HEPATOLOGY

AUTOIMMUNE, CHOLESTATIC AND BILIARY DISEASE | HEPATOLOGY, VOL. 74, NO. 5, 2021

Chyme Reinfusion Restores the Regulatory Bile Salt–FGF19 Axis in Patients With Intestinal Failure

Kiran V.K. Koelfat [1,2*] Denis Picot,[3*] Xinwei Chang,[1,2*] Mireille Desille-Dugast,[4] Hans M. van Eijk,[1,2] Sander M.J. van Kuijk,[5] Martin Lenicek,[6] Sabrina Layec,[3] Marie Carsin,[3] Laurence Dussaulx,[3] Eloi Seynhaeve,[3] Florence Trivin,[3] Laurence Lacaze,[4] Ronan Thibault,[4**] Frank G. Schaap [1,2,7**] and Steven W.M. Olde Damink[1,2,7**]

BACKGROUND AND AIMS: Automated chyme reinfusion (CR) in patients with intestinal failure (IF) and a temporary double enterostomy (TDE) restores intestinal function and protects against liver injury, but the mechanisms are incompletely understood. The aim was to investigate whether the beneficial effects of CR relate to functional recovery of enterohepatic signaling through the bile salt–FGF19 axis.

APPROACH AND RESULTS: Blood samples were collected from 12 patients, 3 days before, at start, and 1, 3, 5, and 7 weeks after CR initiation. Plasma FGF19, total bile salts (TBS), 7-α-hydroxy-4-cholesten-3-one (C4; a marker of bile salt synthesis), citrulline (CIT), bile salt composition, liver tests, and nutritional risk indices were determined. Paired small bowel biopsies prior to CR and after 21 days were taken, and genes related to bile salt homeostasis and enterocyte function were assessed. CR induced an increase in plasma FGF19 and decreased C4 levels, indicating restored regulation of bile salt synthesis through endocrine FGF19 action. TBS remained unaltered during CR. Intestinal farnesoid X receptor was up-regulated after 21 days of CR. Secondary and deconjugated bile salt fractions were increased after CR, reflecting restored microbial metabolism of host bile salts. Furthermore, CIT and albumin levels gradually rose after CR, while abnormal serum liver tests normalized after CR, indicating restored intestinal function, improved nutritional status, and amelioration of liver injury. CR increased gene transcripts related to enterocyte number, carbohydrate handling, and bile salt homeostasis. Finally, the reciprocal FGF19/C4 response after 7 days predicted the plasma CIT time course.

CONCLUSIONS: CR in patients with IF-TDE restored bile salt–FGF19 signaling and improved gut–liver function. Beneficial effects of CR are partly mediated by recovery of the bile salt–FGF19 axis and subsequent homeostatic regulation of bile salt synthesis. (Hepatology 2021;74:2670-2683).

A temporary double enterostomy (TDE) is most often created to avoid or protect high-risk anastomoses in frail or sick patients. TDEs are often used in the acute setting of intestinal ischemia resulting from, e.g., a thromboembolic event or herniation. Unfortunately, some patients may develop intestinal failure (IF) due to (functional) short bowel syndrome,

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; C4, 7-α-hydroxy-4-cholesten-3-one; cDNA, complementary DNA; CIT, citrulline; CR, chyme reinfusion; CRP, C-reactive protein; CUBN, cubilin; FXR, farnesoid X receptor; GCA, glycocholic acid; GCDCA-3S, glycochenodeoxycholate-3-sulfate; GGT, gamma glutamyl transferase; IF, intestinal failure; IVS, i.v. fluid supplementation; NRI, nutritional risk index; OCT, ornithine carbamoyl transferase; OSTα/β, organic solute transporter subunits-α/β; PN, parenteral nutrition; SI, sucrase-isomaltase; TBS, total bile salts; TDE, temporary double enterostomy; VIL1, villin-1.

Received October 20, 2020; accepted May 22, 2021.

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.32017/suppinfo.

**These authors share co–first authorship.*

***These authors share co–senior authorship.*

Supported by a European Society for Clinical Nutrition and Metabolism research fellowship (2015-16, to K.V.K.K.), the Dutch Society of Gastroenterology (2014-15, to K.V.K.K.), the Netherlands Organization for Scientific Research (NWO 022.003.011, to K.V.K.K.), and the China Scholarship Council (201707040095, to X.C.).

[Correction added September 2, 2021 after first online publication: Corresponding author Ronan Thibault's contact information was updated]

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.32017

Potential conflict of interest: Nothing to report.

requiring parenteral nutrition (PN) and/or i.v. fluid supplementation (IVS) until recovery for surgical reestablishment of intestinal continuity, typically 3-6 months after primary surgery.[1] Prolonged PN dependence is often necessary but is associated with risk of increased morbidity and mortality,[2,3] mainly related to central venous tract complications, catheter-related infections, central venous thrombosis, or catheter replacement. The development of IF-associated liver disease frequently occurs.[1] An alternative treatment is chyme (intestinal or fistula secretions) reinfusion (CR), through an extracorporeal enteral nutrition technique, into the distal part of the small bowel. This method results in improved nutritional status, better intestinal absorptive function, recovery from liver disease, reduced intestinal secretions, and a shorter period to PN independence in retrospective studies.[4–7] The mechanisms underlying the beneficial effects of CR are incompletely understood, but functional recovery of the enterohepatic circulation of bile salts and attendant signaling has been postulated.[4]

FGF19 is a postprandial hormone secreted by ileocytes in a bile salt–stimulated fashion that involves activation of the bile salt–sensing transcription factor farnesoid X receptor (FXR).[8] The key role of FGF19 is to control bile salt homeostasis by negatively regulating bile salt synthesis through repression of the bile salt synthetic enzyme cholesterol-7α-hydroxylase in the liver.[8] Disruption of the negative feedback loop results in unopposed bile salt synthesis. As a result of low circulating FGF19 levels, bile salt overproduction occurs; and this has been associated with pediatric and adult IF-associated liver disease, or primary bile acid diarrhea.[9–15] Currently, pharmacological treatment with FGF19 analogues in patients with hepatobiliary diseases including primary biliary cholangitis and NASH has been evaluated clinically and renders promising therapeutic effects.[16,17]

In the Restored Enterohepatic Signaling: Chyme Reinfusion Therapy (RESCUE) study presented here, we test the hypothesis that functional recovery of the enterohepatic circulation by CR and attendant restoration of the regulatory bile salt–FGF19 axis in patients with IF and a TDE is associated with CR-related beneficial effects.

Patients and Methods

STUDY DESIGN AND PARTICIPANTS

The RESCUE study is a prospective study with a preintervention and postintervention design conducted at Rennes University Hospital (CHU Rennes) and Clinique Saint Yves, Rennes, France (a tertiary referral center for intestinal rehabilitation of patients prior to restorative surgery), from January 2017 to July 2018. The study was registered at clinicaltrials.gov (NCT02990195). Eligible participants were adult

ARTICLE INFORMATION:

From the [1]Department of Surgery, Maastricht University Medical Center, Maastricht, the Netherlands; [2]NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands; [3]Department of Nutritional and Digestive Rehabilitation, Clinique Saint Yves, Rennes, France; [4]INSERM, INRAE, University of Rennes, Nutrition Metabolisms and Cancer, NuMeCan, Nutrition Unit, CRB Santé, CHU Rennes, Rennes, France; [5]Department of Clinical Epidemiology and Medical Technology Assessment, Maastricht University Medical Center, Maastricht, the Netherlands; [6]Institute of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic; [7]Department of General, Visceral and Transplantation Surgery, RWTH University Hospital Aachen, Aachen, Germany.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Xinwei Chang, M.D.
Department of Surgery, Maastricht University Medical Center
Universiteitssingel 50 6229 ER, PO Box 6200 MD, Maastricht
The Netherlands
E-mail: x.chang@maastrichtuniversity.nl
Tel.: +31 43 388 1499
or
Ronan Thibault, M.D., PhD.
Centre labellisé de nutrition parentérale à domicile, CHU Rennes
2, rue Henri Le Guilloux, 35000 Rennes, France
E-mail: ronan.thibault@chu-rennes.fr
Tel.: +33 2 99 28 96 46 – FAX: +33 2 99 28 24 34
or
Kiran V.K. Koelfat, M.D., M.Sc.
Department of Surgery, Maastricht University Medical Center
Universiteitssingel 50 6229 ER, P.O. Box 6200 MD, Maastricht
The Netherlands
E-mail: k.koelfat@maastrichtuniversity.nl
Tel.: +31 43 388 1499

patients with IF aged 18 years or above with a TDE intended to receive CR as primary care. Further inclusion criteria were a minimum of 25 cm of healthy distal small intestine that was suitable for CR. We excluded patients with mental disability, pregnancy or lactation, HCC, autoimmune disorders, blood coagulation disorders, or shock of any cause. None of the patients had known viral or genetic liver disease prior to the double enterostomy. We obtained written informed consent from all participants. This study was approved by the institutional review board of Rennes University Hospital, Rennes, France (2016-A01156-45).

CR was performed at Clinique Saint Yves using the Enteromate II system (Labodial, Les Clayes Sous Bois, France) as described.[18] In brief, proximal effluent was continuously pumped into a 30-mL collection container equipped with weight monitoring. When the collected volume exceeded 10 mL, the second pump started and reinfused the collected chyme into the distal part of the small bowel. In patients with inadequate oral intake, an additional enteroclysis was applied to deliver enteral nutrition to the reinfusion tube in the efferent small bowel. CR was initiated approximately 1 week after referral of the patient to Clinique Saint Yves and continuously applied for 2-3 months. Afterward, patients underwent surgical reestablishment of intestinal continuity. Blood samples were collected 3 days before CR (day −3), at the day of CR initiation (day 0), and during the course of CR (after 1, 3, 5, and 7 weeks). Patients underwent endoscopic biopsy of the distal small intestine 3 days before and 3 weeks after CR at CHU Rennes by an experienced gastroenterologist. The study design is shown in Supporting Fig. S1.

DATA COLLECTION AND DEFINITIONS

Data were collected and managed using a customized Access Database (Microsoft, Redmond, WA). The following items were recorded: gender, age, height, body weight, body mass index, underlying diseases, afferent small intestinal length (from duodenojejunal flexure to the proximal stoma), efferent small intestinal length (from distal stoma to the terminal part of the remnant efferent small intestine), resected length, efferent small intestinal anatomy, energy (kilocalories per kilogram) and protein (grams per kilogram) intake (parenteral, oral, and enteral) adjusted by body weight, and duration of CR and PN. We measured proximal stoma or fecal output daily in all patients before and after CR initiation, respectively. Percent weight loss was assessed as follows: $\text{Weight loss}\,(\%) = 100 * \frac{(\text{usual weight} - \text{present weight})}{\text{usual weight}}$. None of the patients was diagnosed with ascites during CR treatment. Nutritional Risk Index (NRI) was determined as follows: $\text{NRI} = 1.519 * \text{serum albumin}\,(\text{g/L}) + 41.77 * \frac{\text{present weight}}{\text{usual weight}}$.[19] NRI-based risk groups were as follows: absence of malnutrition risk (NRI > 97.5), moderate malnutrition risk (83.5 ≤ NRI ≤ 97.5), and severe malnutrition risk (NRI < 83.5).[19]

BLOOD SAMPLES AND ANALYSES

Blood samples were collected from patients in the morning and after an overnight fast in three 8-mL vacuum tubes (serum, EDTA, and lithium heparin tubes). Serum tubes were left at room temperature for 30 minutes, and plasma tubes were put on ice immediately after collection. After 30 minutes, all blood samples were centrifuged at 4,000 rpm for 10 minutes at 4°C. The supernatant was collected, divided into aliquots, and stored at −80°C until transport to Maastricht University (Maastricht, the Netherlands) for further analysis. Plasma levels of FGF19 and proinflammatory cytokines (IL-6 and TNFα) were assessed by sandwich ELISA as reported.[20] Plasma citrulline (CIT; a marker of the mass of functional enterocytes) was determined by a high-performance liquid chromatography method.[6,21] Plasma 7-α-hydroxy-4-cholesten-3-one (C4; a blood marker of bile salt synthesis),[22] total bile salts (TBS), and bile salt composition (in both plasma and chyme) were assayed by liquid chromatography-mass spectrometry (Schaap and OLDE Damink, manuscript in preparation). Serum liver tests (alanine aminotransferase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], γ-glutamyltransferase [GGT], and total bilirubin), albumin, and C-reactive protein (CRP) were evaluated by routine assay at the Department of Clinical Biochemistry at Maastricht University Medical Center. For baseline comparisons of TBS, FGF19, and C4 levels, our own published data on 12 healthy controls (mean age 55 years, 33% female) were used.[23]

GENE EXPRESSION ANALYSIS

Ileal biopsies were obtained through routine endoscopic procedures and immediately preserved in

RNAlater (Thermo Scientific, Waltham, MA) for 24 hours at 4°C and then stored at −80°C (Rennes University Hospital). Biopsies were ultimately transported to Maastricht University Medical Center for transcript analyses. Here, total RNA was isolated using the RNeasy Plus Mini Kit according to the manufacturer's protocol (Qiagen, Hilden, Germany). Final RNA concentration and purity were determined by spectrophotometry. A total of 750 ng RNA was converted to complementary DNA (cDNA) using the SensiFAST cDNA Synthesis Kit (Bioline, London, UK). For the quantitative RT-PCR, we used the SensiFAST SYBR Hi-ROX kit (Bioline) and cDNA as template on a LightCycler 480 SW 1.5 system (Roche, Basel, Switzerland). Quantitative RT-PCR data were analyzed using LinRegPCR software,[24] and gene expression levels were normalized to the geometric mean of four reference genes (*36B4*, proteasome 20S subunit beta 4, receptor accessory protein 5, and hypoxanthine phosphoribosyltransferase 1). Employed primer sequences are provided in Supporting Table S1. Paired ileal biopsies were available from 7 of 12 patients. In the remaining patients, (paired) biopsies could not be gathered for logistic reasons (n = 2), last-minute patient refusal (n = 1), or because transcript data were excluded from analyses due to a nonenterocytic transcript signature (n = 2).

STATISTICAL ANALYSIS

Initially, 20 patients were planned for enrollment in this study. A planned interim analysis was performed after enrollment of two thirds of patients (n = 13). One patient was not included in the study after enrollment because he received enteral nutrition as enteroclysis into the downstream small bowel, before starting the protocol. On the interim analysis (n = 12), the primary endpoint (plasma FGF19 levels) had significantly changed ($P < 0.005$), justifying early termination of the study. For blood analyses, five out of 72 time points were missing (6.9%). Multiple data imputation was used to handle the missing data. The means of day −3 and day 0 measurements were taken as baseline values. Data are expressed as mean ± SD, median (interquartile range), or frequency (percentage) where appropriate. Graphical data are expressed as mean ± 95% CI. Schematic overview was created with Adobe Illustrator and BioRender.com. Differences between patients with IF and healthy controls were evaluated by Mann-Whitney U test. Correlations were evaluated by Pearson's (r) or Spearman's (ρ) correlation coefficient depending on data distribution. For continuous variables, changes during CR were evaluated by paired-samples t test or Wilcoxon matched-pairs signed ranks sum test depending on the data distribution. Categorical variables were analyzed using Fisher's exact test. To evaluate longitudinal changes, ANOVA or Friedman's test for repeated measurements, corrected using Dunn's multiple comparisons test, was used. Linear mixed models were performed to identify whether the FGF19 or C4 response predicts the evolution of markers for intestinal absorptive function (CIT) and liver injury (ALP and GGT). Akaike's information criterion was used to compare the best fit of a covariance structure for the linear mixed models. $P < 0.05$ was considered statistically significant. SPSS statistics, version 26.0 (IBM, Chicago, IL), was used for all statistical analyses.

Results

PATIENT CHARACTERISTICS

Twelve patients (mean age 68 years, 42% females) were included in the study (Table 1). Despite the fact that features of IF necessitating supplementation with PN were apparent in all surgical patients, 10 patients actually received PN and/or i.v. hydration at admission. Ten patients remained in the hospital during the 7-week course of CR, and 2 patients received home CR after specific training and education. Major underlying causes for small intestinal resection and/or creation of a TDE were mechanical obstruction and intestinal ischemia. Patients underwent small intestinal resection (n = 8) or received a terminal colostomy on the transverse (n = 3) or right colon (n = 1). Small bowel resection was combined with a partial gastrectomy in a single patient. Additional individual patient data are summarized in Supporting Table S2.

DYSREGULATED BILE Salt–FGF19 AXIS IN PATIENTS WITH IF-TDE

First, we investigated whether the bile salt–FGF19 axis was disturbed in patients with IF-TDE prior to CR. Baseline levels of TBS, FGF19, and C4 of healthy controls and patients with IF are shown in Fig. 1A. Demographic information on healthy controls is

TABLE 1. Patient demographics and surgical characteristics at baseline

Variable	Study Cohort
Patients, n*	12
Age, years	67.7 ± 14.6
Male/female	7/5
Underlying etiology, n (%)	
Mechanical occlusion	3 (25)
Ischemia	4 (33)
Peritonitis	5 (42)
PN dependence, n (%)	10 (83)
Jejunal efflux (mL/24 hours)	2,245 (1,366-2,913)
Jejunal efflux (mL • weight^{-1} • 24 hours^{-1})	39 (22-52)
Hospital admission time before referral (days)	28 (18-59)
Plasma CIT ≤ 20 μmol/L, n (%)	7 (58)
SB resection ≥ 30 cm, n (%)	5 (42)
Resection SB length if resection (n = 6), cm	105 (45-135)
Upstream SB length (n = 9), cm	120 (100-200)
Upstream SB length ≤ 100 cm, n (%)	4 (44)
Downstream SB length (n = 11), cm	160 (120-225)
Theoretical total SB length† (n = 8), cm	315 (268-353)
Downstream SB anatomy, n (%)	
Ileo-colon-rectum	8 (67)
Ileo-colon-stoma	4 (33)
Serum liver tests (IU/L)	
ALP	216 (163-291)
GGT	176 (73-252)
ALT	66 (45-109)
AST	37 (29-57)
Total bilirubin, μmol/L	6.2 (3.7-9.3)
Serum CRP (mg/L)	6.3 (2.1-36.0)

*If data are missing, numbers in parentheses indicate the number of assessments for a particular factor. Data are depicted as percentages =, mean ± SD, or median (interquartile range).
†Theoretical total SB length = upstream + downstream SB length.
Abbreviation: SB, small bowel.

provided in Supporting Table S3. TBS levels in patients with IF were not different compared to healthy controls (1.9 [1.4-3.0] vs. 1.6 [0.9-2.4] μmol/L, P = 0.288). In line with earlier observations in patients with chronic IF,[14] baseline FGF19 levels were markedly lower compared to healthy controls (0.023 [0.012-0.061] vs. 0.098 [0.071-0.136] ng/mL, P < 0.001) (Fig. 1A). Conversely, high baseline C4 levels were observed in patients with IF compared to controls (95 [38-169] vs. 19 [10-31] ng/mL, P = 0.002), indicating dysregulated control of bile salt synthesis (Fig. 1A). Indeed, FGF19 showed a strong negative correlation with C4 at baseline (ρ = −0.86, P < 0.001) (Fig. 1B). Furthermore, baseline C4 levels were positively related to 24-hour jejunal output adjusted by baseline weight (ρ = −0.80, P = 0.002) (Fig. 1C), indicating a choleretic effect before CR that is linked to enhanced bile salt synthesis.[25] FGF19 levels were not related to jejunal efflux (ρ = −0.44, P = 0.155) (Fig. 1C). Of note, baseline C4 levels were higher in women compared to men (179 [95-274] vs. 55 [28-103] ng/mL, P = 0.017), but C4 levels of both sex groups of patients were significantly higher compared to healthy controls (data not shown). No clinical or biochemical explanation was found for the sex difference. None of the other laboratory parameters were different between men and women.

CR RESTORES THE REGULATORY BILE Salt–FGF19 AXIS

Reinfusion of proximal small intestinal secretions into the distal small bowel allows enterohepatic recycling of bile salts to the liver and may accordingly reinstate the gut–liver axis of bile salt–FGF19 signaling. For this reason, we studied the effect of CR on the time courses of plasma TBS, FGF19, and C4. The individual responses are shown in Supporting Fig. 2A,B. Systemic TBS levels did not change significantly after introducing CR (P = 0.112) (Fig. 2A). FGF19 levels increased after CR initiation (P = 0.002), with a transient peak after 7 days, reaching a new steady-state level that was higher than at baseline thereafter (Fig. 2A). In line with elevated FGF19 levels, plasma C4 levels were reduced after the start of CR (P < 0.001) (Fig. 2A), indicating reduced bile salt synthesis during CR. The percentage change from baseline is shown in Fig. 2B. Most pronounced responses of FGF19 and C4 were seen after 7 days of CR (Fig. 2B). TBS levels were unaltered after 7 days of CR (1.9 [1.4-3.0] vs. 1.2 [0.5-3.6] μmol/L, P = 0.773) (Fig. 2C). FGF19 levels were higher for all but 1 patient after 7 days compared to baseline values (0.023 [0.012-0.061] vs. 0.128 [0.077-0.208] ng/mL, P = 0.003) (Fig. 2C). Conversely, and in line with FGF19 function, C4 levels were consistently lower after 7 days compared to baseline values (95 [38–196] vs. [18 [11-26] ng/mL, P < 0.001) (Fig. 2C).

Further, gene expression of *FXR* and the FXR-regulated organic solute transporter subunits *α/β* (*OSTα/β*, engaged in export of bile salts from the enterocyte to the venous capillaries) was up-regulated after CR (P < 0.05 for all) (Fig. 2D). Transcripts of the apical sodium-dependent bile salt transporter,

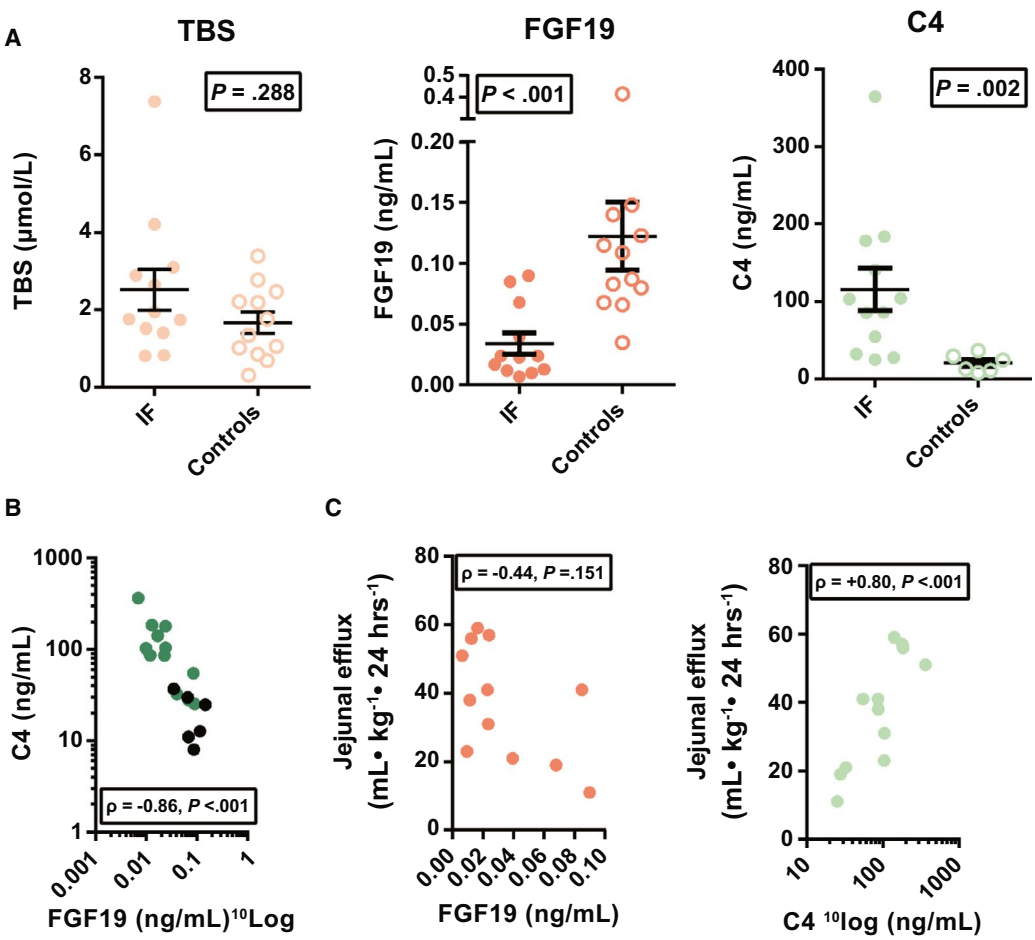


FIG. 1. Dysregulated bile salt synthesis in patients with IF-TDE prior to CR. (A) Baseline values of TBS, FGF19, and C4 of patients with IF-TDE (n = 12) compared with controls (n = 12). Note, C4 levels were available for 6 out of 12 healthy volunteers. (B) Correlation between FGF19 and C4 levels in patients with IF-TDE (blue circles) and controls (black circles). (C) Correlation between jejunal efflux adjusted by weight and FGF19 or C4. Data are depicted as mean ± 95% CI. Differences were evaluated by Mann-Whitney U test. Correlations were evaluated with Spearman's (ρ) correlation coefficient. *P* values are depicted.

implicated in uptake of bile salts from the intestinal lumen, were not altered after CR (P = 0.612) (Fig. 2D). Expression of *DIET1*, a modulator of FGF19 secretion,[26] was significantly increased after 21 days (Fig. 2D). For reasons unknown, *FGF19* mRNA was not detected in the majority of specimens, irrespective of sampling prior to or after 3 weeks of CR (data not shown).

Eleven patients (92%) had jejunal effluxes above 1,200 g/24 hours, and a single patient had an output of 678 g/24 hours prior to CR. Inherent to the design of the device for reinfusing chyme, jejunal efflux could not be quantified during the course of CR. All patients developed stools or colonic stoma output after CR, with a single patient having a persistently high fecal output as a consequence of diarrhea. Nevertheless, intestinal output declined significantly during CR (2,245 [1,366-2,913] vs. 203 [125-375] g/24 hours, P < 0.001) (Fig. 2E).

CR ALTERS COMPOSITION OF CIRCULATING BILE SALTS

Reentry of chyme into the distal small bowel and colon restores interaction between the gut microbiota and host bile salts and results in microbial bile salt metabolism.[27] At baseline, gut microbiota–derived secondary bile salts and deconjugated bile salt species were virtually absent (Fig. 3A,B). The molar fractions of secondary and deconjugated bile salt species increased

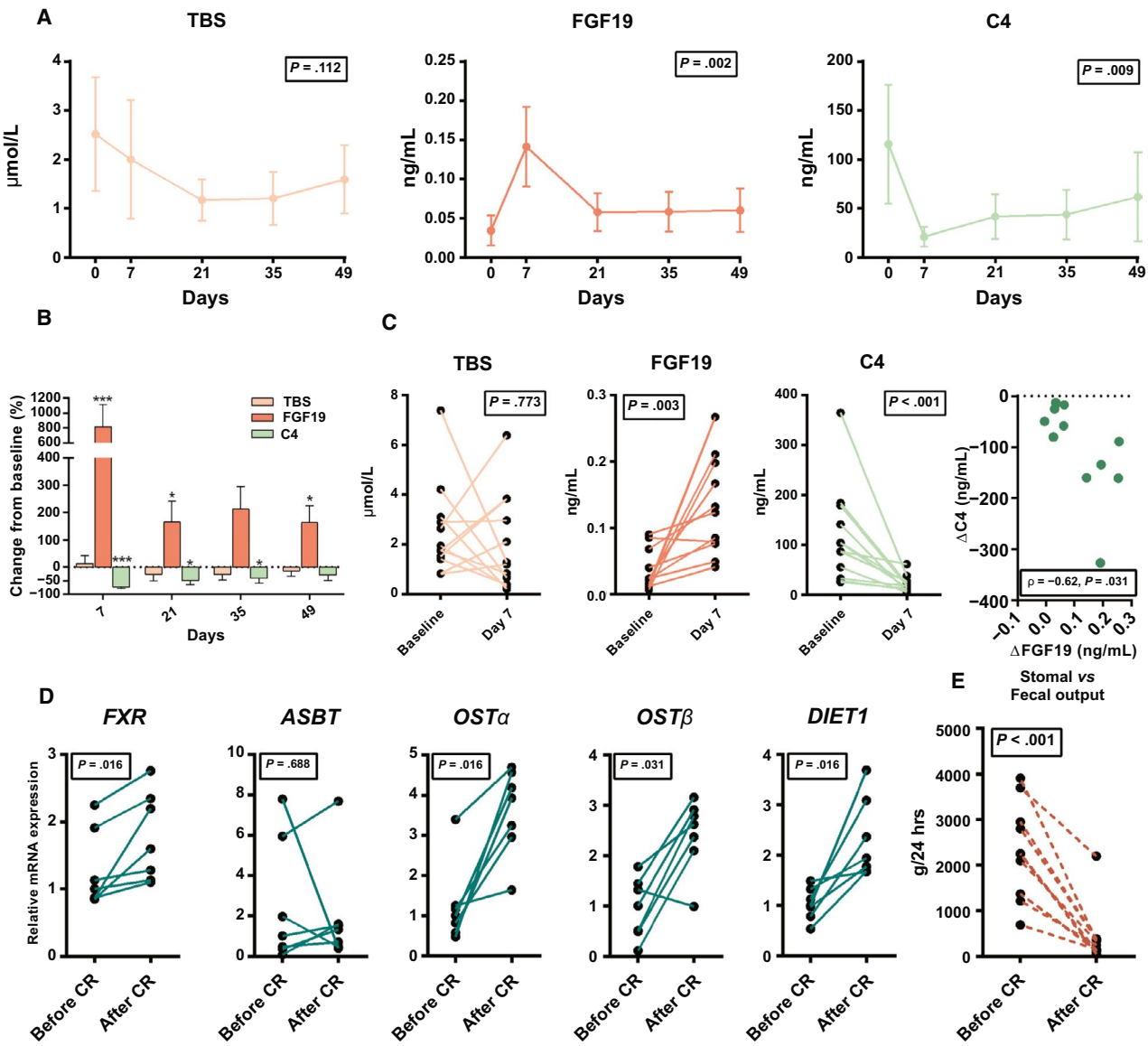
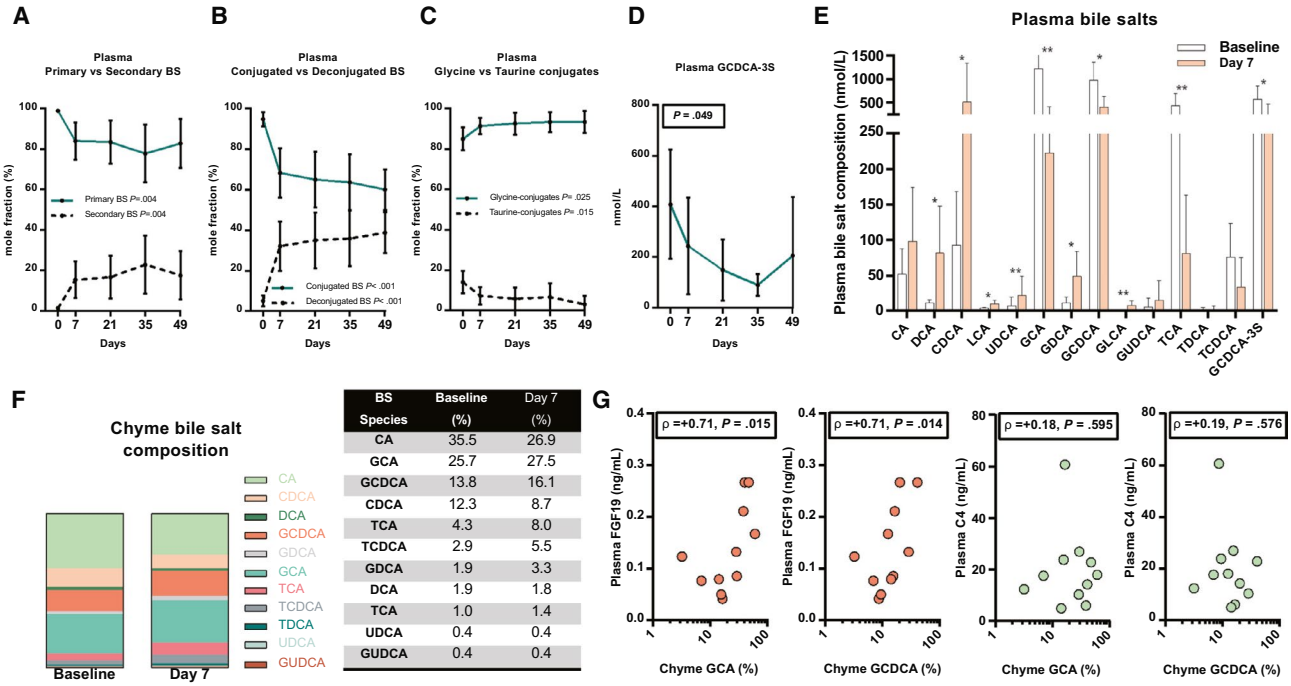


FIG. 2. CR restores homeostatic control of bile salt synthesis within 7 days. Twelve patients with a TDE on PN underwent CR for up to 7 weeks. Blood was sampled at the depicted time points before and after start of CR and analyzed for (A) TBS, FGF19, and C4. (B) Relative change from baseline over time for TBS, FGF19, and C4. (C) Individual changes of TBS, FGF19, and C4 in the first week of CR. (D) Paired small intestinal biopsies were studied (n = 7 patients) at 3 days before CR and 21 days after CR and analyzed for mRNA expression of *FXR* and genes related to bile salt transport. (E) Proximal jejunal output at start of CR and stomal or fecal output at discharge from the clinic/end of CR were assessed to address intestinal output changes. Data are depicted as mean ± 95% CI. Trends in time were evaluated by ANOVA with repeated measures. mRNA expression and intestinal output differences were evaluated by Wilcoxon matched-pairs signed ranks sum test. *P* values are depicted. Asterisks indicate significance levels: **P* < 0.05, ***P* < 0.01, and ****P* < 0.001. Abbreviation: ASBT, apical sodium-dependent bile salt transporter.

during CR treatment ($P = 0.004$ and $P < 0.001$, respectively) and reflect the restored contact between bile salts and bile salt–metabolizing gut microbes (Fig. 3A,B). Moreover, the fractions of glycine-conjugated bile salts were elevated after CR ($P = 0.025$) (Fig. 3C), also mirrored by a lower ratio of taurine-conjugated to glycine-conjugated bile salts ($P = 0.008$) (Supporting Fig. S3A). Individual plasma bile salt species are reported in Fig. 3E. Absolute plasma levels of a number of secondary bile salts ([glyco]deoxycholate, [glyco]lithocholate,



BS Species	Baseline (%)	Day 7 (%)
CA	35.5	26.9
GCA	25.7	27.5
GCDCA	13.8	16.1
CDCA	12.3	8.7
TCA	4.3	8.0
TCDCA	2.9	5.5
GDCA	1.9	3.3
DCA	1.9	1.8
TCA	1.0	1.4
UDCA	0.4	0.4
GUDCA	0.4	0.4

FIG. 3. Alteration of serum bile salt composition after CR initiation. Time course of circulating bile salt composition during CR (n = 12). Graphs show time courses of (A) primary and secondary bile salts, (B) deconjugated and conjugated bile salts, (C) glycine-conjugated and taurine-conjugated bile salts, and (D) the sulfated bile salt species GCDCA-3S. (E) Individual plasma bile salt species. (F) Chyme bile salt composition—molar fraction of individual bile salt species as percentage of total moles of bile salt. (G) Correlation between chyme GCA and GCDCA and plasma levels of FGF19 and C4. Data are depicted as mean ± 95% CI. Trends in time were evaluated by ANOVA with repeated measures. *P* values are depicted. Asterisks indicate significance levels: **P* < 0.05 and ***P* < 0.01. Abbreviations: BS, bile salt; CA, cholic acid; CDCA, chenodeoxycholic acid; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid; GLCA, glycolithocholic acid; GUDCA, glycoursodeoxycholic acid; LCA, lithocholic acid; TCA, taurocholic acid; TCDCA, taurochenodeoxycholic acid; TDCA, taurodeoxycholic acid; UDCA, ursodeoxycholic acid.

and ursodeoxycholate; all $P < 0.05$) were significantly increased after 7 days of CR treatment (Fig. 3E). An overview of absolute levels of all detected bile salt species at the respective time points is provided in Supporting Table S4. Absolute levels of the most abundant sulfated bile salt species (i.e., glycochenodeoxycholate-3-sulfate [GCDCA-3S]), which are typically formed under conditions of cholestasis,[28] were reduced after CR ($P = 0.049$) (Fig. 3D) and showed a positive correlation with the hepatobiliary injury markers ALP and GGT ($\rho = +0.65$, $P = 0.026$ and $\rho = +0.50$, $P = 0.104$, respectively) (Supporting Fig. S3B). Finally, GCDCA, glycocholic acid (GCA), and taurocholic acid were strongly correlated to ALT ($\rho = +0.66$, $P = 0.024$, $\rho = +0.83$, $P = 0.002$, and $\rho = +0.76$, $P = 0.006$, respectively) (Supporting Fig. S3C).

Furthermore, we analyzed individual chyme bile salt species at baseline and after 7 days of CR treatment. Mole fractions of individual bile salt species in chyme did not show major shifts (Fig. 3F). Note the high fraction of unconjugated bile salt species (50.1%). Intriguingly, the total fraction of unconjugated bile salt species in chyme (i.e., cholic acid, chenodeoxycholic acid, and deoxycholic acid) was unexpectedly high, considering that >99% of biliary bile salts are conjugated.[29] A possible explanation could be bacterial overgrowth in the proximal small intestine and attendant microbial bile salt deconjugation.

Reintroducing bile salts in the distal small bowel supposedly leads to FGF19 production. In support of this notion, mole fractions of GCA ($\rho = +0.71$, $P = 0.015$) and GCDCA ($\rho = +0.71$, $P = 0.014$) were strongly associated with plasma FGF19 levels (Fig. 3G); but significant associations with plasma C4 levels were not found (Fig. 3G).

CR RESTORES INTESTINAL FUNCTION AND PROTECTS AGAINST LIVER INJURY

CR restored endocrine regulation of bile salt synthesis and allowed microbial metabolism of bile salts. Next, we studied whether CR recovered intestinal function and ameliorated liver injury. We first assessed whether CR could improve intestinal function. At baseline, 8 patients had plasma CIT concentrations ≤20 μmol/L (Fig. 4A), indicating impaired intestinal function.[30] The remaining 4 patients (2 on PN) with

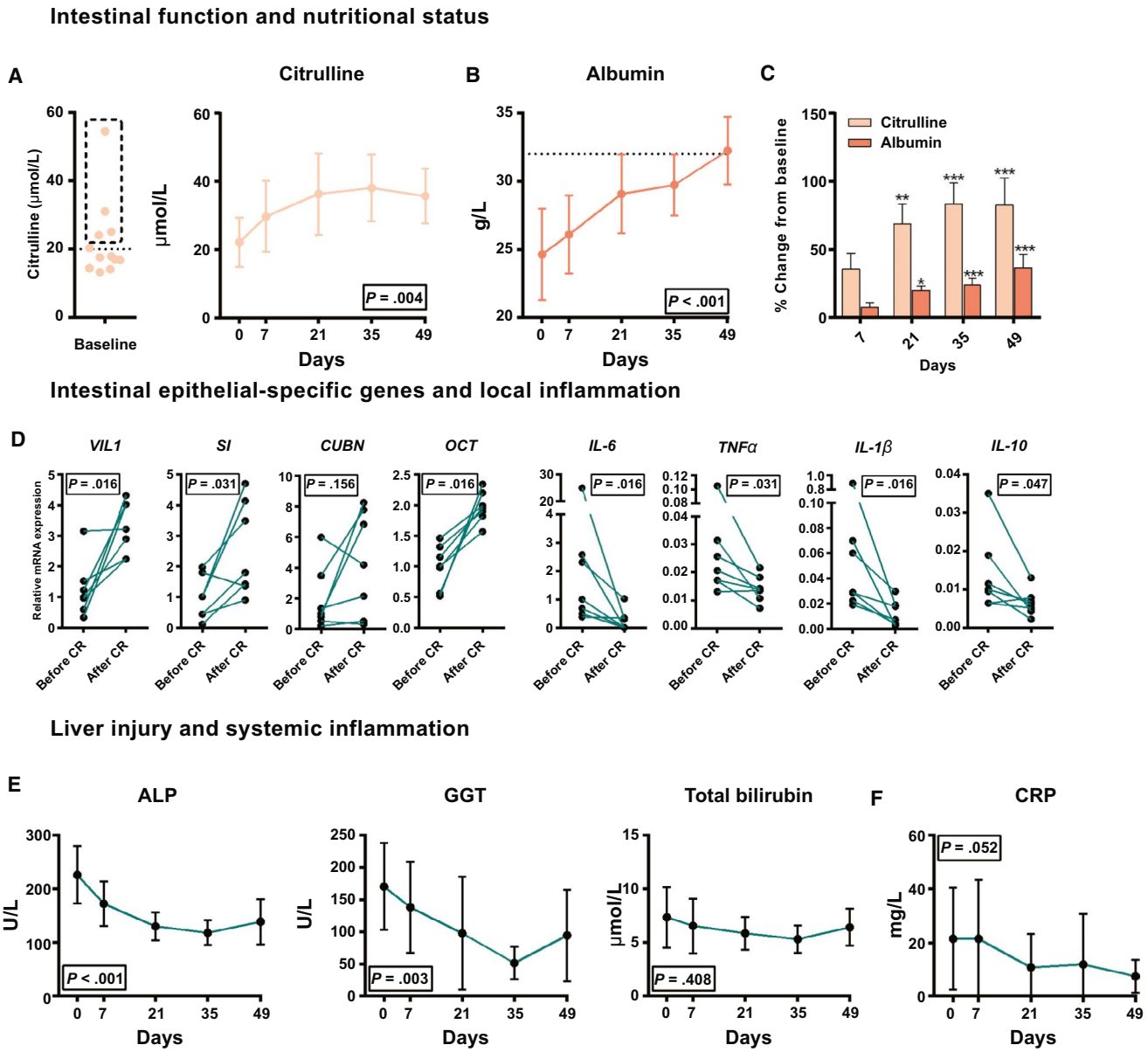


FIG. 4. CR recovers intestinal absorptive function and ameliorates liver injury. Time course of markers for intestinal absorptive function and nutritional status during CR. (A) CIT values of individual patients at baseline (left panel) with values >20 μmol/L in a dashed box and time course of CIT (right panel). (B) Time course of albumin (dashed line represents normal value). (C) Relative change from baseline over time for CIT and albumin. (D) Paired mRNA expression of epithelium-specific genes (*VIL-1*, *SI*, *CUBN*, and *OCT*) and local inflammation *(IL-6*, *TNFα*, *IL1-β*, and *IL-10*) at baseline and 3 weeks after CR. (E) Time course of liver injury markers (ALP, GGT, and total bilirubin) and (F) systemic inflammation (CRP). Data are depicted as mean ± 95% CI. Trends in time were evaluated by ANOVA with repeated measures. Gene expression differences were evaluated by Wilcoxon matched-pairs signed ranks sum test. *P* values are depicted.

higher CIT levels had a combination of persistent high jejunal efflux (median 2,170 mL) and long period of hospital admission (median 47 days) prior to CR, justifying CR treatment. CIT concentrations went up after CR initiation (P = 0.004), and levels considered normal[30] were attained after 5 weeks in all patients (Fig. 4A). Serum albumin levels increased after CR initiation (P < 0.001) and were in the normal range for 6 of 12 patients (50%) after 7 weeks (Fig. 4B). Individual plasma CIT and albumin time courses are depicted in Supporting Fig. S4. Expression of the epithelium-specific genes villin-1 (*VIL1*, cytoskeletal component), sucrase-isomaltase (*SI*, carbohydrate digestive enzyme), and cubilin (*CUBN*, engaged in vitamin B_{12} absorption) were determined to study the impact of CR on enterocyte function. CR significantly increased *VIL1* (P = 0.016) and *SI* (P = 0.031) mRNA levels, whereas *CUBN* expression levels were not altered during CR (P = 0.156) (Fig. 4D). Further, the mRNA level of ornithine carbamoyl transferase (*OCT*, involved in CIT synthesis) was increased after CR (P = 0.016), in line with increased plasma CIT levels after 21 days of CR (Fig. 4A,C). We evaluated the local inflammatory response (small intestinal *IL6* mRNA level). *IL6* mRNA expression was decreased after CR (P < 0.001). In addition, transcript levels of proinflammatory cytokines including *TNFα* and *IL-1β* (Fig. 4D), *IL-8*, and *IL-17* (Supporting Fig. S5) were decreased after 3 weeks of CR treatment (all P < 0.05). mRNA levels of the anti-inflammatory cytokine *IL-10* were also decreased after 3 weeks of CR treatment (Fig. 4D).

Secondly, we studied the effect of CR on liver injury. Serum baseline elevations of ALP and GGT were significantly reduced after 49 days of CR (P < 0.001 and P = 0.003, respectively), approaching the respective normal ranges after 35 days (Fig. 4E). Total bilirubin levels were within the normal range at baseline and were not affected by CR (P = 0.408) (Fig. 4E). ALT and AST levels were above the normal range at baseline; although the time course displayed declining trends in time, these were not significant (P = 0.150 and P = 0.189, respectively) (Supporting Fig. S6).

Finally, we evaluated the systemic inflammatory response (CRP and proinflammatory cytokine levels). Serum CRP was at relatively low levels and decreased nonsignificantly during CR (P = 0.052) (Fig. 4F). We also tested systemic levels of proinflammatory cytokines IL6 and TNF-α but found levels to be below the detection limit of the immune assays for 10 and 12 out of 12 patients, respectively (data not shown), highlighting the noninflammatory state of the patients before and during CR.

IMPROVEMENT OF NUTRITIONAL STATUS BY CR

During CR, the oral feeding met the recommended dietary allowance for energy and protein intake. Three patients with residual malabsorption received additional enteral nutrition by "en Y" enteroclysis. Consequently, PN and/or IVS could be stopped in 9 out of 10 patients (90%) within a median of 3 (range 1-9) days after the start of CR. Most patients (n = 8) had poor nutritional status before CR, but this improved during CR (Supporting Table S5).

THE FGF19 RESPONSE AFTER 7 DAYS, DEPENDENT ON THE C4 RESPONSE, HIGHLY PREDICTS THE COURSE OF CIT

Evaluation of plasma time courses of FGF19 and C4 showed that the reciprocal response in the first week was followed by a gradual increase of CIT levels and decrease of liver injury markers (ALP and GGT) (Figs. 1 and 4). Therefore, we constructed a linear mixed model with random intercepts to evaluate the association between the change in FGF19 and C4 after 7 days of CR (Δ) and alterations in markers of intestinal function (CIT) and liver injury (ALP and GGT) from day 7 onward. First, the reciprocal interaction between $\Delta_{FG19}{}^{*}\Delta_{C4}$ significantly predicted CIT values over time (estimate, −0.54; 95% CI, −0.91 to −0.18; P = 0.007). The interaction plot (R^2 = +0.67, P = 0.007) is depicted in Fig. 5 and demonstrates that the FGF19 response within the first 7 days, dependent on the C4 response, predicts CIT values over time. Finally, the Δ_{FGF19} (per 0.1 unit increase) predicts the course of ALP (estimate, −22.0; 95% CI, −40.2 to −3.8; P = 0.022) and tends to predict GGT (estimate, −26.8; 95% CI, −58.3 to 47.6; P = 0.095) (data not shown).

Discussion

CR reestablishes small intestinal continuity and hence functionally restores the enterohepatic circulation of bile salts. In this prospective study, the key

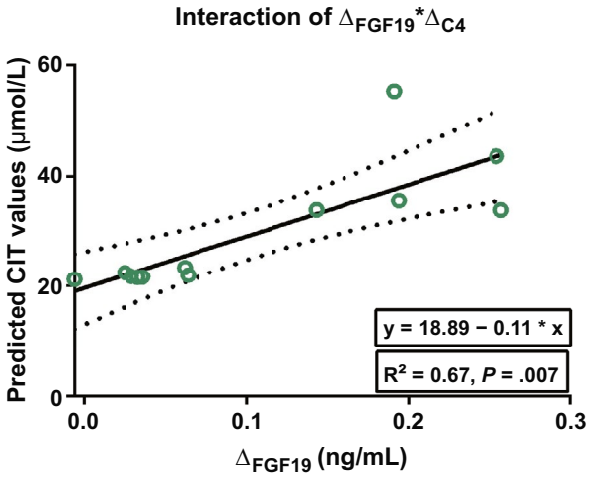


FIG. 5. The FGF19 response, dependent on the C4 response, after 7 days predicts the plasma course of CIT over time. A linear mixed effects model was constructed to evaluate whether the response of FGF19 and C4 (difference between 7 days and baseline values = Δ) predicts the course of CIT over time. Interaction plot depicting the association between the interaction of Δ_{FGF19}*Δ_{C4} after 7 days and predicted CIT values over time (from 7 days onward). The Δ_{FGF19} is depicted on the *x*-axis. The *y*-axis shows the predicted CIT values. The regression line is shown with 95% CI. R^2 and *P* values are depicted.

finding is that CR rapidly reinstates the regulatory bile salt–FGF19 axis and further restores intestinal function, reverses liver injury, and contributes to the improvement of the nutritional status in patients with IF-TDE. Our findings support a role for recovery of homeostatic control of bile salt synthesis by CR in patients with IF-TDE. The pathophysiology and mechanistic findings are schematically summarized in Fig. 6.

To our knowledge the bile salt-FGF19 axis was not previously investigated in patients with IF undergoing extracorporeal recovery of intestinal continuity. In our study, we observed that endocrine control of bile salt synthesis was recovered after 7 days of CR, followed by a new equilibrium phase with higher FGF19 levels compared to the pre-CR period. This is in line with studies showing that administration of an FGF19 analogue strongly suppressed serum C4 levels in healthy human volunteers within 7 days and in patients with chronic liver disease after 4 or 12 weeks of treatment.[31–33] The restored regulatory bile salt–FGF19 axis, evidenced by a decline in C4 levels, was an anticipated effect of reinstating the enterohepatic circulation of bile salts. Furthermore, functional recovery of the enterohepatic circulation of bile salts was also

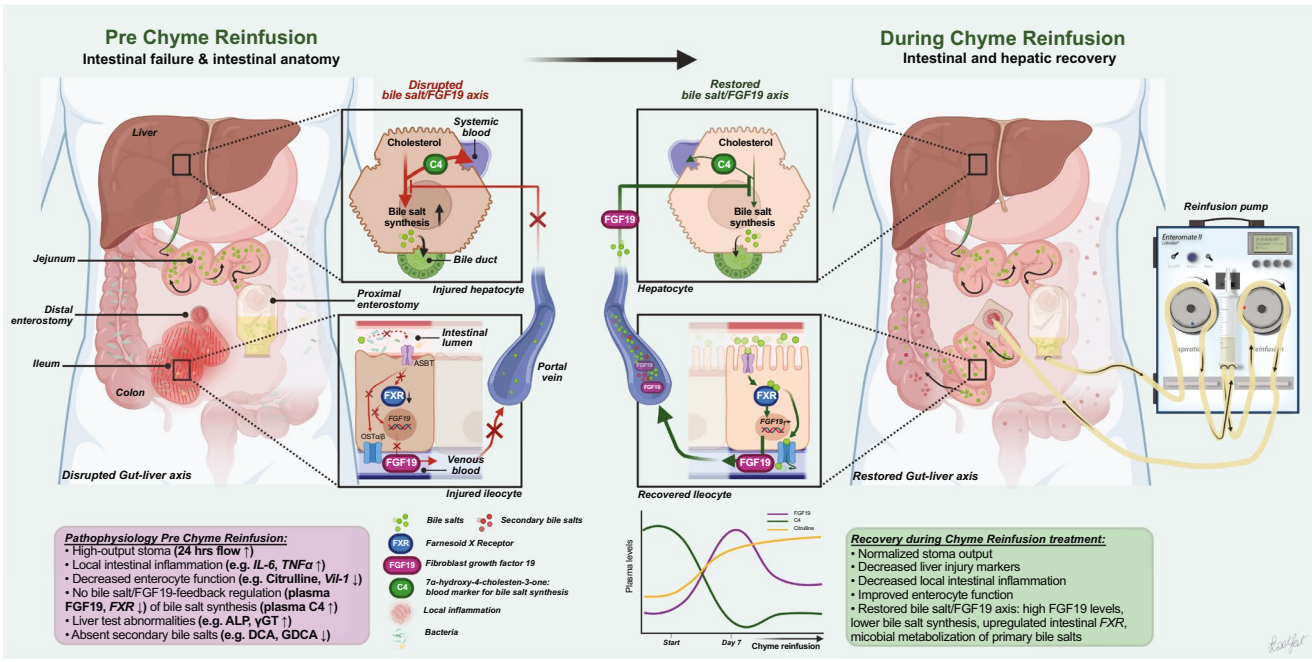


FIG. 6. Schematic summary of the pathophysiology and mechanistic findings before and during CR in patients with IF and a double enterostomy. Abbreviations: ASBT, apical sodium-dependent bile salt transporter; DCA, deoxycholic acid; GDCA, glycodeoxycholic acid.

reflected by larger abundance of microbiome-derived secondary and deconjugated bile salt species, indicating restored microbial metabolism of the gut.[16]

The initial response of FGF19 and C4 levels after 7 days of CR was followed by gradually increasing CIT levels, indicating that restoration of the bile salt–FGF19 axis preceded recovery of intestinal function. Interestingly, the initial FGF19 response, dependent on C4, was highly associated with the later course of CIT over time. This would imply that restoring endocrine control of bile salt synthesis is an early step in functional recovery of the intestine. A recent study demonstrated involvement of FGF19 in preserving intestinal barrier integrity and protection against intestinal inflammation mediated by FXR.[34]

Our findings also demonstrated up-regulated mRNA expression of the bile salt sensor FXR, the bile salt transporters *OSTα/β*, and the epithelium specific genes *VIL-1*, *SI*, and *OCT* in the distal small intestine after 21 days of CR. Interestingly, *FXR* expression was negatively related to the proinflammatory cytokine (*IL6*) and positively related to intestinal epithelium-specific genes (*VIL1*, *SI*, *CUBN*, and *OCT*) (data not shown). Mechanistically, this may be attributed to normalized transepithelial bile salt flux facilitated by CR, hence intestinal FXR activation. Bile salts play a direct role in preserving the intestinal barrier function and protecting against intestinal inflammation, effects mediated by FXR.[35]

Remarkably, findings in the present study showed that jejunal output prior to CR was strongly related to systemic C4 levels. A plausible explanation could be that enhanced synthesis and biliary secretion of bile salts induces a choleretic effect and eventually elevated jejunal output.[36] Because the enterohepatic circulation of bile salts was recovered and C4 levels were normalized after CR, it is likely that reduced stomal or fecal output after CR treatment was, in part, due to restoration of the ileal brake,[6] a direct consequence of recovery of the bile salt–FGF19 axis. Indeed, patients with chronic primary bile acid diarrhea have high C4 levels.[37] Note, a single patient continued to have a high fecal output (>2 L/day) after 49 days of CR treatment. This patient had persistently the lowest FGF19 and highest C4 levels during the course of CR. Intriguingly, this patient underwent a partial gastrectomy prior to CR. High gastrin levels are observed after gastrectomy and are related to increased choleresis.[38,39]

The major strength of our study was long-term follow-up with multiple sampling points, which allowed us to analyze the time course of homeostatic control of bile salt synthesis. This study has certain limitations. First, considering the complexity of IF, we designed a preintervention and postintervention study without any control group without CR treatment. Importantly, CR is recommended, whenever feasible, by the American Society for Parenteral and Enteral Nutrition guidelines and the European Society for Clinical Nutrition and Metabolism guidelines. Withholding this effective treatment for the purpose of a controlled study would have been unethical. In addition, patients already had a median pre-CR period of 4 weeks with unsuccessful treatment with PN or IVS. Second, ileal biopsies were taken 3 days before and 3 weeks after CR. However, changes in FGF19 and C4 levels were rapidly seen after 7 days of CR treatment. Thus, sequential biopsies taken at each corresponding time point of blood collection would be more informative about transcriptional changes during CR. Obviously, frequent biopsying is a huge burden for patients and not justified by clinical necessity. Nonetheless, alterations in circulating FGF19 and C4 levels correspond well to transcriptional changes.[40,41]

The findings of this study make it conceivable that the beneficial effects of CR are mediated in part through the restored bile salt–FGF19 signaling axis. Clinically, CR in surgical patients with a distal entrance of the small bowel should be considered in case IF develops because CR might also benefit postoperative outcomes considering the persistent effects on intestinal function. In addition, in case patients need to undergo a temporary proximal enterostomy (e.g., during acute mesenteric ischemia), establishment of a double-barreled enterostomy or placement of a distal entrance of the small bowel should be considered. Finally, an important clinical implication of our study is that administration of FGF19 analogues could be considered in patients with IF without an accessible distal small bowel.

In conclusion, CR improves intestinal and liver function in patients with IF-TDE. The beneficial effects of CR are partly mediated by activation of bile salt–FGF19 signaling, leading to restored regulation of hepatic bile salt synthesis, and associated with improved gut–liver health. CR needs to be considered as first-line treatment in patients with IF-TDE. Further, therapeutic approaches with FGF19

analogues might be promising in the management of IF and related complications in case a distal intestine is not accessible.

Acknowledgment: We are grateful to Loes Nijssen for laboratory assistance. We thank all the staff from the Department of Digestive Surgery, the Direction de la Recherche Clinique et de l'innovation, and the Centre de Ressources biologiques of Rennes University Hospital for their help in the performance of the study. We are very grateful to Mr. Michel Fournier, former director of the Société Labodial and creator of the Enteromate, which for 30 years was the only device available for CR.

Author Contributions: K.V.K.K., D.P., R.T., F.G.S., and S.W.M.O.D. were responsible for study concept and design. K.V.K.K., D.P., X.C., M.D., S.L., M.C., L.D., E.S., F.T., L.L., and R.T. were responsible for acquisition of data. K.V.K.K., X.C., and S.M.J.v.K. were responsible for statistical analysis. K.V.K.K., D.P., X.C., R.T., F.S., and S.W.M.O.D. were responsible for interpretation of data. K.V.K.K. and X.C. were responsible for drafting of the manuscript. D.P., R.T., F.S., and S.W.M.O.D. were responsible for critical revision of the manuscript for important intellectual content. All authors were responsible for final critical revision and editing.

REFERENCES

1) Pironi L, Arends J, Baxter J, Bozzetti F, Peláez RB, Cuerda C, et al. ESPEN endorsed recommendations. Definition and classification of intestinal failure in adults. Clin Nutr 2015;34:171-180.
2) Oterdoom LH, ten Dam SM, de Groot SDW, Arjaans W, van Bodegraven AA, et al. Limited long-term survival after in-hospital intestinal failure requiring total parenteral nutrition. Am J Clin Nutr 2014;100:1102-1107.
3) Burden S, Hemstock M, Taylor M, Teubner A, Roskell N, MacCulloch A, et al. The impact of home parenteral nutrition on the burden of disease including morbidity, mortality and rate of hospitalisations. Clin Nutr ESPEN 2018;28:222-227.
4) Rinsema W, Gouma DJ, von Meyenfeldt MF, Soeters PB. Reinfusion of secretions from high-output proximal stomas or fistulas. Surg Gynecol Obstet 1988;167:372-376.
5) Lévy É, Palmer DL, Frileux P, Parc R, Huguet C, Loygue J, et al. Inhibition of upper gastrointestinal secretions by reinfusion of *Succus entericus* into the distal small bowel. A clinical study of 30 patients with peritonitis and temporary enterostomy. Ann Surg 1983;198:596-600.
6) Picot D, Garin L, Trivin F, Kossovsky MP, Darmaun D, Thibault R, et al. Plasma citrulline is a marker of absorptive small bowel length in patients with transient enterostomy and acute intestinal failure. Clin Nutr 2010;29:235-242.
7) Picot D, Layec S, Seynhaeve E, Dussaulx L, Trivin F, Carsin-Mahe M, et al. Chyme reinfusion in intestinal failure related to temporary double enterostomies and enteroatmospheric fistulas. Nutrients 2020;12:1376.
8) Inagaki T, Choi M, Moschetta A, Peng LI, Cummins CL, McDonald JG, et al. Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. Cell Metab 2005;2:217-225.
9) Cai S-Y, Ouyang X, Chen Y, Soroka CJ, Wang J, Mennone A, et al. Bile acids initiate cholestatic liver injury by triggering a hepatocyte-specific inflammatory response. JCI Insight 2017;2:e90780.
10) Pereira-Fantini PM, Lapthorne S, Joyce SA, Dellios NL, Wilson G, Fouhy F, et al. Altered FXR signalling is associated with bile acid dysmetabolism in short bowel syndrome–associated liver disease. J Hepatol 2014;61:1115-1125.
11) Xiao Y-T, Cao YI, Zhou K-J, Lu L-N, Cai W. Altered systemic bile acid homeostasis contributes to liver disease in pediatric patients with intestinal failure. Sci Rep 2016;6:39264.
12) Koelfat KVK, Visschers RGJ, Hodin C, de Waart DR, van Gemert WG, Cleutjens JPM, et al. FXR agonism protects against liver injury in a rat model of intestinal failure–associated liver disease. J Clin Transl Res 2018;3:318-327.
13) Mutanen A, Lohi J, Heikkilä P, Jalanko H, Pakarinen MP. Loss of ileum decreases serum fibroblast growth factor 19 in relation to liver inflammation and fibrosis in pediatric onset intestinal failure. J Hepatol 2015;62:1391-1397.
14) **Koelfat KVK**, **Huijbers A**, Schaap FG, van Kuijk SMJ, Lenicek M, Soeters MR, et al. Low circulating concentrations of citrulline and FGF19 predict chronic cholestasis and poor survival in adult patients with chronic intestinal failure: development of a Model for End-Stage Intestinal Failure (MESIF risk score). Am J Clin Nutr 2019;109:1620-1629.
15) Walters JRF, Tasleem AM, Omer OS, Brydon WG, Dew T, le Roux CW, et al. A new mechanism for bile acid diarrhea: defective feedback inhibition of bile acid biosynthesis. Clin Gastroenterol Hepatol 2009;7:1189-1194.
16) Hirschfield GM, Chazouillères O, Drenth JP, Thorburn D, Harrison SA, Landis CS, et al. Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: a multicenter, randomized, double-blind, placebo-controlled phase II trial. J Hepatol 2019;70:483-493.
17) Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, et al. NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2018;391:1174-1185.
18) Picot D, Layec S, Dussaulx L, Trivin F, Thibault R. Chyme reinfusion in patients with intestinal failure due to temporary double enterostomy: a 15-year prospective cohort in a referral centre. Clin Nutr 2017;36:593-600.
19) Aziz EF, Javed F, Pratap B, Musat D, Nader A, Pulimi S, et al. Malnutrition as assessed by nutritional risk index is associated with worse outcome in patients admitted with acute decompensated heart failure: an ACAP-HF data analysis. Heart Int 2011;6:e2.
20) Schaap FG, van der Gaag NA, Gouma DJ, Jansen PLM. High expression of the bile salt-homeostatic hormone fibroblast growth factor 19 in the liver of patients with extrahepatic cholestasis. Hepatology 2009;49:1228-1235.
21) van Eijk HM, Rooyakkers DR, Deutz NE. Rapid routine determination of amino acids in plasma by high-performance liquid chromatography with a 2–3 microns Spherisorb ODS II column. J Chromatogr 1993;620:143-148.
22) Axelson M, Aly A, Sjovall J. Levels of 7 alpha-hydroxy-4-cholesten-3-one in plasma reflect rates of bile acid synthesis in man. FEBS Lett 1988;239:324-328.

23) Koelfat KVK, Plummer MP, Schaap FG, Lenicek M, Jansen PLM, Deane AM, et al. Gallbladder dyskinesia is associated with an impaired postprandial fibroblast growth factor 19 response in critically ill patients. Hepatology 2019;70:308-318.
24) Ruijter JM, Ramakers C, Hoogaars WMH, Karlen Y, Bakker O, van den Hoff MJB, et al. Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. Nucleic Acids Res 2009;37:e45.
25) Eusufzai S, Axelson M, Angelin B, Einarsson K. Serum 7 alpha-hydroxy-4-cholesten-3-one concentrations in the evaluation of bile acid malabsorption in patients with diarrhoea: correlation to SeHCAT test. Gut 1993;34:698-701.
26) **Vergnes L**, **Lee J**, Chin R, Auwerx J, Reue K. Diet1 functions in the FGF15/19 enterohepatic signaling axis to modulate bile acid and lipid levels. Cell Metab 2013;17:916-928.
27) Sayin S, Wahlström A, Felin J, Jäntti S, Marschall H-U, Bamberg K, et al. Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist. Cell Metab 2013;17:225-235.
28) Masubuchi N, Sugihara M, Sugita T, Amano K, Nakano M, Matsuura T, et al. Oxidative stress markers, secondary bile acids and sulfated bile acids classify the clinical liver injury type: promising diagnostic biomarkers for cholestasis. Chem Biol Interact 2016;255:83-91.
29) Hofmann A. The continuing importance of bile acids in liver and intestinal disease. Arch Intern Med 1999;159:2647.
30) Crenn P, Coudray–Lucas C, Thuillier F, Cynober L, Messing B. Postabsorptive plasma citrulline concentration is a marker of absorptive enterocyte mass and intestinal failure in humans. Gastroenterology 2000;119:1496-1505.
31) Hirschfield GM, Chazouilleres O, Drenth JP, Thorburn D, Harrison SA, Landis CS, et al. Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: a multicenter, randomized, double-blind, placebo-controlled phase II trial. J Hepatol 2019;70:483-493.
32) Luo J, Ko B, Elliott M, Zhou M, Lindhout DA, Phung V, et al. A nontumorigenic variant of FGF19 treats cholestatic liver diseases. Sci Transl Med 2014;6:247ra100.
33) Mayo MJ, Wigg AJ, Leggett BA, Arnold H, Thompson AJ, Weltman M, et al. NGM282 for treatment of patients with primary biliary cholangitis: a multicenter, randomized, double-blind, placebo-controlled trial. Hepatol Commun 2018;2:1037-1050.
34) Gadaleta RM, Garcia-Irigoyen O, Cariello M, Scialpi N, Peres C, Vetrano S, et al. Fibroblast growth factor 19 modulates intestinal microbiota and inflammation in presence of farnesoid X receptor. EBioMedicine 2020;54:102719.
35) Gadaleta RM, van Erpecum KJ, Oldenburg B, Willemsen ECL, Renooij W, Murzilli S, et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut 2011;60:463-472.
36) Erlinger S, Dhumeaux D. Mechanisms and control of secretion of bile water and electrolytes. Gastroenterology 1974;66: 281-304.
37) Walters JRF, Johnston IM, Nolan JD, Vassie C, Pruzanski ME, Shapiro DA, et al. The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. Aliment Pharmacol Ther 2015;41:54-64.
38) Wilson SD, Schulte WJ, Meade RC. Longevity studies following total gastrectomy in children with the Zollinger-Ellison syndrome. Arch Surg 1971;103:108-115.
39) Zaterka S, Grossman MI. The effect of gastrin and histamine on secretion of bile. Gastroenterology 1966;50:500-505.
40) **Zhang JH**, **Nolan JD**, Kennie SL, Johnston IM, Dew T, Dixon PH, et al. Potent stimulation of fibroblast growth factor 19 expression in the human ileum by bile acids. Am J Physiol Gastrointest Liver Physiol 2013;304:G940-G948.
41) Galman C, Arvidsson I, Angelin B, Rudling M. Monitoring hepatic cholesterol 7alpha-hydroxylase activity by assay of the stable bile acid intermediate 7alpha-hydroxy-4-cholesten-3-one in peripheral blood. J Lipid Res 2003;44:859-866.

Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.32017/suppinfo.