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Parenteral Plant Sterols Accumulate in the Liver Reflecting Their Increased Serum Levels and Portal Inflammation in Children With Intestinal Failure

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Abstract

Background: Parenteral plant sterols (PSs) are considered hepatotoxic; however, liver PSs and their associations with liver injury in patients with intestinal failure (IF) have not been reported. **Materials and Methods:** We analyzed liver and serum PS (avenasterol, campesterol, sitosterol, and stigmasterol) concentrations and ratios to cholesterol and their associations with biochemical and histologic liver damage in children with IF during ($n = 7$) parenteral nutrition (PN) and after weaning off it ($n = 9$), including vegetable oil–based lipid emulsions. **Results:** Liver avenasterol, sitosterol, and total PS concentrations and cholesterol ratios were 2.4-fold to 5.6-fold higher in PN-dependent patients ($P < .05$). Parenteral PS delivery reflected liver avenasterol and sitosterol ratios to cholesterol ($r = 0.83$ – 0.89 , $P = .02$ – $.04$), while serum and liver total PS levels were positively interrelated ($r = 0.98$, $P < .01$). Any liver histopathology was equally common while portal inflammation more frequent (57 vs 0%, $P = .02$) in PN-dependent patients. All liver PS fractions correlated positively with histologic portal inflammation ($r = 0.53$ – 0.66 , $P < .05$), and their total concentration was significantly ($P = .01$) higher among patients with versus without portal inflammation. In PN-dependent patients, liver fibrosis and any histopathology correlated with liver campesterol and stigmasterol levels ($r = 0.79$ – 0.87 , $P \leq .03$). **Conclusion:** Among children with IF, parenteral PSs accumulate in the liver, reflect their increased serum levels, and relate with biochemical liver injury, portal inflammation, and liver fibrosis, thus supporting their role in promoting liver damage. (*JPEN J Parenter Enteral Nutr.* 2017;41:1014–1022)

Keywords

intestinal failure-associated liver disease; parenteral nutrition; phytosterols; lipid emulsions; short bowel syndrome

Clinical Relevancy Statement

Although parenteral plant sterols are assumed to accumulate in the liver, current knowledge on their distribution in the human body is based on serum plant sterol measurements. This is the first study demonstrating that liver plant sterol concentrations are elevated during parenteral nutrition and that their increased levels relate with biochemical and histological liver injury in children with intestinal failure.

Introduction

Parenteral nutrition (PN) is a life-saving therapy for patients with intestinal failure (IF). PN dependence, however, predisposes to IF-associated liver disease (IFALD), the most significant and life-threatening complication of IF.^{1–3} IFALD is the most common indication for intestinal transplantation in children, among whom young gestational age, extensive bowel resection, and lack of enteral nutrition further increase the risk of liver damage.^{1,4} Early histopathologic changes characteristic to IFALD include portal inflammation (PI) and cholestasis, while fibrosis and steatosis develop with time and persist after weaning off PN.^{2,5}

Vegetable oil–based PN solutions contain variable amounts of plant sterols (PSs), equivalent to cholesterol in mammals, which

cannot be synthesized in the human body.^{6,7} Current research evidence suggests that PSs significantly contribute to IFALD

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Conflicts of interest: None declared.

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development.^{4,6-10} In healthy individuals, the small amount of absorbed dietary PS is excreted into bile, and serum PS levels remain low.^{6,7} As liver capacity to metabolize PS is limited,¹¹ intravenously administered soy oil-based and olive oil-based PN emulsions substantially increase serum PS concentrations. Elevated serum PS levels during PN are associated with biochemical cholestasis, increased plasma liver enzyme levels, and IFALD-related liver histopathology, whereas reducing PN lipid dose decreases serum PS levels and improves liver dysfunction.^{6-9,12-15} The finding that substituting soy oil-based PN with fish oil-based emulsion devoid of PS can reverse liver injury further supports the role of PS in IFALD development.^{1,16} In animal models, PS levels increase rapidly following PN initiation, not only in serum, but also in the liver.^{4,7,10,17} The ensuing cholestasis and hepatocyte damage have been demonstrated in vitro to be mediated by PSs, which inhibit the nuclear farnesoid X receptor (FXR), a bile acid sensor and a key regulator of bile acid and sterol homeostasis in the liver.^{4,10} Importantly, PSs may also promote hepatic inflammation by suppressing FXR and directly activating liver macrophages.⁴ Parenteral PSs are assumed to act in the human body similarly as in animal models; however, no direct evidence on their hepatic accumulation and associated effects exists in IF patients. In this study, we aimed to find out whether PSs accumulate in the liver during PN, whether serum and liver PS levels are correlated, and whether liver PSs associate with liver histopathology. To meet these aims, we compared serum and liver PS levels between patients currently receiving PN and those weaned off PN, analyzed their interrelations, and studied their associations with PN characteristics, biochemical markers of liver function, and liver histopathology in children with IF.

Methods

Patients and Study Design

For this study, children with IF managed by our intestinal rehabilitation program who were receiving PN ($n = 7$) or were weaned off PN ($n = 9$) were enrolled during 2011–2015.¹⁸ IF was defined as PN requirement for >3 consecutive months or small bowel resection >50% of age-adjusted bowel length.^{3,19} Routine liver biopsies were taken in PN-dependent patients if PN duration exceeded 1 month or progressive liver dysfunction was observed. Follow-up biopsies after weaning off PN were scheduled if liver histopathology was present in a previous biopsy or if biochemical liver function parameters deteriorated over time.²⁰ Patients underwent liver biopsy and serum sampling after an overnight fast during the same day. Core needle liver biopsies were obtained during intestinal surgery or under ultrasound guidance during general anesthesia for gastroscopy.⁵ As serum PS concentrations are known to remain elevated for some weeks after PN cessation,⁸ patients weaned from PN were included if they had not received parenteral support for at least 3 months prior the study.

Medical records were reviewed for baseline diagnoses, previous surgical procedures, and intestinal anatomy. Length of

the remaining small bowel was expressed as a percentage of the age-adjusted reference values.¹⁹ Growth parameters and PN duration were recorded. Detailed PN data were collected for those receiving PN at the time of biopsy, including the number of weekly PN infusions as well as intake of PN energy (kcal/kg/d and as proportion to total energy intake), glucose (g/kg/d), fat (g/kg/d and as proportion of PN energy), cholesterol ($\mu\text{g/kg/d}$), and total PSs ($\mu\text{g/kg/d}$).^{15,21} Routine biochemical liver tests, including alanine aminotransferase, aspartate aminotransferase, glutamyl transferase (GT), bilirubin, bile acids, albumin, and prothrombin time, as well as serum and lipoprotein lipids, were analyzed by our hospital laboratory.

Liver Biopsies

The liver biopsy material was divided between histologic stainings and PS analysis. Histologic specimens were fixed in formalin, embedded in paraffin, sliced, and stained with hematoxylin and eosin. Additional stainings included reticulin, periodic acid–Schiff, copper, iron, and immunostaining for a biliary epithelial marker cytokeratin 7.⁵ Liver histology was evaluated by experienced pediatric pathologists blinded from other study variables. Biopsy specimens were considered representative, as they contained a median of 11 portal tracts (interquartile range [IQR], 10–15). Fibrosis was graded according to Metavir stage as follows: no fibrosis (F0), portal fibrosis without septa (F1), portal fibrosis and few septa (F2), numerous septa without cirrhosis (F3), or cirrhosis (F4).²² PI was defined as abnormal inflammatory cell infiltration in portal areas. Cholestasis was graded as absent or present, and steatosis was present if the proportion of hepatocytes with fat deposits exceeded 10%.⁵

PS Analyses

Serum and liver cholesterol and PS fractions (campesterol, sitosterol, stigmasterol, and avenasterol) were measured with gas-liquid chromatography on a 50-m-long SE-30 nonpolar capillary column (Ultra 2 Column; Agilent Technologies, Palo Alto, CA), with 5 α -cholestane as internal standard.²³ Serum PS concentrations were expressed as $\mu\text{g/dL}$ and as a ratio to the cholesterol concentration of the same gas-liquid chromatography run ($100 \times \mu\text{g/mg}$ of cholesterol, called *ratios* in the text). The liver biopsy material used for PS analysis, weighing a median of 4.3 mg (IQR, 2.3–6.1 mg), was snap frozen and stored at -20°C until analyzed. The results were expressed as $\mu\text{g/100 g}$ of liver tissue and as ratio to the cholesterol concentration of liver tissue of the same gas-liquid chromatography run ($100 \times \mu\text{g/mg}$ of cholesterol, called *ratios* in the text). Serum PS data were missing for 1 patient.

Statistical Analyses

Data are expressed as medians (IQRs) or as frequencies. Mann-Whitney *U* test was used to compare continuous variables and

Fisher exact test to compare frequencies between groups. Spearman rank correlation was used to examine associations between variables. Simple linear regression was used to analyze the value of serum PSs to predict liver PS concentrations. All analyses were carried out with SPSS 22 (SPSS Inc, Chicago, IL).

Ethic

This study was approved by the Helsinki University Central Hospital ethics committee (2/13/03/03/2010) and institutional review board (57/2010, 12/2013). Written informed consent was received from all patients or their caregivers before any procedures.

Results

Patient Characteristics

Of the 15 patients (53% males, $n = 8$), 1 had chronic intestinal pseudo-obstruction, while 14 had short bowel syndrome (SBS) due to necrotizing enterocolitis ($n = 6$), small bowel atresia ($n = 4$), midgut volvulus ($n = 3$), or intestinal resection for Hirschsprung's disease extending to duodenojejunal flexure ($n = 1$). Compared with age-adjusted reference values,¹⁹ patients had 25% (17%–31%) of small bowel and 75% (40%–100%) of colon remaining (no difference between patients receiving PN and not). The ileum was missing in 6 patients and the ileocecal valve in 7.

Growth data, liver biochemistry, and liver histology are shown in Table 1. Higher plasma GT was observed in patients receiving PN versus not. One-third to two-thirds of PN-dependent patients had increased transaminases, GT, and bile acids, while all patients had normal total bilirubin. Histological fibrosis (F1, F2) and any liver histopathology (steatosis, fibrosis, cholestasis, or PI) were equally common between subgroups, while PI was more frequent in patients receiving PN versus not.

Nutrition

At time of liver biopsy, 9 patients had weaned off PN 67 (9.6–99) months earlier after receiving PN for 11 (4.9–14) months (Table 2). The remaining subjects ($n = 7$) had been receiving PN for 11 (5.5–16) months, currently receiving 7 (6.5–7) PN infusions/week and 50% (33%–83%) of total daily calories parenterally, 13% (13%–21%) of which composed of fat. A combination of olive oil, soy oil, medium-chained triglycerides, and fish oil (SMOFlipid; Fresenius Kabi) was used in 1 patient; soy- and olive oil (20%/80%)–based lipid emulsion (Clinoleic, Baxter) in 5; and a combination of soy, olive (5%/22%; Clinoleic), and fish oil (73%; Omegaven, Fresenius Kabi) in 1.

Serum Cholesterol and PSs

Serum cholesterol was comparable between subgroups (Table 3). Concentrations and ratios of sitosterol, stigmasterol, and total PS

Table 1. Age, Growth, Liver Biochemistry, Serum Lipids, and Liver Histology in Patients With Intestinal Failure Who Were Receiving PN and Weaned Off PN.^a

Variable	Patients Receiving PN (n = 7)	Patients Weaned Off PN (n = 9)
Age, y	1.7 (0.83, 5.55)	8.0 (1.2, 10.9)
Height for age, SD	−2.2 (−2.5, −0.6)	−1.7 (−2.4, −0.2)
Weight for height, percentiles	−5 (−9, −4)	−6 (−9, −3)
ALT, IU/L	34 (24, 71)	30 (21, 34)
Off reference limits	2 (29)	2 (22)
AST, IU/L	41 (36, 61)	35 (32, 39)
Off reference limits	3 (43)	0
GT, IU/L	29 (20, 56)	15 (11, 16) ^b
Off reference limits	2 (29)	0
Total bilirubin, $\mu\text{mol/L}$	3 (2.5, 4)	6 (5, 12) ^c
Off reference limits	0	0
Bile acids, $\mu\text{mol/L}$	6.5 (5.0, 13)	13 (8.4, 18) ^d
Off reference limits	4 (57)	2 (67) ^d
Albumin, g/L	33 (28, 37)	41 (40, 42)
Off reference limits	6 (86)	1 (11) ^c
Prothrombin time	73 (63, 79)	83 (58, 111)
Off reference limits	3 (43)	3 (33)
Cholesterol, mmol/L	2.9 (2.4, 3.7)	3.3 (2.4, 3.8)
LDL	1.5 (1.2, 1.6)	1.3 (1.0, 1.7)
HDL	1.0 (0.8, 1.3)	1.1 (0.9, 1.6)
Triglycerides	0.76 (0.54, 1.1)	0.91 (0.52, 1.2)
Liver histology		
Cholestasis	1 (14)	0
Portal inflammation	4 (57)	0 ^c
Steatosis	3 (43)	4 (44)
Fibrosis	5 (71)	5 (56)
Any histopathology	5 (71)	7 (78)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GT, gamma-glutamyl transferase; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PN, parenteral nutrition.

^aData are presented as medians (interquartile range) or n (%). P values for the differences between subgroups were calculated by Mann-Whitney U test for continuous variables and Fisher exact test for frequencies.

^b $P \leq .01$.

^c $P \leq .05$.

^dMeasured for 3 patients weaned off PN.

as well as avenasterol ratios were higher in patients receiving PN than not (Table 3, Figure 1A). Sitosterol constituted 78% (71%–79%) of serum total PS in PN-dependent patients, while campesterol was the most abundant individual serum PS in patients weaned off PN, at 51% (49%–59%) of total PS. Parenteral lipid energy percentage was related with serum sitosterol concentration and ratio, stigmasterol ratio, avenasterol ratio, and total PS concentration and ratio ($r = 0.81$ – 0.84 , $P \leq .05$ for all). Daily fat intake (g/kg) and number of weekly PN infusions correlated with serum stigmasterol concentration and ratio ($r = 0.83$ – 0.94 ,

Table 2. Characteristics of PN Preceding Liver Biopsy.

Characteristic	Patients Receiving PN (n = 7)
PN lipid emulsion, n	
Clinoleic (soy and olive oil)	5
SMOF (soy, olive, fish oil, and medium- chained triglycerides)	1
Clinoleic + Omegaven (soy, olive, and fish oil)	1
PN, median (interquartile range)	
Duration, mo	11 (5.5–16)
Infusions/wk	7 (6.5–7)
Total calories/d	617 (482–789)
Calories/kg/d	51 (37–65)
Proportion of total daily calories	50 (33–83)
Glucose, g/kg/d	8.1 (7.8–12.2)
Fat, g/kg/d ^a	1.1 (0.47–1.7)
Cholesterol, µg/kg/d ^a	906 (805–959)
Plant sterols, µg/kg/d ^a	1522 (464–2287)

PN, parenteral nutrition.

^aDetailed parenteral lipid dosage was not available for 1 patient (n = 6).

$P < .01$ – $.04$ for all). Daily PS intake (µg/kg) correlated with serum sitosterol concentration and ratio ($r = 0.83$, $P = .04$ for both), stigmasterol concentration ($r = 0.89$, $P = .02$), and total PS concentration and ratio ($r = 0.83$, $P = .04$ for both).

Liver Cholesterol and PSs

Liver cholesterol concentrations were similar, while concentrations and ratios of sitosterol, avenasterol, and total PS were higher in patients receiving PN versus not (Table 3, Figure 1B). In PN-dependent patients, sitosterol was the most abundant individual liver PS, composing 55% (52%–59%) of total PS, whereas in patients off PN, campesterol constituted 35% (32%–46%) and sitosterol 28% (25%–29%) of liver PS. Time having been off PN correlated negatively while parenteral lipid energy percentage correlated positively with sitosterol concentration ($r = -0.58$, $P = .02$; $r = 0.76$, $P = .05$) and ratio ($r = -0.74$, $P < .01$; $r = 0.81$, $P = .05$), avenasterol ratio ($r = -0.54$, $P = .03$; $r = 0.81$, $P = .05$), and total liver PS ratio ($r = -0.70$, $P < .01$; $r = 0.84$, $P = .04$, respectively). Among PN-dependent patients, number of weekly PN infusions correlated positively with sitosterol and total liver PS concentrations ($r = 0.76$, $P = .05$ for both) and PS intake with liver ratios of sitosterol and avenasterol ($r = 0.83$, $P = .04$; $r = 0.87$, $P = .02$).

Interrelations Between Serum and Liver PSs

The linear regression model suggested that total serum PS was a strong predictor of liver PS levels among all patients ($r = 0.83$, $P < .01$ for absolute concentrations; $r = 0.98$, $P < .01$ for ratios to cholesterol; Figure 1C and 1D). Of different PS

Table 3. Serum and Liver Plant Sterol Concentrations and Ratios to Cholesterol.^a

Concentration: Ratio	Patients Receiving PN	Patients Weaned Off PN
Serum, n	6	9
Cholesterol, mg/dL	110 (89–137)	122 (90–140)
Campesterol, µg/dL	351 (176–458)	254 (157–418)
100 × µg/mg of cholesterol	256 (180–514)	181 (129–343)
Stigmasterol, µg/dL	78 (33–134)	18 (12–22) ^b
100 × µg/mg of cholesterol	65 (40–128)	14 (13–17) ^b
Sitosterol, µg/dL	1713 (593–2769)	186 (85–191) ^b
100 × µg/mg of cholesterol	1364 (609–3119)	114 (85–157) ^b
Avenasterol, µg/dL	92 (49–141)	41 (38–67)
100 × µg/mg of cholesterol	66 (53–156)	40 (36–42) ^c
Total plant sterols, µg/dL	2193 (837–3480)	512 (322–675) ^c
100 × µg/mg of cholesterol	1750 (859–3920)	340 (279–554) ^b
Liver, n	7	9
Cholesterol, mg/100 g	366 (338–478)	312 (295–323)
Campesterol, µg/100 g	1298 (818–2423)	1014 (591–1923)
100 × µg/mg of cholesterol	355 (261–572)	234 (209–442)
Stigmasterol, µg/100 g	319 (267–1434)	385 (240–429)
100 × µg/mg of cholesterol	120 (81–248)	103 (70–133)
Sitosterol, µg/100 g	3933 (2731–9186)	754 (515–1000) ^b
100 × µg/mg of cholesterol	1100 (733–2289)	196 (182–309) ^b
Avenasterol, µg/100 g	2156 (1089–3541)	692 (455–1000) ^c
100 × µg/mg of cholesterol	575 (390–676)	222 (144–247) ^c
Total plant sterols, µg/100 g	7134 (4933–16,584)	3000 (2189–3943) ^c
100 × µg/mg of cholesterol	1996 (1524–3926)	708 (672–1082) ^b

PN, parenteral nutrition.

^aCholesterol and plant sterol concentrations assessed with gas-liquid chromatography. Unless noted otherwise, data are presented as medians (interquartile range). P values for the differences between subgroups were calculated by Mann-Whitney U test.

^b $P \leq .01$.^c $P \leq .05$.

fractions, serum/liver concentrations and ratios of sitosterol and avenasterol were positively interrelated in PN-dependent patients. In patients weaned off PN, serum/liver ratios of campesterol were positively and ratios of stigmasterol negatively interrelated (Table 4).

Serum and Liver PSs in Relation to Biochemical Liver Function

Serum stigmasterol concentration and ratio correlated positively with GT ($r = 0.61$, $P = .02$; $r = 0.59$, $P = .02$, respectively), and sitosterol concentration with aspartate

Figure 1. Subgroup comparison of total plant sterol (PS) ratios to cholesterol ($100 \times \mu\text{g}/\text{mg}$ of cholesterol) in (A) serum and (B) liver. The box plots display median, interquartile range, and range. The round dot represents a value larger than the upper quartile plus 1.5 times the interquartile range. P values for the differences between subgroups were calculated by Mann-Whitney U test. Regression plots between serum and liver total PS (C) ratios to cholesterol ($100 \times \mu\text{g}/\text{mg}$ of cholesterol) and (D) concentrations ($\mu\text{g}/100 \text{ g}$ of liver tissue). PN, parenteral nutrition.

aminotransferase ($r = 0.53$, $P = .04$). Liver total PS ratio correlated positively with GT ($r = 0.52$, $P = .04$) and liver sitosterol ratio with GT ($r = 0.63$, $P = .01$) and albumin ($r = -0.52$, $P = .04$).

Cholesterol and Liver Histology

In all patients, serum cholesterol was inversely related with histologic steatosis ($r = -0.66$, $P < .01$). Among PN-dependent patients, serum cholesterol correlated negatively with steatosis ($r = -0.83$, $P = .04$) and parenteral cholesterol intake with fibrosis, steatosis, and any liver histopathology ($r = -0.83$,

$P = .04$ for all). Among patients weaned off PN, serum cholesterol correlated negatively with the presence of any liver histopathology ($r = -0.73$, $P = .03$).

Serum and Liver PSs in Relation to Liver Histology

Histologic PI correlated positively with GT ($r = 0.65$, $P < .01$) and serum avenasterol concentration and ratio ($r = 0.58$, $P = .02$; $r = 0.54$, $P = .04$, respectively). Liver concentrations of all PS fractions were significantly higher in patients with versus without PI (Figure 2). PI also correlated positively with all

Table 4. Spearman Rank Correlations and Their *P* Values Between Serum and Liver Plant Sterol Concentrations and Ratios to Cholesterol.

Plant Sterol	Correlation (<i>P</i> Value)	
	PN-Dependent Patients (n = 6)	Patients Weaned Off PN (n = 9)
Campesterol concentration	0.54 (.27)	0.40 (.29)
Ratio to cholesterol	0.71 (.11)	0.90 (<.01)
Stigmasterol concentration	0.41 (.43)	−0.04 (.91)
Ratio to cholesterol	0.49 (.33)	−0.87 (<.01)
Sitosterol concentration	0.83 (.04)	0.24 (.53)
Ratio to cholesterol	1.00 (.01)	0.38 (.31)
Avenasterol concentration	0.83 (.04)	0.10 (.80)
Ratio to cholesterol	0.83 (.04)	0.59 (.10)
Total plant sterol concentration	0.83 (.04)	0.45 (.22)
Ratio to cholesterol	0.87 (.02)	0.72 (.03)

PN, parenteral nutrition.

liver PS concentrations and, apart from avenasterol, with their ratios to cholesterol: sitosterol ($r = 0.66$, $P < .01$ for both), avenasterol ($r = 0.56$, $P = .02$), stigmasterol ($r = 0.53$, $P = .03$; $r = 0.56$, $P = .02$), campesterol ($r = 0.53$, $P = .03$; $r = 0.50$, $P < .05$), and liver total PS ($r = 0.63$, $P < .01$; $r = 0.66$, $P < .01$, respectively).

Among PN-dependent patients, liver campesterol concentration and ratio correlated positively with liver fibrosis ($r = 0.79$, $P = .03$ for both), PI ($r = 0.87$, $P = .01$ for both), and any histopathology ($r = 0.79$, $P = .03$ for both), whereas liver stigmasterol concentration and ratio correlated with fibrosis ($r = 0.79$, $P = .03$ for both) and any liver histopathology ($r = 0.79$, $P = .03$ for both). Table 5 displays individual liver biopsy findings in relation to liver PS content, parenteral PS delivery, and other patient characteristics among patients receiving PN. PI and fibrosis tended to occur in SBS children with the highest liver PS content, while the patient with chronic intestinal pseudo-obstruction with nearly an entire small intestine remaining had no evidence of liver histopathology, despite receiving the highest amounts of parenteral fat and energy.

Discussion

Although increasing evidence suggests that parenteral PSs are hepatotoxic, knowledge on their tissue distribution and metabolic effects is mainly based on animal models and remains largely unexplored in humans.^{4,7,10} This is the first study demonstrating that liver PS levels are markedly higher during PN than after weaning off PN and associate closely with parenteral delivery of vegetable oil-based lipid emulsions, suggesting that parenteral PSs accumulate in the liver during PN delivery in patients with IF. Serum PS levels were highly predictive of liver PS load. In patients receiving lipid emulsions with low stigmasterol and campesterol contents,²¹ parenteral PS intake

also reflected liver sitosterol and avenasterol ratios to cholesterol. Serum and liver PS levels correlated with plasma GT, and all liver PS fractions associated with histologic PI, although none of the patients had biochemical cholestasis.

Hepatic accumulation of PSs during PN is thought to result from both their excess parenteral administration as well as their ability to suppress canalicular sterol transporters through inhibition of FXR.^{4,7,10} Accordingly, we found the highest liver concentrations for sitosterol, composing 70%–90% of total PSs in commercial parenteral vegetable oil lipid solutions.^{21,24} Compared with soy oil-based PN, the mixture of 80% olive oil and 20% soy oil has a similar sitosterol content while remarkably lower campesterol and stigmasterol contents.^{21,24} Sitosterol is secreted to bile more effectively than other PS fractions,¹¹ possibly contributing to the decline of liver and serum PS levels after PN cessation in our patients. Indeed, sitosterol composed up to 90% of total PS intake in our patients,^{21,24} yet it made up 55% and 25% of total liver PS in patients receiving and weaned off PN, respectively. Liver stigmasterol and campesterol concentrations, instead, did not differ in relation to PN status, and serum and liver stigmasterol were negatively correlated, suggesting that these PS fractions are probably excreted at a slower rate.¹¹ The finding that transition from soy oil-based to olive oil-based PN may improve IFALD^{14,15,25} may in part be explained by the different PS compositions of these solutions.

Delivery of fish oil-based PN associates with reversal of IFALD.^{1,16} Although this has been proposed to be mediated by its fatty acid composition and antioxidative characteristics,²⁶ another possible explanation is the absence of PS in fish oil, supported by studies showing that adding stigmasterol to fish oil and administering fish oil with soy oil can result in similar serum PS levels and hepatotoxicity than soy oil alone.^{4,9,13,16,27} Another potential benefit of fish oil is its higher cholesterol content versus soy and olive oil.²¹ Children receiving PN frequently present with abnormally low serum cholesterol, which, however, is essential for normal growth and development.^{15,28} PSs derived from PN are able to displace cell membrane cholesterol, which may decrease tissue elasticity and contribute to hepatocyte damage.^{7,29} Indeed, we found low cholesterol intake and low serum cholesterol to relate with PN duration as well as with liver fibrosis and steatosis. As serum and liver sterol proportions are distorted during PN, with high PS and low cholesterol levels reflecting the lipid profile of PN solutions, the ratio of each PS fraction to cholesterol probably mirrors the metabolic effects of PS better than their absolute concentrations.

Serum PS concentrations paralleled liver PS, and both reflected parenteral PS intake in piglets after only 2 weeks receiving PN.¹⁰ Accordingly, in addition to the observed correlation between PS intake and accumulation, we found serum total PS determination to be a useful method to estimate liver PS load, as these parameters were closely interrelated. No human data exist on the rate of hepatic PS accumulation after

Figure 2. Comparisons of liver plant sterol (PS) concentrations (μg/100 g of liver tissue) between patients with (n = 4) and without (n = 12) histologic portal inflammation (PI). Box plots display median, upper and lower interquartile range, and range. The round dot represents a value larger than the upper quartile plus 1.5 times the interquartile range. *P* values for the differences between subgroups were calculated by Mann-Whitney *U* test.

initiation of PN. However, if this occurs as rapidly as suggested by animal studies, it could explain why PN-related cholestasis occasionally manifests after few weeks of PN.^{2,30} Apart from stigmasterol and campesterol, liver PS levels were lower after weaning off versus during PN, suggesting that accumulated PSs are eliminated after PN cessation. Moreover, serum PS levels of patients weaned off PN were similar to those previously reported among healthy children.^{8,12} Advanced liver damage could relate with protracted PS secretion⁹; however, advanced fibrosis or PI was not observed in any patient weaned off PN, although as many as 78% presented with IFALD-related liver histology. Liver steatosis and fibrosis are known

to persist after PN cessation,⁵ and our results suggest that this occurs regardless of dissolution of liver PS.

Experimental studies have demonstrated that sitosterol, stigmasterol, and campesterol downregulate FXR and decrease transcription of its target genes,^{4,10,31} which are involved in synthesis, uptake, and excretion of bile acids as well as excretion of sterols and phospholipids. Antagonism of FXR may promote cholestasis through all these mechanisms.^{4,10} In line with earlier studies,^{6-9,12-15} we found serum and liver PSs to correlate with GT, a sensitive marker of cholestasis and biliary injury.³² In PN-dependent patients, liver campesterol and stigmasterol also correlated with liver fibrosis. Furthermore, PS

Table 5. IF Etiology, Age at Liver Biopsy, Characteristics of PN Preceding Liver Biopsy, Liver Total PSs, and Liver Biopsy Findings Presented Individually for PN-Dependent Patients (n = 7).

Patient	IF Etiology	Age, y	Energy, %	Lipid Emulsion	PN				Liver Total PSs		Histologic	
					Duration, mo	Lipid Calories, %	Lipid, g/kg/d	Plant Sterols, µg/kg/d	µg/100 g of Liver	100 × µg/mg of Cholesterol	Portal Inflammation	Fibrosis
1	CIPO	5.53	90	Clinoleic	11	21	3.5	4841	7130	1996	No	No
2	SBS	18.6	45	Clinoleic	1.6	— ^a	— ^a	— ^a	19,130	3816	Yes	Yes
3	SBS	1.74	50	Clinoleic	21	29	1.5	2014	14,040	4420	Yes	Yes
4	SBS	0.20	100	Clinoleic	0.7	15	1.7	2287	49,530	4036	Yes	Yes
5	SBS	5.58	20	Clinoleic	61	13	0.47	1031	3920	1762	No	Yes
6	SBS	0.76	17	SMOF	9.3	11	0.40	413	4700	1285	Yes	Yes
7	SBS	0.90	75	Clinoleic + Omegaven	11	13	0.79	464	5160	1137	No	No

CIPO, chronic intestinal pseudo obstruction; IF, intestinal failure; PN, parenteral nutrition; PS, plant sterol; SBS, short bowel syndrome; SMOF, soy, olive, fish oil, and medium-chain triglycerides.

^aDetailed parenteral lipid dosage was not available for 1 patient.

may advocate hepatic inflammation by downregulating FXR and by activating liver macrophages.⁴ Accordingly, all liver PS fractions were increased in patients with PI, which is a common early histopathologic finding in IFALD^{30,33} and associates with cholestasis⁵ as well as with elevated serum stigmaterol and avenasterol ratios in IF children.¹² We found PI to associate more strongly with liver than serum PS levels, suggesting that the underlying cause would be the hepatic accumulation of PS rather than PN administration as such.

Another important mechanism in the pathogenesis of IFALD is proinflammatory signaling through permeable bowel wall, which likely promotes hepatic inflammation.^{4,9} Indeed, stigmaterol induced hepatocyte injury and inflammation in mice only when intestinal integrity was disrupted.³⁴ Moreover, SBS children are known to be more prone to IFALD than dysmotility patients.³⁵ Also, in our study, the child with chronic intestinal pseudo-obstruction and nearly an entire small intestine remaining had no histologic liver injury, despite receiving higher doses of parenteral lipid and PSs than any patient with SBS. Once an inflammatory state has been established, the increased cytokine signaling may further suppress FXR expression and exacerbate hepatocyte damage, suggesting that the development of inflammation may be crucial in IFALD progression.^{4,34} These data are also in line with our finding that liver fibrosis occurred during PN almost exclusively in the presence of PI.

The small sample size, use of olive oil-based and fish oil-based PN emulsions instead of pure soy oil, and relatively well-preserved liver function of the study patients may partly explain the lacking correlations between PS levels and some markers of liver function and cholestasis, including transaminases and bilirubin. Furthermore, serum and liver PSs were measured cross sectionally, and no entire PN history was available for all patients. Despite these limitations, this is the first study demonstrating that in patients with IF, liver PS levels increase during

parenteral delivery of vegetable oil-based lipid emulsions and relate with biochemical and histologic liver damage. Although our results do not confirm a causal relationship between liver PS accumulation and IFALD nor rule out other PN components contributing to liver injury, they support the increasingly favored concept proposing that PSs play a key role in the development of IFALD.

In conclusion, significantly higher liver and serum PS levels were found in children with IF who were noncholestatic during PN than after weaning off PN. Liver PS levels were related with parenteral lipid and PS administration and correlated strongly with serum PS concentration. Elevated liver PS levels associated with GT and histologic PI, as well as with liver fibrosis in PN-dependent patients. In addition to future in vitro works unraveling further molecular mechanisms behind IFALD, more clinical studies are warranted to increase our knowledge on the metabolic effects and distribution of PSs, as well as safe PN administration in patients with IF.

Statement of Authorship

M. P. Pakarinen is responsible for the study design and contributed to data collection, analyses, and interpretation. M. Hukkinen conducted the analyses, wrote the manuscript, and contributed to study design. A. Mutanen participated in data collection. M. Nissinen, H. Gylling, and L. Merras-Salmio contributed to study design. All authors critically revised the manuscript, accepted the final version, and agree to be accountable for all aspects of work ensuring integrity and accuracy.

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