2010). With development of end-stage liver disease, cirrhosis, splenomegaly with thrombocytopenia, ascites, and varices are encountered (Kelly DA 2010).

2.2.5 Liver histology

2.2.5.1 Normal liver histology

Normal liver has a regular structure based on portal tracts, efferent veins, and hepatocytes arranged in plates and separated by a sinusoidal labyrinth. The smallest portal tracts contain portal venules, hepatic arterioles and interlobular bile ducts. Blood from both venules and arterioles passes through the sinusoidal system to the efferent hepatic venules, larger veins, and finally the vena cava. Bile flows from the smallest ducts to larger ducts and through the common bile duct to the small intestine. To describe the functional relationship between the various structures of the liver, the two most widely used models are the classic lobule and the Rappaport's acinus (Figure 5). The classic lobule has an efferent venule in the centre and portal tracts in the periphery. The acinus is based on a terminal portal tract, with blood passing from this, through successive less well-oxygenated parenchymal zones 1, 2 and 3, to efferent venules. Hepatocytes localized around the portal triad (portal vein, bile duct, and artery) display different metabolic activities than those lining the central vein; for example, cells closest to the portal triad perform most of the cholesterol synthesis, have the best circulation, and the most nutrients, but also suffer first from bile flow obstruction (Jungermann K 1996). Pericentrally localized hepatocytes perform glucose metabolism and are most vulnerable to ischemia (Jungermann K 1996). (Lefkowitch JH 2010)

Evaluation of liver histology is performed using several stains after the liver biopsy is fixed in formalin, embedded in paraffin, and sliced. Haematoxylin and eosin (H&E) and Periodic acid-Schiff stains (PAS) are used for screening of basic liver structure, fibrosis, steatosis, and inflammatory infiltrate. Reticulin stain is helpful in the accurate assessment of structural changes and detection of portal and lobular fibrosis. In cholestasis, hepatocellular swelling and pallor and accumulation of copper in affected cells is seen in copper staining. Ductular reaction containing proliferated ductule-like structures is seen in cytokeratin-7 (CK7) staining, normally not demonstrable in hepatocytes, in cholestatic liver. Iron stain enables iron but also bile, lipofuscin and other pigments to be evaluated. (Lefkowitch JH 2010)

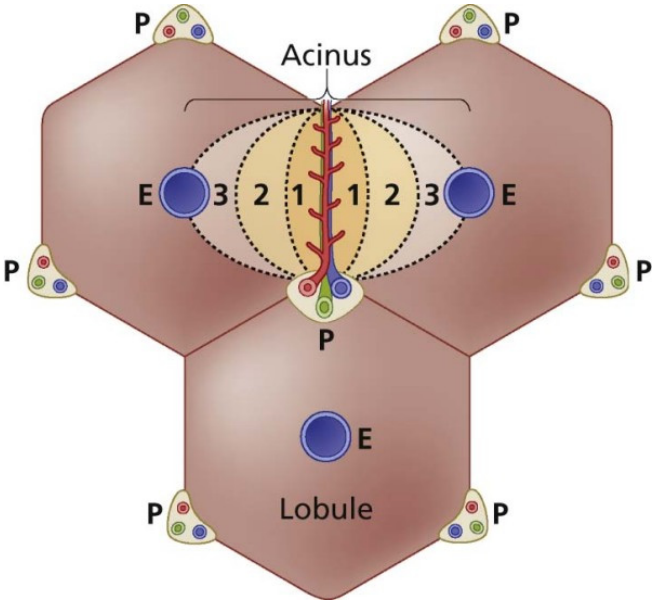

Figure 5. Diagrammatic representation of a simple acinus. The acinus divided into zones 1, 2, and 3, with three adjacent lobules for comparison in the figure. Portal tracts (P) contain bile ducts, arterioles and venules. E, efferent vein (central vein or terminal hepatic venule). Adapted from Lefkowitch JH, eds. Liver biopsy interpretation. Volume 1. 8th ed. Philadelphia: Elsevier Saunders, 2010:18 with permission of Elsevier.

2.2.5.2 Liver histology in IFALD

Data available on liver histology in IFALD is limited as most studies are retrospective and often include patients on PN with other underlying diagnoses than IF (Table 5). The hepatic dysfunction associated with long-term PN differs in adults and children as infants have more histological cholestasis and adults have steatosis (Postuma R 1979, Benjamin DR 1981, Wolfe BM 1988, Mullic FG 1994, Loff S 1999, Cavicchi M 2000). Retrospective studies on children on long-term PN with mixed underlying diagnoses have reported liver steatosis, fibrosis, and cholestasis in up to 68%, 94%, and 100% of patients (Postuma R 1979, Cohen C 1981, Peyret B 2011). In autopsy samples of 31 children on total PN, Cohen and co-workers described the liver damage to progress from steatosis and portal inflammation to canalicular cholestasis and ductular proliferation, further to portal fibrosis, and eventually micronodular

cirrhosis (Cohen C 1981). Longer duration of PN is shown to relate on progression of histological cholestasis (Zambrano E 2004). Fibrosis associates with short remaining small bowel and long duration of PN in children with IF on long-term PN (Peyret B 2011).

Parenteral plant sterols are likely to contribute on IFALD, but only a limited number of animal and human studies linking liver histology and PN plant sterols are available. Loff and co-workers compared liver histology of ten infants on PN to a rabbit model of IF (Loff S 1999). The study revealed similar histological findings, including fibrosis and bile duct proliferation, in neonates and rabbits after 1 to 4 weeks on soy-based PN (Loff S 1999). In neonatal piglets on total PN, including amino acids, dextrose and soy oil-based lipids, liver steatosis and hepatocellular apoptosis was seen after 7 PN days (Wang H 2006). Interestingly, a rabbit model comparing parenteral soy oil-, olive oil- and soy oil-based emulsion with added fish oil showed more fibrosis in rabbits receiving the latter (Kohl M 2007). In children with IF transition from soy-based PN lipids to fish oil results in reversal of biochemical cholestasis, but histological fibrosis is reported to persist (Gura KM 2008, Soden JS 2010).

Although serum liver enzymes usually slowly normalize after weaning off PN, the liver changes may persist or even progress (Rodgers BM 1976, Cohen C 1981, Dahms BB 1981, Moss RL 1993, Hasegava T 2002, Pichler J 2010). Dahms and co-workers described a complete resolution of histological portal inflammation and bile duct proliferation, but persistent cholestasis and periportal fibrosis while serum liver biochemistry normalized in six neonates after weaning off PN (Dahms BB 1981). Moss and co-workers found progression of liver steatosis and fibrosis while weaning off PN (Moss RL 1993). Hasegava and co-workers reported resolution of liver steatosis and cholestasis but persistent fibrosis after isolated small bowel transplantation and weaning off PN in a child with IFALD (Hasegawa T 2002). Case reports of liver malignancy in patients with IFALD have been published (Vileisis RA 1982, Yeop I 2012). However, the type and reversibility of the liver injury and the effect of previously described risk factors of IFALD on liver histology during and after weaning off PN in IF patients are still insufficiently characterized (Table 5).

Table 5. Studies on liver histology in patients on long-term parenteral nutrition.

Reference	Population	n	Study design	Duration of PN	Liver histology		
				Range	Fibrosis	Steatosis	Cholestasis
Rodgers BM 1976	Infants on PN	11*	Retrospective	NR	NR	NR	11/11 (100)
Postuma R 1979	Neonates on TPN	14*	Retrospective	NR	12/14 (86)	NR	12/14 (86)
Cohen C 1981	Infants on TPN	31*	Autopsy samples	1- >150 mo	11/31 (35)	21/31 (68)	19/31 (61)
Dahms BB 1981	Infants on TPN	11*	Retrospective	1.5-4.0 mo	10/11 (91)	NR	11/11 (100)
Moss RL 1993	Infants on TPN	30* †	Retrospective	NR	9/30 (30)	NR	22/30 (73)
	Liver biopsy	23*					
	Autopsy samples	13*					
Misra S 1996	Children on TPN	26*	Retrospective	>36 mo	4/8 (50)	NR	1/8 (13)
	Liver biopsy	8*					
Loff S 1999	Neonates on PN	10*	Retrospective	1-4 mo	NR	NR	10/10 (100)
Cavicchi M 2000	Adults with IF on home PN	57	Prospective cohort	6-198 mo	NR	40/57 (70)	38/57 (67)
Zambrano E 2004	Neonates on TPN	24*	Autopsy samples	0.1-4.4 mo	17/24 (71)	7/24 (29)	19/24 (79)
Fitzgibbons S 2010	Children on PN	66*	Retrospective	3.0-8.4 (mo, IQR)	74/83 (89)	NR	NR
	Liver biopsy	83* †					
Peyret B 2011	Children with IF on home TPN	42	Retrospective	96±9.7 mo (mean, sem)	17/18 (94)	7/18 (39)	7/18 (39)
	Liver biopsy	18					
Naini BV 2012	Children and adults on TPN	89*	Retrospective	7 days-29 years	85/89 (96)	35/89 (39)	72/89 (81)

Data are presented as frequency and percentage (%) of patients examined. mo; months, n; number of patients, IQR; interquartile range, sem; standard error of mean, PN; parenteral nutrition, TPN; total parenteral nutrition, NR; not reported. † Some of the patients had more than one biopsy recorded. * Underlying diagnoses other than intestinal failure, for example congenital heart disease, prolonged endotracheal intubation and intensive care, sepsis, bronchopulmonary dysplasia, esophageal atresia, pancreatitis and ileus, included in the study population.

2.2.6 Serum fibroblast growth factor 21 and liver steatosis

Fibroblast growth 21 (FGF21), a hormone primarily secreted by the liver, regulates glucose and lipid metabolism and relates to liver steatosis in animals and humans (Itoh N 2004, Kharitonenkov A 2005, Kharitonenkov A 2007, Xu J 2009, Fisher FM 2011). In experimental animal models administration of recombinant FGF21 results in reduced levels of blood glucose, insulin, triglycerides, low-density lipoprotein (LDL) cholesterol and high-density lipoprotein (HDL) cholesterol, and reversed fat accumulation in the liver (Kharitonenkov A 2005, Xu J 2009). In humans, increased serum FGF21 levels are associated with obesity, insulin resistance, impaired glucose tolerance, hypertriglyceridemia, and liver steatosis (Chen WW 2008, Zhang X 2008, Chavez AO 2009, Li H 2009, Dushay J 2010, Angelin B 2012). In adults with nonalcoholic fatty liver disease, serum and liver FGF21 concentrations, as well as hepatic expression of FGF21, are increased in parallel with liver steatosis (Chen WW 2008, Dushay J 2010). Serum FGF21 was previously suggested as a biomarker of hepatic lipid accumulation in adults with type 2 diabetes mellitus and nonalcoholic fatty liver disease (Chen WW 2008, Dushay J 2010). The significance of serum FGF21 in IF and associated liver disease remains unclear.

3 Aims of the study

Various risk factors have been linked to IFALD, but the type and reversibility of histological liver injury and its risk factors are insufficiently characterized. In this thesis, studies on the incidence of long-term PN and IFALD, risk factors of IFALD, including parenteral plant sterols, and liver histology were performed to determine the long-term effects of pediatric onset IF on liver function and histology.

The specific aims were:

1) To investigate interrelations between serum non-cholesterol sterols, including plant sterols and cholesterol precursors, PN, liver function and liver histology in pediatric onset IF

2) To study the incidence of long-term PN, associated liver disease, and other associated complications in neonates and children in Finland

3) To characterize the risk factors of IFALD and long-term changes in liver histopathology and evaluate the effects of risk factors of IFALD on liver histology during and after weaning off PN in pediatric onset IF

4) To assess serum FGF21 levels in relation to liver steatosis, serum lipids, and glucose homeostasis in pediatric onset IF

4 Patients and methods

4.1 Patients (I-IV)

Study I

In study I, all patients with pediatric onset IF stabilized on long-term PN treated in Children's Hospital, Helsinki University Central Hospital, in 2010 were invited to participate in the follow-up study. Eleven patients (100%) at mean age 6.3 years (range 1.6-16) were recruited. (Table 6)

Study II

In study II, all pediatric patients (age <17 years) receiving long-term PN (>28 days), including daily infusions of carbohydrates and protein with or without lipids, treated in Finland's five University Hospitals (Helsinki, Tampere, Turku, Kuopio and Oulu) between November 2009 and November 2010 were prospectively identified and recruited in the study. Patients with a malignant primary disease were excluded. The pediatric units of the five University Hospitals treat all prematurely born newborns and pediatric patients on prolonged PN in Finland allowing all-inclusive nationwide patient identification. Altogether 39 patients on long-term PN, including 28 neonates and 11 children, were recruited. At inclusion, the mean ages of neonates and older children were 50 days (range 20-126) and 6.9 years (2.1-16.6). (Table 6)

Studies III and IV

For studies III and IV, the medical records of patients with pediatric onset IF treated in Children's Hospital, Helsinki University Central Hospital, from January 1984 to August 2010 were reviewed. A total of 56 patients were identified, and 52 of them were alive. Eligible patients were invited to participate in the cross-sectional study. Of the eligible patients, 38 patients (73%) at median age of 7.2 years (range 0.2-27) participated in study III, of which 35 (67%) patients also participated in study IV. (Table 6)

4.2 Controls (I-IV)

In study I, II, and IV, healthy day-case surgery patients (i.e. inguinal hernia, umbilical hernia, undescended testes) matched for age and sex without evidence of intestinal disease, diabetes, or dyslipidemia served as controls for serum non-cholesterol sterols and serum FGF21 measurements. In study II, neonates on long-term PN were compared to healthy controls as well as older children on PN. In study III, the liver histology of IF patient was compared to liver biopsies of age-matched liver transplant donors. (Table 6)

4.3 Methods (I-V)

Study design and data collection (I-IV)

Collection of clinical data included underlying diagnoses, gestational age, birth weight and height, surgical procedures and complications, patient outcome, and anatomy of the remaining bowel. Anatomy of the remaining bowel, including length of small bowel, ileum, and colon and presence of ileocaecal valve, was obtained from the original operative records. Age-adjusted bowel length was calculated based on published age-specific normal values (Struijs MC 2009). IF was defined as over 50% resection of small bowel or duration of PN over 30 days. (I-IV)

Study I

During the follow-up (median 207 days, range 97-646), the PN regimen was kept virtually unchanged. Repeated measurement of serum plant sterols, cholesterol precursors and liver biochemistry were performed a median 4 times (range 2-6) prospectively, approximately every other month (range 2 weeks-6 months). At the time of each measurement, the exact composition and amount of PN was recorded between the test points. Liver biopsy was obtained in eight patients. Parenteral and enteral cholesterol and plant sterol intake was calculated based on parenteral and enteral emulsions analyzed for plant sterols, squalene, and cholesterol similar to serum non-cholesterol sterols as described below (see page 51). (Table 6)

Study II

In study II, the patients were followed for one year, including the time of inclusion, 2 and 4 weeks after newly started PN, and there after every 12 weeks during PN for one year. If a patient weaned off PN during the follow-up, the last follow-up point was one month after discontinuation of PN. In every follow-up point, blood samples were collected, the exact composition and amount of PN, medications, antibiotic therapy as intravenous antibiotic days, growth, septic episodes, were prospectively collected. Antimicrobial therapy was recorded as intravenous antibiotic days, including intravenous antibiotic use one month before inclusion in children and between birth and inclusion in neonates. (Table 6)

Studies III and IV

In cross sectional studies III and IV, the duration of PN, composition of PN during three months preceding liver biopsy, number of blood culture positive septic episodes from birth to study date, and surgical procedures, were collected from patient records. The cross-sectional studies included clinical examination, blood samples, abdominal ultrasound, gastroscopy, and liver biopsy. (Table 6)

Clinical examination (III, IV)

Clinical examination, including weight, height and blood pressure measurements, and evaluation of pubertal development according to Tanner staging (Tanner J 1962), was done by the primary researcher. Body mass index (BMI) was calculated as [weight (kg)/ height (m^2)] for adults. In children, body mass index-for-age (ISO-BMI) was calculated (over two year-olds) and height and weight were expressed as Z-scores according to Finnish reference values (Saari A 2011).

Gastroscopy (III)

Gastroscopy, performed for evaluation of possible esophageal varices, was done during the same general anesthesia as the liver biopsies by an experienced endoscopist.

Abdominal ultrasound (III)

Abdominal ultrasound was performed by a pediatric radiologist to evaluate the overall appearance of liver, biliary tract pathology, portal venous flow, and spleen size.

Blood samples (I-IV)

All blood samples were drawn after overnight fast. In studies I and II, repeated measurements of serum fat-soluble vitamins, liver biochemistry, serum cholesterol and non-cholesterol sterols, including plant sterols, cholestanol and cholesterol precursor sterols, and squalene were done. In studies III and IV the blood samples were collected the day before liver biopsy. The biochemical parameters used in studies I-IV are shown in Table 7.

Serum cholesterol and non-cholesterol sterols (I, II, IV)
Serum cholesterol and non-cholesterol sterols, including cholestanol, plant sterols (stigmasterol, avenasterol, sitosterol, campesterol), and cholesterol precursor sterols (cholestenol, lathosterol, desmosterol), and squalene, were measured from nonsaponifable material by gas-liquid chromatography, on a 50-m-long SE-30 non-polar capillary column (Ultra 1 Column, Agilent Technologies, Palo Alto, CA), with 5α-cholestane as the internal standard (Miettinen TA 1983, Miettinen TA 1988). Non-cholesterol sterols and squalene in the serum were expressed as ratios to the cholesterol concentration of the same gas-liquid chromatography run, that is, 100 x µg/mg of cholesterol, to exclude the effects of varying serum lipoprotein concentration. (I, II)

Serum cholesterol and triglyceride levels, and that of HLD-cholesterol, after precipitation of apolipoprotein-B containing lipoproteins, were determined by commercial kits (Boehring Diagnostica, Mannheim, Germany and Wako Chemicals, Neuss, Germany). LDL-cholesterol was calculated according to Friedewald and co-workers (Friedewald WT 1972). (I, II, IV)

Serum FGF21 (IV)

The serum samples for FGF21 were prepared by centrifugation after blood collection and stored at -20˚C until analyzed. FGF21 concentrations were measured by ELISA kit (BioVendor, GmbH, Heidelberg, Germany) according to the manufacturer's instructions. Intraassay coefficient of variation was 3% and interassay coefficient of variation 12.6%. In statistical analyses, the values below the sensitivity of the assay were assigned at value 7 pg/mL, and both actual and log-transformed values were used.

Plasma amino acids (IV)

To determine plasma citrulline concentration, plasma amino acids were measured by using automatic amino acid analyzer (Biocrom 30 Physiological and Midas Autosampler, Biochrom Limited, Cambridge, England). Amino acids, separated by cation-exchange liquid chromatography, were detected spectrophotometrically after reaction with ninhydrin reagent.

Liver biochemistry and other laboratory measures (I-IV)

Serum or plasma ALT, GT, AST, bilirubin, conjugated bilirubin, bile acids, albumin, prealbumin, vitamins A, E and S-25-OHD, platelets and coagulation markers (tromboplastin time; TT, international normalized ratio; INR, activated partial tromboplastin time; APTT), glucose, glycosylated hemoglobin (HbA1c), insulin, leptin and creatinine were measured by routine hospital methods. In study IV, insulin resistance was evaluated with homeostasis model assessment (HOMA-IR) (Matthews DR 1985). The cut-off for the diagnosis of insulin resistance was set at >2.5 (Tresaco B 2005).

$$\text{HOMA-IR} = \frac{\text{Fasting glucose (mmol/L)} / \text{Fasting insulin (mU/L)}}{22.5}$$

For studies III and IV, AST-to-platelet ratio index (APRI) was calculated according to Wai and co-workers (Wai CT 2003).

$$\text{APRI} = \frac{\text{AST level} / \text{Upper limit of normal AST} \times 100}{\text{Platelet count } (10^9/\text{L})}$$

Table 6. Aims, study type and design, patients, controls and data collection in studies I-IV.

	Study I	Study II	Study III	Study IV
Aim of the study	To study interrelations between serum non-cholesterol sterols, liver function and histology, and characteristics of PN in pediatric onset IF	- To evaluate incidence of long-term PN and associated complications - To describe significance of PN plant sterols on neonatal IFALD	To characterize liver histology and evaluate risk factors of IFALD in pediatric onset IF	To evaluate serum FGF21 levels in relation to liver steatosis, serum lipids, glucose homeostasis and renal function in pediatric onset IF
Study type	Prospective follow-up Median 209 days	Prospective follow-up 1 year	Cross-sectional	Cross-sectional
Study design	- Repeated laboratory tests* - Liver biopsy - Prospective collection of clinical data	- Repeated laboratory tests* - Prospective collection of clinical data	- Laboratory tests* - Abdominal ultrasound - Gastroscopy - Liver biopsy - Collection of clinical data	- Laboratory tests* - Liver biopsy - Collection of clinical data
Patients (n)	11	28 neonates 11 children	38	35
Inclusion criteria	Children with IF on long-term PN treated in Children's Hospital, Helsinki University Central Hospital	Children on PN over 28 days without malignant primary disease treated in Finland	IF patients born between 1984-2010 and treated in Children's Hospital, Helsinki University Central Hospital	IF patients born between 1984-2010 and treated in Children's Hospital, Helsinki University Central Hospital
Liver histology (n)	8	0	38	30
Controls (n)	20	32	15	59
Inclusion criteria	Healthy age and sex matched day-case surgery patients served as controls for serum cholesterol and non-cholesterol sterols	- Healthy age and sex matched day-case surgery patients served as controls for serum cholesterol and non-cholesterol sterols - Neonates on long-term PN were compared to older children on long-term PN	Liver biopsies of age-matched liver transplant donors seved as controls for liver histology	Healthy age and sex matched day-case surgery patients served as controls for serum FGF21

IF; intestinal failure, IFALD; intestinal failure associated liver disease, PN; parenteral nutrition, FGF21; fibroblast growth factor 21. * See Table 7 for laboratory tests.

Table 7. Biochemical parameters used in the thesis.

Parameter	Units	Study			
		I	II	III	IV
Serum lipids					
Total cholesterol	mmol/L	x	x		x
High-density lipoprotein cholesterol (HDL)	mmol/L	x	x		x
Low-density lipoprotein cholesterol (LDL)	mmol/L	x	x		x
Triglycerides	mmol/L	x	x		x
Non-cholesterol sterols and squalene	100x µg/mg of cholesterol and µmol/L	x	x		
Liver biochemistry					
Plasma alanine aminotransferase (ALT)	U/L	x	x	x	x
Plasma aspartate aminotransferase (AST)	U/L	x	x	x	x
Plasma glutamyl transferase (GT)	U/L	x	x	x	x
Plasma bilirubin	µmol/L	x	x	x	x
Plasma conjugated bilirubin	µmol/L	x	x	x	x
Serum bile acids	µmol/L		x		
Plasma albumin	g/L	x		x	x
Plasma prealbumin	mg/L	x		x	x
Plasma tromboplastin time (P-TT)	%	x	x	x	x
Plasma activated partial tromboplastin time (P-APTT)	s			x	
International normalized ratio (INR)				x	x
Blood platelets	E9/L			x	x
Fat soluble vitamins					
Serum Vitamin A	µmol/L	x	x		
Serum Vitamin E	µmol/L	x	x		
Serum Vitamin D (25-hydroxy-D vitamin)	nmol/L	x	x		
Glucose homeostasis					
Plasma glucose	mmol/L				x
Plasma glycosylated hemoglobin (HbA1c)	mmol/mol				x
Serum insulin	mU/L				x
Serum leptin	µg/L				x
Plasma creatinine	µmol/L				x
Plasma citrulline	µmol/L				x
Serum Fibroblast growth factor 21 (FGF21)	pg/mL				x

Liver biopsies and histological analyses (I, III, IV)

In study I, liver histology was analyzed based on the original pathology report. Liver fibrosis was scaled from 0 to 3 (0=no fibrosis, 1=fibrosis, 2=portal-portal bridging fibrosis, 3=cirrhosis). Cholestasis, steatosis and inflammation were recorded as absent (=0), mild (=1), moderate (=2) or severe (=3).

For studies III and IV, ultrasound-guided core needle liver biopsies were taken under general anesthesia. Experienced pediatric radiologists performed the core needle liver biopsies, after which patients were followed over night at hospital. After liver biopsy, one complication occurred: a small right-sided pneumo-thorax, which resolved spontaneously. The control liver biopsies were wedge biopsies of age-matched liver transplant donors (III). The biopsies were fixed in formalin, embedded in paraffin, sliced, and stained with H&E. Additional stainings included reticulin, PAS, copper, and iron (Scheuer PJ 2006). Immunostaining for CK-7 was performed using SP52 monoclonal antibody and Ultra View Universal Detector Kit (Vienna, Tuscon, Arizona). Two experienced pediatric liver pathologists and the primary researcher, blinded to clinical data, reviewed the slides together until consensus was reached. The number of portal tracts and overall liver structure were evaluated to determine the adequacy of the biopsy specimen. The liver biopsies were evaluated for fibrosis [lobular, portal, Metavir staging (Bedossa P 1996), Ishak staging (Ishak K 1995)], steatosis, cholestasis, bile ductular proliferation, and portal inflammation as outlined in Table 8. Steatosis was classified as micro- and macrovesicular. Presence of hepatocyte apoptosis, foamy degeneration, Mallory bodies, pseudo-rosettes, and extramedullar hematopoiesis was recorded. For analytical purposes, cholestasis was defined as the highest of the three cholestasis grades. When portal inflammation was present, the distribution of inflammatory cells was recorded. (Cohen C 1981, Scheuer PJ 2006, Peyret B 2011)

Table 8. Grading and staging of histological findings in the liver biopsies used in studies III and IV.

Histological finding	Scale	Definition
Fibrosis		
Lobular	0	Absent
	1	Present
	2	Prominent
Portal	0	Absent
	1	Fibrous expansions of most portal areas
	2	Focal portal-to-portal bridging
	3	Marked bridging
	4	Cirrhosis
Metavir (Bedossa P 1996)	0	No fibrosis
	1	Portal fibrosis without septa
	2	Portal-portal fibrous septa
	3	Portal-portal and portal-central fibrous septa
	4	Cirrhosis
Ishak (Ishak K 1995)	0	No fibrosis
	1	Fibrous expansions of some portal areas, with or without short fibrous septa
	2	Fibrous expansions of most portal areas, with or without short fibrous septa
	3	Fibrous expansions of most portal areas with occasional portal-portal bridging
	4	Fibrous expansions of portal areas with marked bridging portal-portal as well as portal-central
	5	Marked bridging (portal-portal and/or portal-central) with occasional nodules
	6	Cirrhosis
Steatosis		
Proportion of hepatocytes affected	0	Absent
	1	<25% of hepatocytes
	2	25-50% of hepatocytes
	3	>50% of hepatocytes
Foamy degeneration	0	Absent
	1	<25% of hepatocytes
	2	25-50% of hepatocytes
	3	>50% of hepatocytes
Cholestasis		
Intracellular, canalicular and ductular	0	Absent
	1	Minimal
	2	Marked
	3	Prominent
Ductular proliferation	0	Absent
	1	Focal
	2	Generalized
CK7 expression in periportal hepatocytes	0	Absent
	1	Rare
	2	Present
	3	Prominent
	4	Extensive
CK7 positive ductular reaction	0	Absent
	1	Present
	2	Prominent
Portal inflammation	0	Absent
	1	Rare
	2	Present
	3	Prominent
	4	Extensive
Accumulation of copper or iron	0-4	Absent-Extensive

4.4 Statistical analyses (I-IV)

Descriptive statistics were expressed as median or mean and range or as frequencies and percentages. The Kolmogorov-Smirnov test and graphics were used to assess distributions. In cases of non-normal distributions, non-parametric tests were chosen. Differences between two groups were tested for significance using Mann-Whitney U-test (I, II, IV) and independent samples T-test (III), as appropriate. Analysis of variance (one-way ANOVA) was applied to compare more than two groups (IV). Categorical variables were tested with Fisher exact test (III, IV). Correlations were tested by Spearman rank correlation test (I-IV). Multivariate stepwise regression and multivariate logistic regression analyses were done to identify predictors of liver fibrosis in study III. In study IV, a multiple stepwise regression model was performed to identify predictors of serum FGF21 level. All tests were two-tailed and the level of statistical significance was set at 0.05 (I-IV).

4.5 Ethical considerations (I-IV)

The study protocols conform to the ethical guidelines of the 1975 Declaration of Helsinki and were approved by the ethics committee for Pediatric, Adolescent Medicine and Psychiatry in the Hospital District of Helsinki and Uusimaa (I-IV) and by the ethical committees of Tampere, Turku, Kuopio, and Oulu University Hospitals (II). The participants and their parents were fully informed about the study protocol, collection of data and the use of collected data. All patients participated in the studies on a voluntary basis. An informed written consent was received from all patients, controls, and/or their parents. All liver biopsies and gastroscopies of patients were performed during general anesthesia. The participants were informed about the study findings, and, if needed, appropriate care was arranged.

5 Results

5.1 Incidence of long-term PN and IFALD in neonates and children (II, III)

In study II, of all neonates born in Finland during the one year follow-up period, 0.05% (28:60 430, 46:100 000 live births) required long-term PN. Neonates born before 37 weeks of gestation treated in neonatal intensive care units needed long-term PN more often (2.0%, 24/1223) compared to all children born in Finland during the follow-up. (II)

IFALD, defined by liver biochemistry, occured in 27% of older children and in 63% of neonates on long-term PN (II). The overall survival was 89% among neonates on long-term PN as three neonates died due to septic complications (II). In study III, of the 56 identified IF patients, four had died (7%), including end-stage liver disease in two and septic complications in two as a cause of death, and one patient had esophageal varices and portal hypertension as a sign of end-stage liver disease.

5.2 Septic episodes (II, III)

During PN, blood culture positive septic episodes were more frequent among neonates (1.6 septic episodes per patient) than in children (1.0 septic episodes per patient) ($P<0.05$) (II). *Staphylococcus epidermidis* (56% of blood cultures in neonates, 33% of blood cultures in children) was found to be the most prevalent pathogen. Other pathogens found in blood cultures are shown in table 9 (Mutanen A et al, unpublished results). The absolute number of septic episodes and per 1000 catheter days was equal in patients on PN [mean 2.7 septic episodes (range 0-10) and mean 3.6 septic episodes per 1000 catheter days (range 0-20)] and patients weaned off PN [1.2 (0-7) and 2.9 (0-28)] (III).

Table 9. Septic episodes and intravenous antibiotic treatment in neonates and children with IF.

Variable	Neonates	Children	P-value°
Number of patients	21	11	
Number of septic episodes per patient	1.6	1.0	<0.05
Number of patients			
with septic episode	11 (53)	2 (18)	0.066
One septic episode	6 (29)	2 (18)	0.526
≥2 septic episodes	5 (24)	0 (0)	0.083
Antibiotic days before inclusion; median (range)†	30 (7-97)	0 (0-15)	<0.001
Antibiotic days during follow-up; median (range)	5 (0-64)	0 (0-120)	0.367
Microbes (n)			
Staphyolcoccus epidermidis	10	1	
Pseudomonas aeruginosa	3		
Enterococcus faecalis	2		
Stafylococcus, coagulase negative	2		
Acinetobacter baumannii	1		
*Staphylococcus aureus**	1	1	
Escherichia coli **	1		
*Micrococcus species****		1	
*Bifidobacter breve****		1	
*Rhotorula glutinis (fungus)****		1	

Unpublished results from study II (Mutanen et al.). Data are number of cases (percentage). Blood culture results were available in 21 neonates and 11 children. †One month before inclusion in children and between birth and inclusion in neonates. In same blood culture *with *Pseudomonas aeruginosa*, ** with *Acinetobacter baumannii*. ***All tree in same blood culture. °Mann-Whitney U-test for comparison between neonates and children.

5.3 Nutrition (I-IV)

In studies I-IV, the daily parenteral calorie requirements were assessed individually based on weight gain, height-adjusted weight, head circumference, and growth. The patients received PN lipids mainly as an olive oil-based regimen combined with fish oil-based regimen in some. The patients received 2 to 7 cyclic PN infusions per week and were given median of 25 to 69 percent of total energy parenterally. Supplemental fat- and water-soluble vitamins and trace elements were routinely given as part of PN. The amount of parenteral fat was kept below 35% of parenteral energy needs.

Due to the limited maximum volume of daily infusions in neonates they often received a higher amount of total parenteral calories as fat compared to older children. Enteral nutrition was tailored individually based on bowel anatomy and function and was started as early as tolerated while the percentage of parenteral calories was gradually decreased. Enteral nutrient formulas, including primarly hydrolyzed proteins with medium and/or long-chain fatty acids, were used as appropriate. In neonates breast milk was prefered. (Table 10)

Table 10. Composition of parenteral nutrition in patients on parenteral nutrition (PN) in studies I-IV.

	Study				
	I	II (neonates)	II (children)	III	IV
Patients on PN (n, %)	11 (100)	28 (100)	11 (100)	16 (42)	10 (29)
Duration of PN (mo)	33 (18-196)	1.6 (1.0-10)	56 (4.9-212)	44 (2.5-204)	53 (2.8-170)
PN infusions per week (n)	7 (2-7)	7 (7)	7 (2-7)	7 (2-7)	6 (2-7)
PN energy (%)*	48 (8-100)	69 (30-100)	25 (5-100)	50 (6-100)	42 (7-100)
PN glucose (%)**	69 (29-83)	66 (36-77)	76 (50-91)	77 (53-92)	77 (60-90)
PN fat (%)**	29 (3-35)	24 (0-56)	19 (0-34)	13 (0-33)	13 (0-30)
PN lipid emulsion					
Olive oil-based (n)	10	27	10	14	9
g/kg/day	0.9 (0-2.2)	1.6 (0-2.5)	0.6 (0-1.9)	0.5 (0-1.6)	0.3 (0-0.9)
Fish oil-based (n)	1	4	0	4	1
g/kg/day	0.2	1.0 (0-1.5)	0	0.9 (0-1.9)	0.8
Soy oil-based (n)	0	0	1	0	0
g/kg/day	0	0	1.5	0	0

Data are median (range) or frequencies (percentage).*Percentage of parenteral calories of total daily calorie intake. ** Percentage of glucose and fat energy of total parenteral calories.

5.4 Liver biochemistry (I-IV)

Neonates on PN had significantly higher serum GT, conjugated bilirubin, and bile acids compared to older children on PN (II). IFALD, defined by liver biochemistry, occurred more often in neonates (63%, 12/19) than in children (27%, 3/11, $P<0.05$) on long-term PN (II). During PN, serum markers of liver function were unrelated to the amount of PN fat, carbohydrates, and protein in children with IF (I).

One month after weaning off PN, the liver biochemistry tended to normalize, but 25% of neonates still fulfilled the criteria of IFALD (II). In study III, only 18% of children with IF weaned off PN showed abnormal liver biochemistry, including ALT, AST, GT, or conjugated bilirubin, or low plasma albumin, prealbumin or platelets, while 63% of those on PN had abnormal liver biochemistry ($P<0.05$). APRI values were comparable between IF patients on PN and weaned off PN (III).

5.5 Serum fat-soluble vitamins (I, II), glucose homeostasis (IV) and renal function (IV)

In neonates and children on long-term PN, the serum levels of vitamin E and vitamin D were within normal range (II). Serum vitamin A level was close to normal in neonates and in normal range in children during PN (I, II). Overall, glucose tolerance and insulin sensitivity was normal in most of the IF patients, as only one patient with liver steatosis had high fasting plasma glucose and HbA1c and two patients with liver steatosis had HOMA-IR >2.5 (IV). Renal function, measured by serum creatinine [median 37 (range 17-82)], was in normal age-specific range in all patients (IV).

5.6 Serum lipids, lipoprotein and cholesterol (I)

Children with IF on PN had low serum concentration of total cholesterol [median 2.75 mmol/L (range 1.70-3.95) vs. 3.66 (2.30-4.60)], LDL cholesterol [1.40 mmol/L (0.47-2.50) vs. 2.20 (1.10-2.60)] and HDL cholesterol [0.80 mmol/L (0.52-1.48) vs. 1.27 (0.99-1.96)] compared to age- and sex matched healthy controls, most likely reflecting cholesterol malabsorption in IF patients ($P<0.005$ for all). The serum concentration of triglycerides was comparable between IF patients and controls [median 1.15 mmol/L (range 0.52-2.55) vs. 1.24 (0.45-5.94), $P=0.906$].

5.7 Serum non-cholesterol sterols (I, II)

Serum cholesterol precursors during PN (I, II)

During PN, serum cholesterol precursors proportions to cholesterol, including cholestenol (median 45.0 100x µg/mg of cholesterol vs 13.5, P<0.001), lathosterol (261 vs 71.0, P<0.001), and desmosterol (116 vs 86.0, P<0.001) were increased up to 3-fold among children with IF compared to healthy controls (I). In neonates on long-term PN, lathosterol proportion to cholesterol was increased compared to healthy controls, but lower than in older children on PN (P<0.005 for all) (II). In children on PN, serum cholesterol precursors, including cholestenol and lathosterol, were negatively related to individual and total serum plant sterol levels (P<0.05 for all) (I). These findings of increased cholesterol precursor levels in neonates and children with IF on long-term PN presumably reflect increased cholesterol synthesis due to malabsorption of cholesterol and bile acids.

S*erum plants sterols and cholestanol during PN* (I, II)

During PN, the median serum cholestanol and plant sterol levels, including stigmasterol, avenasterol, sitosterol, and total plant sterols, were significantly increased in neonates by 2- to 22-fold and in children by 2- to 11-fold compared to healthy age- and sex-matched controls (P<0.005) (I, II). Campesterol was increased among neonates on PN, but not in children on PN, compared to healthy controls (I, II). Total and individual serum plant sterol ratios to cholesterol were related to percentage of parenteral energy of total daily calories (P<0.05 for all), but not to absolute amounts of parenteral lipids, total or individual plant sterols, carbohydrate or protein (I, II). The total duration of PN reflected serum ratios of cholestanol, stigmasterol, avenasterol, sitosterol, and total plant sterol levels in neonates (P<0.05 for all) (II), but not in children (I).

In neonates with IFALD, the serum stigmasterol, avenasterol, total plant sterol, and sitosterol and cholestanol proportions to cholesterol were increased up to 3.0-, 1.8-, 1.7-, 1.7-, and 1.7-fold compared to neonates without IFALD (P<0.05 for all) (II). Liver biochemistry, including ALT (r=0.557, P<0.05), AST (r=0.483, P<0.05), correlated with the total duration of PN (II). Furthermore, serum ratios of cholestanol, stigmasterol, avenasterol, and sitosterol positively associated with serum AST, ALT,

and conjugated bilirubin (P<0.05) (II). Serum total plant sterol to cholesterol proportion associated with serum ALT (r=0.540, P<0.05) and conjugated bilirubin (r=0.548, P<0.05) levels (II). Serum total plant sterol/PN total plant sterol-ratio correlated with serum ALT and bile acids (I).

Relation of liver histology, liver biochemistry, serum plant sterols and cholestanol during PN (I)

In study I, some degree of liver fibrosis was found in 63% (5/8) of IF patients during PN. Patients with liver fibrosis, the median serum sitosterol, campesterol, avenasterol, stigmasterol, total plant sterol, and cholestanol concentrations were 1.1 to 2.5 times higher compared to patients without histological liver fibrosis. However, the difference of serum plant sterol levels between patients with and without fibrosis was not statistically significant likely due to small number of patients. The serum concentration of total and individual plant sterols tended to correlate with the degree of liver fibrosis (r=0.55-0.60, P=0.16-0.12). The degree of liver fibrosis was not related to liver biochemistry, serum total plant sterols/PN total plant sterol-ratio, or amount of parenteral lipids, plant sterols, or energy.

Relation of *bowel anatomy, liver biochemistry, and serum plant sterols and cholestanol during PN* (II)

In neonates with IF on PN, the remaining age-adjusted small bowel length associated with the serum cholestanol to cholesterol proportion (r=-0.555, P<0.05) and bile acids levels (r=-0.604, P<0.05). Moreover, neonates on PN without an ICV (n=4) had a higher serum cholestanol proportion to cholesterol (median 607 100x µg/mg of cholesterol) than those patients with an ICV (n=14, median 406 100x µg/mg of cholesterol, P<0.01). A negative association was found between the remaining age-adjusted colon length and the highest serum ALT, stigmasterol, avenasterol, sitosterol, and total plant sterol levels (r=-0.539- -0.476, P<0.05).

Serum plant sterol and cholestanol after weaning off PN (II)

One month after weaning off PN, serum cholestanol and plant sterols, including total plant sterols, stigmasterol, avenasterol, sitosterol, remained high in neonates compared to healthy controls (P<0.05 for all). In neonates weaned off PN with persistent IFALD serum cholestanol and stigmasterol proportions to cholesterol were

4.2 and 2.2-fold with simultaneously increased conjugated bilirubin and ALT levels compared to neonates weaned off PN without IFALD (P<0.05 for all).

5.8 Abdominal ultrasound and gastroscopy (III)

In abdominal ultrasound (n=34), abnormal appearance of the liver was seen in 4 patients, including nodularity and increased hepatic echogenity. All patients with abnormal hepatic ultrasound had fibrosis (Metavir stage mean 1.5, range 1-2) and two had steatosis (grade 1 and 3) in liver biopsy. Excluding gallstones in one patient, no other biliary tract abnormalities were observed. Two patients had undergone cholecystectomy previously. In one patient weaned off PN, splenomegaly with esophageal varices (grade 2) in gastroscopy and Metavir stage 2 fibrosis in liver biopsy were found. Esophageal varices were not found in any other patient and all had normal liver vasculature.

5.9 Liver histology in IF (I, III, IV)

In study I on eleven IF patients on PN, most of the liver biopsy samples (66%, 5/8) showed some degree of fibrosis. Other findings were cholestasis (n=3), steatosis (n=1), reactive changes (n=1), and inflammation (n=2).

In study III, liver histology was abnormal in most of the IF patients (84%). Abnormal liver histology was equally common during PN (in 94%) and after weaning off PN (in 77%) (P=0.370). In IF patients, the frequency of liver fibrosis (in 74% of IF patients vs. 0% of controls, P=0.001), steatosis (47% vs. 13%, P=0.028) and portal inflammation (21% vs 0%, P=0.088) was increased compared to control samples of age-matched liver transplant donors (P<0.05 for all). Entirely normal liver histology was found only in six IF patients (16%) with less septic episodes [mean 0.3 septic episodes per patient (range 0-2) vs 2.1 (0-10), P=0.009] and longer remaining age-adjusted small bowel length [mean 79% (range 42-100) vs 35% (3-100), P=0.001] compared to patients with abnormal liver histology. When comparing patients with SBS and patients with intestinal motility disorders, abnormal liver histology (92% vs

71%, P=0.167), including cholestasis (17% vs 14%, P=1.000), steatosis (54% vs 36%, P=0.328) and fibrosis (83% vs 57%, P=0.127) was found equally (Mutanen et al, unpublished results).

Fibrosis (III)

Liver fibrosis similarly governed liver histology in patients on PN (88%) and patients weaned off PN (64%, P=0.143). Fibrosis was focused on portal areas, with occasional lobular fibrosis, in both patient groups (Figure 6). Metavir fibrosis stage was comparable between patients on PN [mean 1.6 (range 0-4)] and patients weaned off PN [1.1 (0-2), P=0.089]. Metavir fibrosis stage associated with age at PN start (r=-0.353, P=0.030), duration of PN (r=0.387, P=0.016), time after weaning off PN (r=-0.384, P=0.017), absolute (r=-0.486, P=0.002) and percentage of age-adjusted small bowel length (r=-0.552, P=0.001), ileum length (r=-0.453, P=0.004), and number of blood culture positive septic episodes (r=0.480, P=0.002). Moreover, patients without ileocaecal valve had more often (20/22 vs 8/16, P=0.008) and more advanced liver fibrosis [Metavir fibrosis stage mean 1.6 (range 0-4) vs 0.9 (0-2), P=0.034] compared to those with preserved ileocaecal valve. Lobular fibrosis grade correlated with ileum length (r=-0.581, P=0.001), duration of PN (r=0.466, P=0.003) and absolute (r=-0.334, P=0.035) and age-adjusted colon length (r=-0.391, P=0.015).

Fibrosis Metavir stage associated with ALT (r=0.333, P=0.041), AST (r=0.396, P=0.014), but not with bilirubin, albumin or prealbumin (Mutanen A et al, unpublished results). Moreover, APRI correlated positively with the Metavir fibrosis stage (r=0.404, P=0.013). Age-adjusted small bowel length (P=0.001), grade of portal inflammation (P=0.030) and absence of ileocaecal valve (P=0.044) were significant predictors of fibrosis Metavir stage in a multivariate stepwise linear regression model (R^2=0.425). In a multiple logistic regression model the strongest independent predictor for fibrosis was absence of ileocaecal valve (OR=8.9, P=0.05).

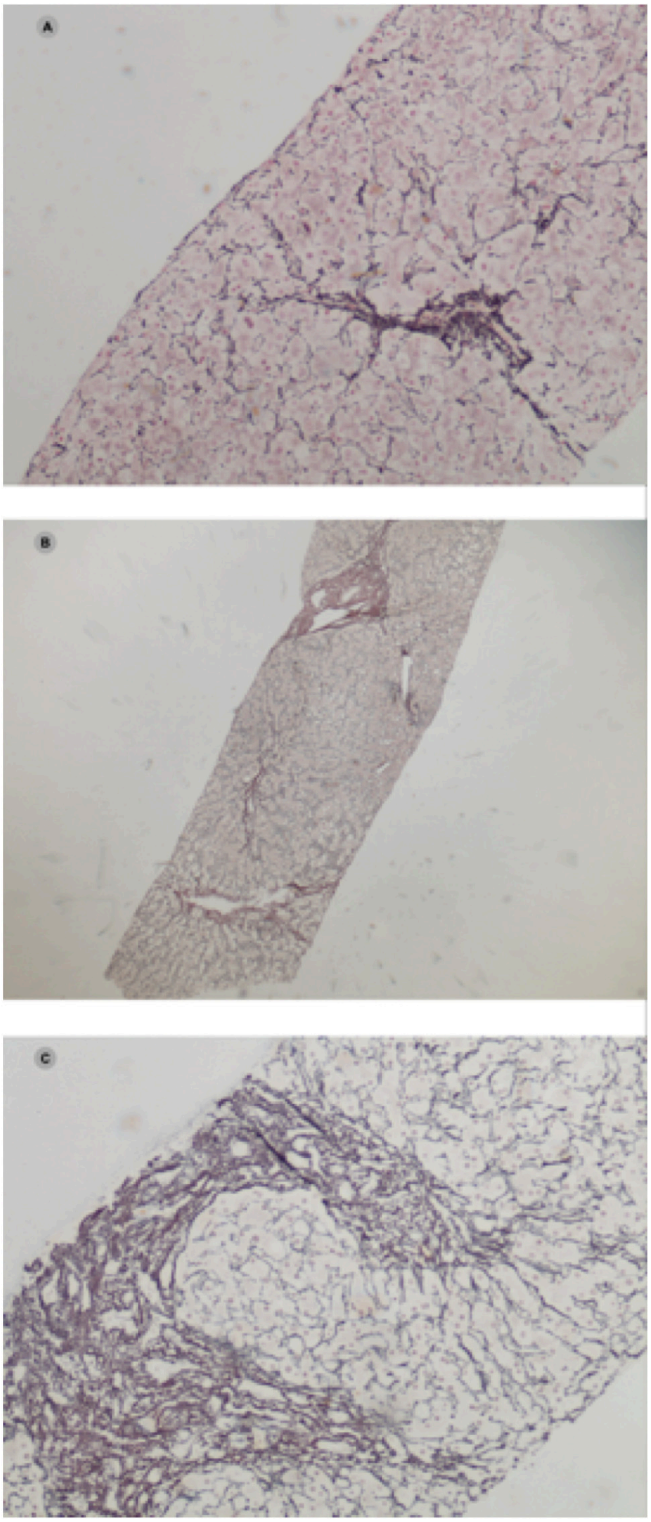

Figure 6. Liver fibrosis in intestinal failure patients is seen in reticulin stainings. Figure A; Fibrosis Metavir stage 1 with portal fibrosis without septa, Figure B; fibrosis Metavir stage 2 with portal-portal fibrous septa, Figure C; fibrosis Metavir stage 3 with portal-portal and portal-central fibrous septa.

Steatosis (III, IV)

Steatosis was equally common during (in 50% to 60%) and after weaning off PN (in 50%, P=0.782), including equal amounts of micro- (50%) and macrovesicular (50%) steatosis in both groups. Furthermore, grade of steatosis [1 (0-3) vs 0.5 (0-3), P=0.571] was comparable during and after weaning off PN (Figure 7). Patients on PN had more foamy degeneration compared to those weaned off PN [grade 0.7 (0-3) vs 0 (0), P=0.044] while no Mallory body formation or hepatocyte apoptosis was observed.

In patients with steatosis, portal fibrosis grade [1.6 (0-4) vs 0.6 (0-3), P=0.013] and Metavir fibrosis stage [1.6 (0-4) vs 0.7 (0-3), P=0.019] was significantly higher compared to those without steatosis while amount of portal inflammation was equal between the two groups (P=0.315). Liver steatosis grade associated with portal fibrosis grade (r=0.433, P=0.017), Metavir fibrosis stage (r=0.411, P=0.024), duration of PN (r=0.471, P=0.009) and absolute (r=-0.502, P=0.005) and age-adjusted (r=-0.604, P<0.001) remaining small bowel length. Steatosis was unrelated to liver biochemistry, weight Z-score, BMI, number of septic episodes, glucose homeostasis or serum lipids.

Portal inflammation (III, IV)

Portal inflammation, consisted mainly of neutrophils and lymphocytes, was more common in patients on PN than patients weaned off PN (P=0.050). Portal inflammation positively associated with cholestasis (r=0.333, P=0.041) and portal fibrosis (r=0.333, P=0.041) but not with steatosis (r=-0.258, P=0.118). Grade of portal inflammation associated with liver biochemistry, including ALT (r=0.43, P=0.005), AST (r=0.538, P=0.001), GT (r=0.487, P=0.002), APRI (r=0.426, P=0.009), albumin (r=-0.465, P=0.005), and prealbumin (r=-0.480, P=0.002), but not with serum lipids, glucose homeostasis, or serum creatinine (Mutanen A et al, unpublished results).

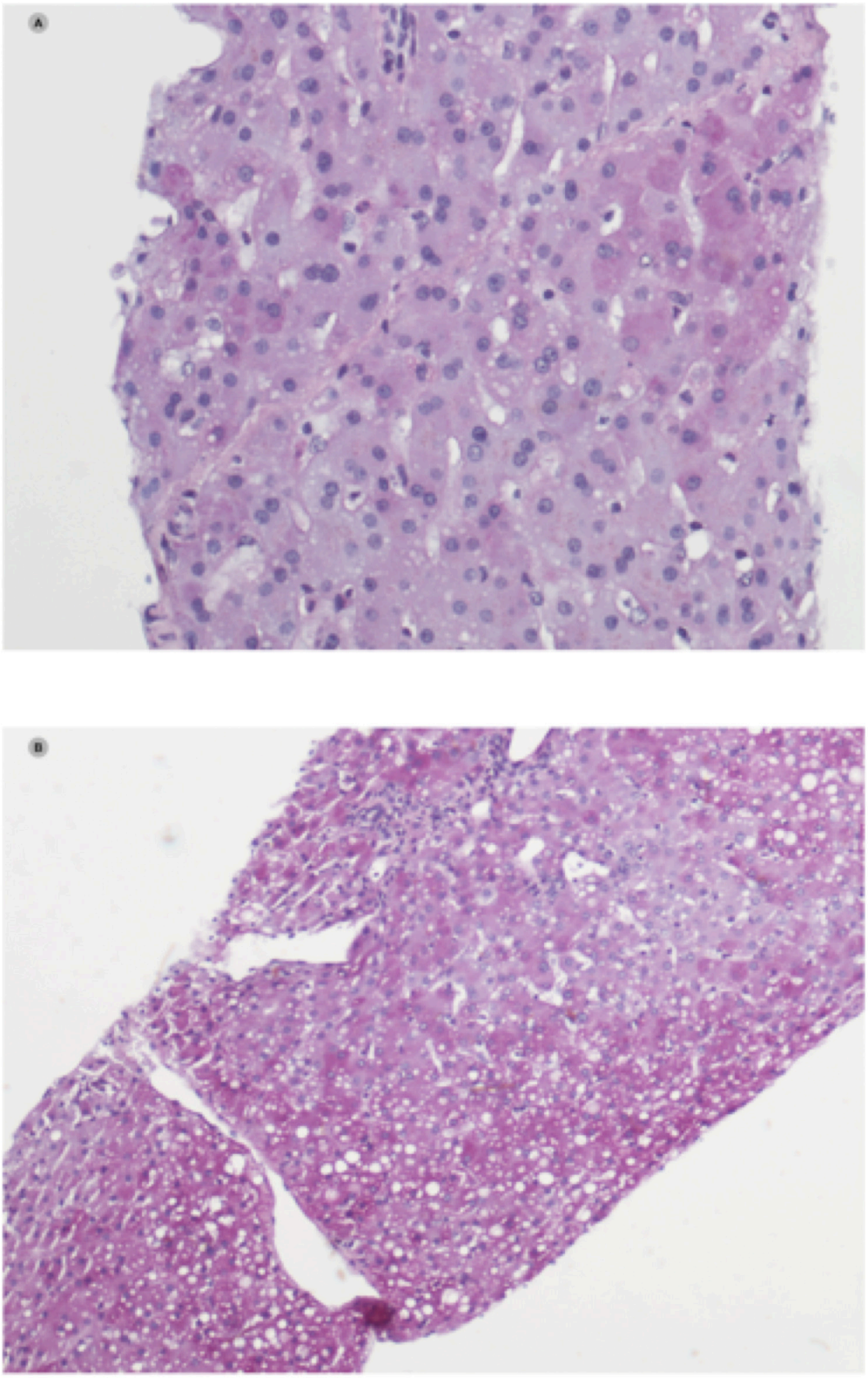

Figure 7. Steatosis in intestinal failure patients is seen in Periodic acid-Schiff (PAS) stainings. Figure A; steatosis grade 1, Figure B; steatosis grade 3.

Cholestasis (III)

Cholestasis (Figure 8), including intracellular cholestasis in five (mean grade 1.8, range 0-3), canalicular cholestasis in five (mean grade 1.5, range 0-3), and ductular cholestasis in one patient (grade 1), was only found in six patients on PN and none of the patients weaned off PN. Time after weaning off PN inversely correlated with cholestasis grade (r=-0.429, P=0.007). In patients on PN, amount of canalicular cholestasis associated with the daily PN glucose dose (g/kg/day; r=0.631, P=0.009), but not with total amount of daily PN calories (kcal/kg/day; r=0.421, P=0.104) or PN fat (g/kg/day; r=0.022, P=0.934). In addition, the number of weekly PN infusions tended to associate with intracellular and intracanalicular cholestasis (r=0.496, P=0.051 for both).

As a sign of cholestasis, expression of CK7 in periportal hepatocytes was increased in patients on PN. Periportal CK7 expression correlated with remaining ileum length (r=-0.347, P=0.041) and number of blood culture positive septic episodes (r=0.421, P=0.013). Intracellular and intracanalicular cholestasis positively correlated with liver biochemistry, including ALT, GT, AST, total bilirubin, and conjugated bilirubin (P<0.05 for all).

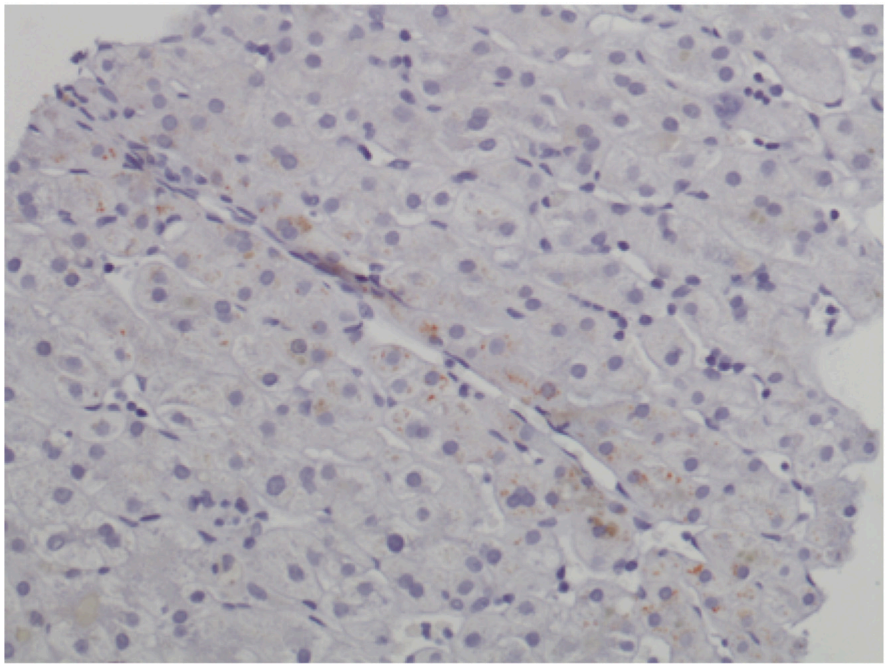

Figure 8. Intracellular cholestasis in intestinal failure patient is seen in Copper staining.

5.10 Serum FGF21 and liver steatosis (IV)

Serum levels of FGF21 were significantly higher in patients [median 299 pg/mL (range 21-20345)] compared to controls [133 pg/mL (7-1607), P=0.018]. Frequency of liver steatosis (60% vs 50%, P=0.709) and serum FGF21 levels [median 642 pg/mL (range 76-8473) vs 163 pg/mL (21-20345), P=0.083] were comparable between patients on PN and patients weaned off PN. In patients with liver steatosis [median 626 pg/mL (range 21-20345)] the serum FGF21 level was six times higher compared to patients without steatosis [108 pg/mL (32-568), P=0.002] or healthy controls [133 pg/mL (7-1607), P<0.001]. Moreover, the serum FGF21 levels of patients without steatosis and healthy controls were comparable (P=0.865).

In patients with mild steatosis (grade 1), serum FGF21 levels were higher [median 627 pg/mL (range 21-2614)] compared to controls [133 (7-1607), P=0.002]. In patients with more advanced liver steatosis (grade 3), the serum FGF21 levels were markedly higher compared to patients with a milder steatosis (grade 0 to 2, P≤0.001 for all). Serum FGF21 level associated to duration of PN (r=0.580, P<0.001), liver steatosis grade (r=0.589, P=0.001), remaining age-adjusted small bowel length (r=-0.368, P=0.029), time after weaning off PN (r=-0.343, P=0.044), prealbumin (r=-0.416, P=0.022), and INR (r=-0.517, P=0.002). In a multiple stepwise linear regression model, including duration of PN, age-adjusted small bowel length and liver steatosis grade, the liver steatosis grade (ß=0.630, P=0.001) was predictive for serum FGF21 level.

Serum FGF21 level did not associate with fibrosis Metavir stage, APRI, or serum citrulline. Furthermore, FGF21 level was unrelated to glucose homeostasis (blood fasting glucose, serum insulin, HbA1c, HOMA-IR), liver biochemistry (ALT, AST, GT, bilirubin), serum leptin or creatinine, BMI, or weight z-score. In addition, serum FGF21 lacked association with the amount of daily parenteral energy (r=0.127, P=0.726), fat (r=0.158, P=0.653) or glucose (r=-0.115, P=0.751) in patients receiving PN. After weaning off PN, plasma triglyceride levels associated to serum FGF21 level (r=-0.412, P=0.036).

6 Discussion

6.1 Methodology

In follow-up study I, all IF patients on long-term PN treated in Children's Hospital, University of Helsinki, volunteered to participate. In follow-up study II, the participation of all five Finnish University Hospitals allowed all-inclusive nationwide patient identification, as these units treat all neonates and children on long-term PN. A high participation rate was reached, as altogether 73% (III) and 67% (IV) of the eligible patients participated in the cross-sectional studies. The participant and nonparticipant groups were comparable in terms of demographic variables and disease characteristics, including age at PN start, duration of PN, remaining bowel anatomy, and number of septic episodes, making significant selection bias unlikely (III, IV).

The study protocols of follow-up studies (I, II) and cross-sectional studies (III, IV) were carefully planned. The procedures needed for sample collection and analysis were done in collaboration with experts from different fields, including pediatric radiologists (abdominal ultrasound and taking liver biopsy), pediatric pathologists (liver biopsy sample evaluation), gastroenterologists (planning serum cholesterol and non-cholesterol sterol measurements), and pediatric surgeons (gastroscopy).

Previous reports and clinical experience in Children's Hospital, Helsinki University Central Hospital, have pointed out that abnormalities in liver histology may persist or even continue to proceed during and in some cases even after weaning off PN in IF patients (Rodgers BM 1976, Cohen C 1981, Dahms BB 1981, Moss RL 1993, Hasegawa T 2002, Fitzgibbons SC 2010, Peyret B 2011). Liver histology was evaluated in studies I, III, and IV. The ultrasound guided percutaneous core needle liver biopsies were taken under general anesthesia, to avoid unnecessary pain, by an experienced pediatric radiologist. Two experienced pediatric liver pathologists and the primary researcher, blinded to clinical data, analyzed the liver biopsies together until consensus was reached to minimize the influence of a single researcher or knowledge of clinical data on results. Liver biopsies were thoroughly evaluated

according to widely accepted scores, including grading for steatosis, fibrosis, cholestasis, bile ductular proliferation, portal inflammation, hepatocyte apoptosis, foamy degeneration, Mallory bodies, pseudo-rosettes and extramedullar hematopoiesis (Cohen C 1981, Ishak K 1995, Bedossa P 1996, Scheuer PJ 2006, Peyret B 2011).

To assess cholesterol metabolism during and after weaning off PN in IF patients, measurements of serum cholesterol and non-cholesterol sterols were performed from nonsaponifable material by gas-liquid chromatography; allowing measurements of markers of cholesterol absorption and synthesis at the same time (I, II). To evaluate both cholesterol synthesis and absorption after weaning off PN, and effects of PN on serum plant sterols in patients on PN, several serum markers were used, as recommended (Miettinen TA 2011). Serum FGF21, previously shown to relate on liver steatosis, glucose and lipid metabolism in adults with nonalcoholic steatohepatitis (NASH) (Itoh N 2004, Kharitonenkov A 2005, Kharitonenkov A 2007, Xu J 2009, Fisher FM 2011), was measured to evaluate its relation to liver steatosis, glucose homeostasis, renal function, and lipid metabolism in pediatric onset IF.

A challenge in the studies was the wide age range of patients. Moreover, the treatment of IF patients has significantly developed over time. The composition of parenteral lipids has changed from soy-based to olive oil- and fish oil-based emulsions containing less or no plant sterols, amount of PN fat is limited, and cyclic PN infusions and early initiation of enteral nutrition are prefered (Mirtallo J 2004, Zambrano E 2004, Pironi L 2012). Furthermore, improved catheter care, awareness and aggressive treatment of septic episodes and SIBO, and new surgical approaches, have significantly changed the treatment and improved outcomes of IF patients (Kelly DA 2006). Although the changes in clinical practice may have modulated some of the results and may hamper their applicability for newly treated children with IF, these studies provide reliable population-based information regarding the current long-term outcomes of liver function and histology in patients with pediatric onset IF.

6.2 Incidence of long-term PN and IFALD in neonates and children in Finland

Study II described, for the first time, the incidence of long-term PN and IFALD in neonates in a prospective nationwide study population. Of all babies born during the follow-up time in Finland 0.05% and off those born before 37 weeks of gestation treated in Finnish neonatal intensive care units 2.0% required long-term PN.

IFALD, defined by abnormal liver biochemistry, was found more frequently in neonates (63%) compared to children (27%) on long-term PN. The overall survival was 89% among neonates on long-term PN as three neonates died from septic complications (II). In study III, four of the 56 identified IF patients had died (7%), including end-stage liver disease in two and septic complications in two as a cause of death, and one patient had esophageal varices and portal hypertension as a sign of end-stage liver disease. These results support the previously reported incidence and survival rates of PNALD in children on PN, including higher incidence of IFALD in neonates (Kelly DA 2006, Pironi L 2012).

6.3 Effects of long-term PN on serum lipids, cholesterol and non-cholesterol sterols, and liver histology in pediatric onset IF

Serum plant sterols during PN in pediatric onset IF

As described by Clayton and others, patients receiving PN containing plant sterols exhibit increased serum concentrations of plant sterols (Clayton PT 1993, Iyer KR 1998, Hallikainen M 2008, Llop M 2008). The findings in studies I and II, including higher serum plant sterol levels, namely sitosterol, stigmasterol, and avenasterol in both children and neonates during PN compared to healthy controls, parallel those previously described. The sitosterol levels of children with IF on PN were lower compared to those seen in patients with homozygous phytosterolemia, a rare familial lipid storage disease leading to accelerated atherosclerosis and sometimes liver failure with sitosterol levels up to 1500 µmol/L, but clearly higher than in patients with heterozygous phytosterolemia, a clinically symptomless condition with serum sitosterol levels <30 µmol/L (Salen G 1992, Miettinen TA 2006). Furthermore, neonates on PN had higher serum plant sterol levels compared to older children on PN.

The distribution of individual plant sterols in the serum closely paralleled that of infused lipid regimen in older children with IF during PN. In contrast, in neonates with IFALD, the serum levels of plant sterols were weighted with high stigmasterol levels during and also after weaning off PN. The parenteral percentage of energy in children, and duration of PN in neonates, positively related to serum plant sterol levels, namely stigmasterol and avenasterol. In neonates on PN, associations between serum plant sterol levels and serum ALT, AST, and direct bilirubin, were identified. In contrast, the serum plant sterol levels were unrelated to the amount of plant sterols or lipids infused both in children and neonates on PN. Similar findings were reported by Ellegård et al (Ellegård L 2005) in adult SBS patients on PN, showing high serum levels of plant sterols but no correlation between administration of plant sterols and serum plant sterol levels.

PNALD is linked to defects in the bile canalicular ABC-transporter family proteins, including the bile salt export pump (BSEP) (Abcb11) and multi-drug resistance 1 and 2 (mdr1, mdr2) genes encoding P-glycoproteins, responsible for transport of bile acids and phospholipids out of hepatocytes (Trauner M 1998, Carter BA 2007, Tazuke Y 2009). On the basis of in vitro studies, plant sterol stigmasterol is suggested to promote cholestasis through inhibition of nuclear receptor FXR, which would further result in reduced hepatocyte expression of a wide variety of FXR-dependent genes, including Abcb11/BSEP and conjugated bilirubin transporter Abcc2 (Trauner M 1998, Carter BA 2007). Moreover, gene knockout mice lacking the FXR hepatoprotective mechanisms are ultrasensitive to bile acid-induced liver injury and treatment of rats with FXR-agonist protects against cholestasis (Sinal CJ 2000, Liu Y 2003). In a mouse model of PNALD combining PN infusions with intestinal injury, El Kasmi and co-workers demonstrated that stigmasterol specifically promotes cholestasis, liver injury, and liver macrophage activation (El Kasmi KC 2013). These changes are likely mediated through suppression of canalicular bile transporter expression (Abcb11/BSEP, Abcc2/MRP2) via antagonism of the nuclear receptors FXR and LXR and failure of up-regulation of the hepatic sterol exporters (ABCG5/8) (El Kasmi KC 2013). The result of studies I and II, showing high levels of serum plant sterols, especially stigmasterol during PN, support these in vitro and animal studies and further outline the role of parenteral plant sterols, especially stigmasterol, as a major contributor in the pathogenesis of IFALD in neonates and children.

Because serum plant sterols reflect the balance between input and biliary excretion, plant sterols are expected to accumulate in liver damage (Hazard SE 2007). In end-stage primary biliary cirrhosis, the inability of damaged liver to efficiently excrete plant sterols or synthesize cholestanol has been reported (Nikkilä K 2005). High cholesterol synthesis is associated with increased biliary cholesterol secretion paralleling biliary secretion of sitosterol and campesterol (Sudhop T 2002). In study II, children on PN had more increased cholesterol synthesis than neonates on PN, which further may have promoted the total increase of serum plant sterols in neonates by decreasing their secretion in to bile. In addition, the serum levels of plant sterols were more strikingly high when comparing neonates with IFALD and neonates without IFALD. After weaning off PN, serum stigmasterol and cholestanol levels remained high in neonates with persistent IFALD. These findings further emphasise the role of parenteral stigmasterol as a liver damage promoting factor in IFALD and suggest that plant sterols, including stigmasterol, accumulate in neonates on PN, presumably due to insufficient hepatobiliary excretion capacity.

Serum cholestanol during PN in pediatric onset IF

Cholestanol is a noncholesterol sterol metabolite of cholesterol that reflects cholesterol absorption under physiological conditions and is shown to be a sensitive indicator of cholestasis in primary biliary cirrhosis and biliary atresia (Miettinen TA 1989, Nikkilä K 1991, Gylling H 1996, Nikkilä K 2008, Nissinen MJ 2008, Pakarinen MP 2010). In study II on neonates on PN, the relationship between the serum cholestanol proportion to cholesterol and bilirubin supports the previous findings, suggesting that the serum cholestanol/cholesterol-ratio is a useful marker of cholestasis also in IFALD.

Serum cholesterol and cholesterol precursors levels during PN

Serum cholesterol precursors, including lathosterol, desmosterol and cholestenol, are, particularly when expressed as proportions to cholesterol, surrogate markers of cholesterol synthesis (Miettinen TA 1990, Nissinen MJ 2008). In study I, the serum levels of total cholesterol, LDL-cholesterol, and HDL-cholesterol of children with IF were low during PN. At the same time, serum levels of cholesterol precursors, including cholestenol, desmosterol, and lathosterol, were high in children with IF on

PN compared to healthy controls. In study II on neonates on long-term PN, lathosterol to cholesterol proportion was increased compared to healthy controls, but lower than in older children on PN. These findings most likely reflect increased cholesterol synthesis due to malabsorption of cholesterol and bile acids, and insufficiency of PN provided cholesterol to compensate for the intestinal losses in IF.

6.4 Characterization of liver histology in pediatric onset IF

Liver histology during PN

Initially the hepatic histopathology secondary to PN is characterized by cholestasis with variable degree of fibrosis and steatosis (Postuma R 1979, Benjamin DR 1981, Hodes JE 1982, Quigley EM 1993, Buchman A 2002). In study III, liver histology was abnormal in 94% of patients on PN and weighted with cholestasis, portal inflammation, fibrosis, and steatosis. Cholestasis, found only in patients on PN, was closely associated with portal inflammation, which further outlines the close relationship of cholestasis and inflammation in the pathogenesis of liver fibrosis in IFALD. Significant or severe fibrosis (Metavir stage ≥2), accompanied by deranged liver biochemistry, was found in over half of IF patients on long-term PN. During PN, equal amounts of micro- and macrovesicular steatosis, with no associated Mallory body formation or apoptosis, were found. Collectively, these findings suggest that during PN, histopathological liver changes occur in most patients with pediatric onset IF and are characterized by cholestasis, portal infalammation, fibrosis, and steatosis.

Liver histology after weaning off PN

In most cases, biochemical cholestasis slowly resolves after weaning off PN (Pichler J 2010). Previous retrospective studies and case reports on mixed patient populations suggest that the liver histology may remain abnormal or even continue to progress after weaning off PN (Rodgers BM 1976, Cohen C 1981, Dahms BB 1981, Moss RL 1993, Hasegawa T 2002). Results of study II showed, that one month after weaning of PN 25% of neonates fulfilled the criteria for IFALD with increased serum stigmasterol and cholestanol levels. In study III, abnormal liver histology was found in 77% of IF patients after weaning off PN an average 8.8 years before. After weaning off PN, despite diminishing portal inflammation and resolution of cholestasis, liver

fibrosis and steatosis persisted. Interestingly, liver fibrosis was equally common during and after weaning off PN with comparable lobular and portal fibrosis grade, Metavir fibrosis stage, and steatosis grade. A weak inverse correlation between Metavir stage and time after weaning off PN was found suggesting that some resolution of fibrosis may occur. Altogether, based on the results of studies III-IV, a majority of patients have persistent abnormal liver histology characterized by fibrosis and steatosis after weaning off PN.

6.5 Serum FGF21 in pediatric onset IF

Serum FGF21 and liver steatosis in pediatric onset IF

Serum FGF21, a member of the fibroblast growth factor family and a hormone with circadian rhythm primarily secreted by the liver, has been shown to regulate glucose and lipid metabolism and associate with liver steatosis in experimental animal models and in humans (Itoh N 2004, Kharitonenkov A 2005, Kharitonenkov A 2007, Xu J 2009, Fisher FM 2011, Yu H 2011). In adults with nonalcoholic fatty liver disease concentration of circulating FGF21 as well as hepatic FGF21 mRNA and protein levels are previously reported to associate with the degree of liver steatosis, supporting the role of FGF21 as a biomarker of hepatic lipid accumulation (Li H 2010). In study IV, serum FGF21 levels reflected hepatic fat accumulation paralleling the steatosis grade as patients with more advanced steatosis had higher serum FGF21 levels compared to patients with milder steatosis. Patients with steatosis had five to six times higher serum FGF21 levels compared to those without steatosis or healthy controls. Both serum FGF21 and liver steatosis associated with the duration of PN and remaining small bowel length. Liver steatosis stage was found to be the only predictor of serum FGF21 level in a multiple regression model. Serum FGF21 levels and liver steatosis grade were comparable during and after weaning off PN. However, time after weaning off PN negatively correlated with serum FGF21 level. This suggests, that a recent exposure to PN may relate to higher serum FGF21 levels. Although, serum FGF21 levels have previously been suggested to decrease in advanced liver injury along with progression from steatosis to fibrosis in NASH (Dushay J 2010), in pediatric onset IF serum, FGF21 concentration correlated positively with INR and negatively with prealbumin suggesting that impaired hepatic

function, reflecting hepatocyte injury, was associated with increased serum FGF21 levels in pediatric onset IF. These findings suggest that increased serum FGF21 levels reflect liver steatosis, and are associated to the duration of PN and remaining small bowel length and may also be a useful marker of liver steatosis in IF associated liver steatosis.

Relation of serum FGF21 on glucose homeostasis, serum lipids, renal function and liver biochemistry

In primary human adipocyte cultures, FGF21 has been shown as a potent regulator of glucose uptake and insulin sensitivity (Arner P 2008). In patients with impaired glucose tolerance, increased FGF21 levels seem to be more closely related to lipid metabolism instead of insulin secretion and sensitivity (Li H 2009). FGF21 levels have also been shown to depend on renal function in chronic kidney disease (Stein S 2009, Lin Z 2011). In contrast to previous findings in humans, study IV showed that serum levels of FGF21 were unrelated to glucose homeostasis, including fasting glucose, serum insulin, HbA1c, and HOMA-IR, or renal function in patients with pediatric onset IF. Of note, glucose tolerance, insulin sensitivity and renal function were normal in most of IF patients.

In patients with impaired glucose tolerance or NASH, increased serum levels of FGF21 are associated with biochemical markers of liver injury, including GT and ALT (Li H 2009, Dasarathy S 2011). Moreover, elevated serum FGF21 levels are related to hepatocyte apoptosis, increased plasma bile acids and leptin in adult patients with NASH (Dasarathy S 2011). In difference to patients with NASH, liver biochemistry or serum leptin were not related to serum FGF21 in IF patients.

Previously, serum FGF21 levels have been reported to correlate positively with plasma triglycerides and BMI in adult patients with impaired glucose tolerance (Chavez AO 2009, Li H 2009). Serum lipids, including cholesterol and triglyceride levels, were in low normal range, reflecting most likely cholesterol and fat malabsorption in IF patients in studies I and IV. Quite surprisingly, the inverse correlation between serum FGF21 and triglyceride levels after weaning off PN was the only association between serum lipids and FGF21 in IF patients. In patients with IF, FGF21 level was unrelated to weight Z-score or BMI. Based on our findings,

serum FGF21 levels are not related to glucose metabolism, renal function, liver biochemistry, serum lipids or weight, reinforcing an independent association between FGF21 and liver steatosis in patients with pediatric onset IF.

6.6 Assessment of liver damage in IF

Liver histology is abnormal in most IF patients during PN and also after weaning off PN suggesting that patients with IF need close follow-up considering associated liver disease during and after weaning off PN. Although APRI correlated with histological liver fibrosis, any of the conventional liver biochemistry, including bilirubin, ALT, AST, GT, albumin, prealbumin, blood platelets, INR, were off age-specific normal limits only in 63% of patients on PN and in 18% of patients weaned off PN, while liver histology was abnormal in 94% of patients on PN and 77% of patients weaned off PN in study III. Abdominal ultrasound was abnormal in only four patients. Based on these findings, conventional liver biochemistry and abdominal ultrasound alone are insufficient for reliable evaluation of liver damage in pediatric onset IF. Liver biopsy remains to be the golden standard for evaluation of liver damage in IF during and after weaning off PN. Moreover, as shown in study IV, serum FGF21 assay, being simple and non-invasive, may be useful in diagnosing IF-associated liver steatosis, especially in patients with more advanced steatosis.

6.7 Risk factors and prevention of IFALD

Etiology of IFALD is proposed as multifactorial (Kelly DA 2006, Carter BA 2007). Various risk factors have been linked to IFALD, but no previous studies are available linking association of risk factors of IFALD and histological changes in the liver during and after weaning off PN in pediatric onset IF. Based on findings in studies I-IV, the etiology of IFALD is multifactorial, including duration, amount and composition of PN, parenteral plant sterols, small bowel length, presense/absence of ileocaecal valve, septic episodes, and prematurity. (Figure 9)

Parenteral plant sterols

Previous studies have linked high serum levels of plant sterols to IFALD both in adults and in children (Clayton PT 1993, Clayton PT 1998, Ellegård L 2005, Llop M 2008, Hallikainen M 2008), whereas extensive PN fat dose overcoming the ability of the liver to clear phospholipids and fatty acids can lead to steatosis, cholestasis, and eventually fibrosis (Fizgibbons SC 2010). A lipid dose <1 g/kg/day is found to be beneficial in preventing IFALD (Cavicchi M 2000, Mirtallo J 2004). Studies on mice have demonstrated that infusion of PN solutions devoid of plant sterols prevent cholestasis and liver injury (El Kasmi KC 2013). In contrast, infusion with PN emulsion containing stigmasterol as a sole plant sterol produced liver injury and cholestasis, despite combination with fish oil (El Kasmi KC 2013). Gene expressions of the canalicular bile acid transporter Abcb11 and conjugated bilirubin transporter Abcc2 were reduced in mice infused with plant sterol containing PN emulsions while serum stigmasterol level correlated with the severity of cholestasis (El Kasmi KC 2013). In study II, neonates and children with IF receiving olive oil-based PN had high levels of serum plant sterols, compared to healthy controls. The serum plant sterol distribution reflected their contents in the parenteral lipid emulsion in children on PN. In neonates with IFALD, serum levels of plant sterols were high during PN. Moreover, after weaning off PN serum stigmasterol and cholestanol levels remained high in those neonates with persistent IFALD. Despite the fact that liver biochemistry often remained close to normal, the majority of patients had abnormal liver histology with high levels of serum plant sterols. A positive correlation was found between serum plant sterols and GT and between serum total plant sterols/PN total plant sterol-ratio and ALT and bile acids. These results provide evidence that significantly increased plant sterol levels, especially that of stigmasterol, may play a significant role in pathogenesis of IFALD. The usage of PN lipid emulsions with low plant sterol contents, for example, combining olive oil-based and fish oil-based lipid emulsions, may be advisable. Serum plant sterol levels can be used to monitor and guide PN lipid dosage. A further refinement of diagnostic strategies and mechanisms behind IFALD is needed to differentiate the significance of the total PN lipid dosage and total and individual plant sterols.

Bacterial overgrowth and septic episodes

During and after weaning off PN, SIBO, epithelial changes, and impaired local immunity of the small intestine are suggested to cause and maintain liver injury in IFALD (Deitch EA 1992, DiBaise JK 2006, Cesaro C 2011). SIBO is induced by the loss of barrier function of the ileocaecal valve, dilated bowel segments, and impaired motility in patients with intestinal motility disorders or after massive intestinal resection (DiBaise JK 2006, Goulet O 2006). PN is shown to increase intestinal permeability, promote lipopolysaccharide-activated Toll-like receptor 4-dependent Kuppfer cell activation, also suppressing FXR dependent gene pathways, and liver injury, presumably caused by bacterial translocation, in mouse model of IF (El Kasmi KC 2012). Interestingly, neither intestinal injury nor PN alone was sufficient to induce liver injury in the mouse model (El Kasmi KC 2012). Based on results in a mouse model, components within the PN solution are suggested to synergize with hepatic inflammatory pathways and cytokines that are induced by Toll like receptor agonists, such as lipopolysacharides absorbed through an injured intestine with SIBO and reduced barrier function, and that activation of Toll like receptor 4-signalling in hepatic macrophages leading to generation of proinflammatory cytokines is an important component of liver injury in IF (El Kasmi KC 2013). Furthermore, IFALD has been shown to be more common in children with recurrent septic episodes related to either central line infections or bacterial translocation (Candusso M 2002, Heine RG 2002, Kelly DA 2010). In study III, the number of blood culture-positive septic episodes, reflecting both central venous catheter and bacterial translocation-related septic episodes, correlated positively with liver fibrosis and chronic cholestasis. Moreover, the patients with the shortest remaining small bowel length and those without an ileocaecal valve had the most advanced liver fibrosis. These findings outline the contribution of SIBO and septic episodes causing and maintaining histological liver damage in IF patients. To preserve liver function and prevent IFALD, preserved ileocaecal valve, longer small bowel length, and awareness and aggressive treatment of septic episodes and SIBO are beneficial in IF.

Remaining bowel length

Small intestine exerts liver protection most likely by multifactorial mechanisms, including enterohepatic circulation of bile acids (Cavicci M 1998). Short remaining intestine length reflects decreased enteral absorption and long-term PN requirements

(Goulet O 2006). Moreover, ileal resection is poorly tolerated because of resulting malabsorption of bile acids (Youssef NN 2012). In study III, the duration of PN and extensive small bowel resection positively correlated with both histological liver fibrosis and steatosis. In a multivariate analysis, age-adjusted small bowel length, portal inflammation, and absence of an ileocaecal valve were the most significant predictors of Metavir fibrosis stage. Interruption of the hepatic circulation, caused by resection of the terminal ileum and ICV, exacerbates cholestasis by removing negative feedback normally exerted on 7α-hydroxylation of cholesterol, which is the rate limiting step in bile acid production (Russel DW 2003). Furthermore, serum cholestanol, a marker of cholestasis, correlated positively with serum AST, ALT, direct bilirubin, and negatively with the remaining age-adjusted small bowel length in neonates on PN in study II supporting this concept.

Prematurity

In study II, IFALD was more frequent in neonates, especially in those born before 37 weeks of gestation, compared to children during long-term PN. Parenteral plant sterols, especially stigmasterol, seem to accumulate in neonates. Moreover, one month after weaning off PN 25% of neonates had persistent IFALD with high stigmasterol and cholestanol levels. In study III, liver fibrosis stage was inversely related to young starting age of PN. Our findings in neonates are likely to reflect the vulnerability and insufficient hepatobiliary excretion capacity of the immature liver of the newborn and further support prematurity as risk factor for IFALD.

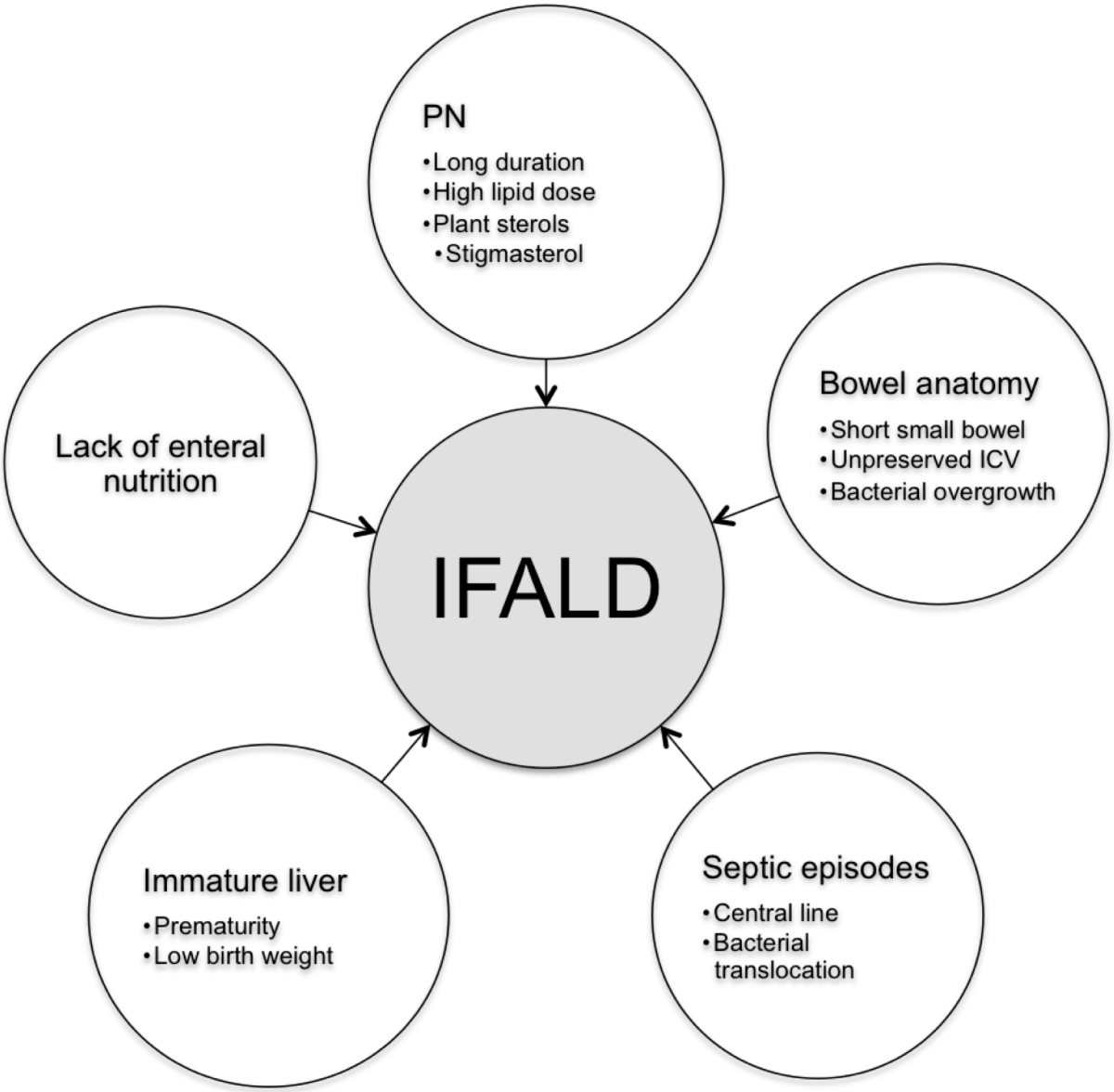

Figure 9. Factors linked to development of intestinal failure associated liver disease (IFALD). PN; parenteral nutrition, ICV; ileocaecal valve.

6.8 Future considerations

These studies have showed that a majority of patients with pediatric onset IF have abnormal liver histology, including fibrosis and steatosis, during PN but also years after weaning off PN. Further studies on the regulation of fibrogenesis and steatosis, including studies on matrix metalloproteases and their tissue inhibitors, and microarray studies on liver tissue samples, may help to better understand the type of the liver injury and to unravel mechanisms causing and maintaining the liver damage in IF (Han YP 2006, Hannivoort RA 2012). Studies with longer follow-up and serial liver biopsies would help to evaluate the natural history of the liver damage.

Based on the findings of this thesis, conventional liver biochemistry and abdominal ultrasound are insufficient for evaluating IFALD. Further prospective studies in patients with pediatric onset IF are needed to find noninvasive methods for evaluating the liver damage in neonates and children with IF. In this respect, liver fibrosis measurement by elastography (FibroScan) may present a promising option (de Lédinghen V 2007). Furthermore, studies on neonates and children with IF on serum FGF21, liver histology, and hepatic expression of key regulators of lipid metabolism are needed.

7 Conclusions

IF and long-term PN are associated with potential life-threatening complications, such as IFALD. The aims of these studies were to study epidemiology of long-term PN and IFALD, to characterize liver histopathology, and evaluate risk factors of IFALD in pediatric onset IF. The effects of parenteral plant sterols on IFALD in neonates and children were assessed in two follow-up studies. In the cross-sectional studies liver histology was assessed during and after weaning off PN in patients with pediatric onset IF. Based on the results of these studies, the following conclusions are presented:

1. Of all neonates born in Finland during the one year follow-up period, 0.05% required long-term PN. IFALD, defined by abnormal liver biochemistry, was found more frequently in neonates (63%) compared to children (27%) on long-term PN. (II)

2. During PN, children with IF have high serum plant sterol levels compared to healthy controls. Serum plant sterol distribution reflects their contents in the PN lipid emulsions. (I)

3. IFALD is frequent in neonates during PN with an association to markedly increased serum plant sterols compared with healthy neonatal controls and children on PN with more mature liver function. The high level of serum plant sterols suggest that plant sterols, especially stigmasterol, accumulate in neonates on PN, presumably due to insufficient hepatobiliary excretion capacity. After discontinuation of PN, serum stigmasterol level remains high in neonates with IFALD. Elevated serum plant sterol levels, especially stigmasterol, may be an independent risk factor for IFALD. (II)

4. During PN, liver histology is characterized by cholestasis, portal inflammation, liver fibrosis and steatosis in patients with pediatric onset IF. Over half of the patients on long-term PN have significant or severe liver fibrosis accompanied with abnormal liver biochemistry. (III)

5. Despite diminishing portal inflammation and resolution of cholestasis, liver histology remains abnormal up to 9 years after weaning off PN in the majority of IF patients. After weaning off PN, the liver histology is characterized by liver fibrosis and steatosis. (III)

6. In addition to duration of PN, extensive small intestinal resection and loss of ileocaecal valve as well as septic episodes are major risk factors of histological liver fibrosis. (III)

7. Increased serum FGF21 levels reflect the presence and the degree of liver steatosis in patients with pediatric onset IF. Both serum FGF21 levels and liver steatosis exclusively associate with the duration of PN and remaining small bowel length; whereas hepatic fat accumulation is a major predictor of increased serum FGF21 concentration. (IV)

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