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Chyme reinfusion restores the regulatory bile salt-FGF19 axis in intestinal failure patients

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Abbreviations used in this paper: TDE, temporary double enterostomy; IF, intestinal failure; PN, parenteral nutrition; IVS, intravenous fluid supplementation; IFALD, intestinal-failure associated liver disease; CR, chyme reinfusion; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; CYP7A1, cholesterol-7- α -hydroxylase; BMI, body mass index; NRI, nutritional risk index; CIT, citrulline; C4, 7 α -hydroxy-4-cholesten-3-one; TBS, total bile salts; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyl transferase; CRP, C-reactive protein; OST α/β , organic solute transporter subunits- α/β ; GCDCA-3S, glycochenodeoxycholate-3-sulfate;

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ABSTRACT

Automated chyme reinfusion (CR) in intestinal failure (IF) patients with 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 beneficial effects of CR relate to functional recovery of enterohepatic signaling via the bile salt-FGF19 axis. 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), C4 (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 via endocrine FGF19 action. TBS remained unaltered during CR. Intestinal FXR was upregulated 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 were gradually rising 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 IF-TDE patients 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.

Keywords: gut-liver axis; intestinal failure; FGF19; bile salts

INTRODUCTION

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 ischaemia resulting from e.g. a thrombo-embolic event or herniation. Unfortunately, some patients may develop intestinal failure (IF) due to (functional) short bowel syndrome, requiring parenteral nutrition (PN) and/or intravenous fluid supplementation (IVS) until recovery for surgical re-establishment of intestinal continuity, typically 3 to 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 related to catheter replacement. The development of IF-associated liver disease (IFALD) 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 shorter period to PN independence in retrospective studies.⁴⁻⁷ The mechanisms underlying the beneficial effects of chyme reinfusion are incompletely understood, but functional recovery of the enterohepatic circulation of bile salts and attendant signaling has been postulated.⁴

Fibroblast growth factor (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 via repression of the bile salt-synthetic enzyme cholesterol-7 α -hydroxylase (*CYP7A1*) 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 IFALD, or primary bile acid diarrhea.⁹⁻¹⁵ Currently, pharmacological treatment with FGF19 analogues in patients with hepatobiliary diseases including primary biliary cholangitis or non-alcoholic steatohepatitis has been evaluated clinically and renders promising therapeutic effects.^{16, 17}

In the 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 IF patients with a TDE, is associated with CR-related beneficial effects.

METHODS

Study design and participants

The RESCUE study (restored enterohepatic signaling: chyme reinfusion therapy) is a prospective study with a pre- and post-intervention 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 IF patients aged 18 years or older with a temporary double enterostomy

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, hepatocellular carcinoma, auto-immune 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 previously.¹⁸ In brief, proximal effluent was continuously pumped into a 30 mL collection container equipped with weight-monitoring. When 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 in approximately one week after referral of the patient to Clinique Saint Yves and continuously applied for two to three months. Afterwards, patients underwent surgical re-establishment of intestinal continuity. Blood samples were collected at three 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 three days before and three weeks after CR at CHU Rennes by an experienced gastroenterologist. Study design is shown in **Supplementary Figure S1**.

Data collection and definitions

Data were collected and managed using a customized Access Database (Microsoft, Redmond, WA, USA). The following items were recorded: gender, age, height, body weight, body mass index (BMI), underlying diseases, afferent small intestinal length (from duodeno-jejunal flexure to the proximal stoma), efferent small intestinal length (from distal stoma to terminal part of the remnant efferent small intestine), resected length, efferent small intestinal anatomy, energy (kcal/kg) and protein (g/kg) 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:

$Weight\ loss\ (\%) = 100 * \frac{(usual\ weight - present\ weight)}{usual\ weight}$. None of the patients was diagnosed with ascites during CR treatment. Nutritional Risk Index (NRI) was determined as follows:

$NRI = 1.519 * serum\ albumin\ (g/L) + 41.77 * \frac{present\ weight}{usual\ weight}$.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 min,

and plasma tubes were put on ice immediately after collection. After 30 min, all blood samples were centrifuged at 4000 rpm for 10 min 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 pro-inflammatory cytokines (interleukin-6, IL-6 and tumor necrosis factor-alpha, TNFα) were assessed by sandwich ELISA as reported previously.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, blood marker of bile salt synthesis)²², total bile salts (TBS), and bile salt composition (both in 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-glutamyl transferase, 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 (Maastricht, The Netherlands). For baseline comparisons of TBS, FGF19 and C4 levels, own published data on twelve healthy controls (mean age 55 years, 33% female) was used.²³

Gene expression analysis

Ileal biopsies were obtained through routine endoscopic procedures and immediately preserved in RNeasy Lysis Buffer (Qiagen, Crawley, UK) for 24 hours at 4°C and then stored at -80°C (Rennes University Hospital, Rennes, France). Biopsies were ultimately transported to Maastricht University Medical Center (Maastricht, The Netherlands) for transcript analyses. Hereto, 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 cDNA using SensiFAST™ cDNA Synthesis Kit (Bioline, London, UK). For the quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), we used SensiFAST™ SYBR Hi-ROX kit (Bioline, London, UK) and cDNA as template on a LightCycler® 480 SW 1.5 system (Roche, Basel, Switzerland). qRT-PCR data was analyzed using LinRegPCR software,²⁴ and gene expression levels were normalized to the geometric mean of 4 reference genes (*36B4*, *PSMB4*, *REEP5*, and *HPRT1*). Employed primer sequences are provided in **Supplementary Table S1**. Paired ileal biopsies were available from seven of twelve patients. In remaining patients, (paired) biopsies could not be gathered for logistic reasons (n = 2), last-minute patient refusal (n = 1), or transcript data was excluded from analyses due to a non-enterocytic transcript signature (n = 2).

Statistical analysis

Initially, twenty 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 (FGF19 level) had significantly changed ($P < .005$) justifying early termination of the study. For blood analyses, five out of seventy-two time

points were missing (6.9%). Multiple data imputation was used to handle the missing data. The mean of day -3 and day 0 measurements were taken as baseline values. Data are expressed as mean \pm standard deviation (SD), median (interquartile range), or frequency (percentage) where appropriate. Graphical data are expressed as mean \pm 95% confidence interval (CI). Differences between IF patients and healthy controls were evaluated by Mann-Whitney U test. Correlations were evaluated by the Pearson (r) or Spearman's (ρ) correlation coefficient dependent on data distribution. For continuous variables, changes during CR were evaluated by paired-samples *t* test or Wilcoxon matched-pairs signed ranks sum test dependent 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, were 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). The Akaike's Information Criterion was used to compare the best fit of a covariance structure for the linear mixed models. *P* value $< .05$ was considered statistically significant. SPSS statistics version 26.0 (IBM Corporation, Chicago, IL, USA) 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, ten patients actually received PN and/or intravenous (IV) hydration at admission. Ten patients remained in the hospital during the 7 weeks course of CR, and two patients received home CR after specific training and education. Major underlying causes for small intestine 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 **Supplementary Table S2**.

Dysregulated bile salt-FGF19 axis in intestinal failure patients with a temporary double enterostomy

First, we investigated whether the bile salt-FGF19 axis was disturbed in IF-TDE patients prior to CR. Baseline levels of TBS, FGF19 and C4 of healthy controls and IF-patients are shown in **Figure 1A**. Note, demographic information on healthy controls is provided in **Supplementary Table S3**. TBS levels in IF patients were not different compared to healthy controls ($1.9 [1.4 \text{ to } 3.0]$ vs $1.6 [0.9 \text{ to } 2.4]$ $\mu\text{mol/L}$, $P = .288$). In line with earlier observations in patients with chronic IF¹⁴, baseline FGF19 levels were markedly lower compared to healthy controls ($0.023 [0.012 \text{ to } 0.061]$ vs $0.098 [0.071 \text{ to } 0.136]$ ng/mL , $P < .001$) (**Figure 1A**). Conversely, high baseline C4 levels were observed in IF patients compared to controls ($95 [38 \text{ to } 169]$ vs $19 [10 \text{ to } 31]$ ng/mL , $P = .002$), indicating dysregulated control of bile salt synthesis (**Figure 1A**). Indeed, FGF19 showed a strong negative correlation with C4 at baseline ($\rho = -0.86$, $P < .001$) (**Figure 1B**). Furthermore, baseline C4 levels were positively related to 24 hrs jejunal output adjusted by baseline weight ($\rho = -0.80$, $P = .002$) (**Figure 1C**), indicating a choleric effect before CR that is linked to enhanced bile salt synthesis.²⁵ FGF19 levels were not related to jejunal efflux ($\rho = -0.44$, $P = .155$) (**Figure 1C**). Of note, baseline C4 levels were higher in females compared to males ($179 [95 \text{ to } 274]$ vs $55 [28 \text{ to } 103]$ ng/mL , $P = .017$), 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 males and females.

Chyme reinfusion (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 **Supplementary Figures 2A & 2B**. Systemic TBS levels did not change significantly after introducing CR ($P = .112$) (**Figure 2A**). FGF19 levels increased after CR initiation ($P = .002$) with a transient peak after 7 days, and reaching a new steady-state level that was higher than at baseline, thereafter

(**Figure 2A**). In line with elevated FGF19 levels, plasma C4 levels were reduced after start of CR ($P < .001$) (**Figure 2A**), indicating reduced bile salt synthesis during CR. The percentage change from baseline is shown in **Figure 2B**. Most pronounced responses of FGF19 and C4 were seen after 7 days of CR (**Figure 2B**). TBS levels were unaltered after 7 days of CR (1.9 [1.4 to 3.0] vs 1.2 [0.5 to 3.6] $\mu\text{mol/L}$, $P = .773$) (**Figure 2C**). FGF19 levels were higher for all but one patient after 7 days compared to baseline values (0.023 [0.012 to 0.061] vs 0.128 [0.077 to 0.208] ng/mL, $P = .003$) (**Figure 2C**). Conversely, and in line with FGF19 function, C4 levels were consistently lower after 7 days compared to baseline values (95 [38 to 196] vs [18 [11 to 26] ng/mL, $P < .001$) (**Figure 2C**).

Further, gene expressions of *FXR* and the *FXR*-regulated organic solute transporter subunits α/β (*OST α/β* , engaged in export of bile salts from the enterocyte to the venous capillaries) were upregulated after CR ($P < .05$ for all) (**Figure 2D**). Transcripts of the apical sodium-dependent bile salt transporter (*ASBT*), implicated in uptake of bile salts from the intestinal lumen were not altered after CR ($P = .612$) (**Figure 2D**). Expression of *DIET1*, a modulator of FGF19 secretion²⁶, was significantly increased after 21 days (**Figure 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 1200 g/24 hrs, and a single patient had an output of 678 g/24 hrs 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 persistently a high fecal output as a consequence of diarrhea. Nevertheless, intestinal output declined significantly during CR (2245 [1366 to 2913] vs 203 [125 to 375] g/24 hrs, $P < .001$) (**Figure 2E**).

Chyme reinfusion alters composition of circulating bile salts

Re-entry 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 (**Figure 3A & 3B**). The molar fractions of secondary and deconjugated bile salt species increased during CR treatment ($P = .004$ and $P < .001$, respectively) and reflect the restored contact between bile salts and bile salt-metabolizing gut microbes (**Figure 3A & 3B**). Moreover, the fractions of glycine-conjugated bile salts were elevated after CR ($P = .025$) (**Figure 3C**), also mirrored by lower ratio of taurine- to glycine-conjugated bile salts ($P = .008$) (**Supplementary Figure S3A**). Individual plasma bile salt species are reported in **Figure 3E**. Note, absolute plasma levels of a number of secondary bile salts ((glyco)deoxycholate, (glyco)lithocholate and ursodeoxycholate) (all $P < .05$) were significantly increased after 7 days of CR treatment (**Figure 3E**). An overview of absolute levels of all detected bile salt species at the respective time points, is provided in **Supplementary 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 cholestasis²⁸, were reduced after CR ($P = .049$) (**Figure 3D**), and showed

a positive correlation with hepatobiliary injury markers ALP and GGT ($\rho = +0.65$, $P = .026$ and $\rho = +0.50$, $P = .104$, respectively) (**Supplementary Figure S3B**). Finally, GCDCA, GCA and TCA were strongly correlated to ALT ($\rho = +0.66$, $P = .024$, $\rho = +0.83$, $P = .002$ and $\rho = +0.76$, $P = .006$, respectively) (**Supplementary Figure 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 (**Figure 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. CA, CDCA and DCA) 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.

Re-introducing bile salts in the distal small bowel supposedly leads to FGF19 production. In support of this notion, mole fractions of GCA ($\rho = +0.71$, $P = .015$) and GCDCA ($\rho = +0.71$, $P = .014$) were strongly associated with plasma FGF19 levels (**Figure 3G**), but significant associations with plasma C4 levels were not found (**Figure 3G**).

Chyme reinfusion 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 $\mu\text{mol/L}$ (**Figure 4A**), indicating impaired intestinal function.³⁰ Remaining 4 patients (2 on PN) with higher CIT levels had a combination of persistent high jejunal efflux (median 2170 mL) and long period of hospital admission (median 47 days) prior to CR, justifying CR treatment. CIT concentrations went up after CR initiation ($P = .004$) and levels considered normal³⁰ were attained after 5 weeks in all patients (**Figure 4A**). Serum albumin levels increased after CR initiation ($P < .001$) and were in the normal range for 6 of 12 patients (50%) after 7 weeks (**Figure 4B**). Individual plasma CIT and albumin time courses are depicted in **Supplementary Figure S4**. Expression of the epithelium-specific genes villin-1 (*VIL1*, cytoskeletal component), sucrase-isomaltase (*SI*, carbohydrate digestive enzyme) and cubilin (*CUBN*, engaged in vitamin B12 absorption) were determined to study the impact of CR on enterocyte function. CR significantly increased *VIL1* ($P = .016$) and *SI* ($P = .031$) mRNA levels, whereas *CUBN* expression levels were not altered during CR ($P = .156$) (**Figure 4D**). Further, the mRNA level of ornithine carbamoyl transferase (*OCT*, involved in CIT synthesis) was increased after CR ($P = .016$), in line with increased plasma CIT levels after 21 days of CR (**Figure 4A & 4C**). We evaluated the local inflammatory response (small intestinal *IL6* mRNA level). *IL6* mRNA expression was decreased after CR ($P < .001$). In addition, transcript levels of pro-inflammatory cytokines including *TNF α* and *IL-1 β* (**Figure 4D**), *IL-8* and *IL-17* (**Supplementary Figure S5**) were decreased after three weeks of CR

treatment (all $P < .05$). mRNA levels of the anti-inflammatory cytokine *IL-10* was also decreased after three weeks of CR treatment (**Figure 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 ($P < .001$ and $P = .003$, respectively), approaching the respective normal ranges after 35 days (**Figure 4E**). Total bilirubin levels were within the normal range at baseline and were not affected by CR ($P = .408$) (**Figure 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 ($P = .150$ and $P = .189$, respectively) (**Supplementary Figure S6**).

Finally, we evaluated the systemic inflammatory response (CRP and pro-inflammatory cytokine levels). Serum CRP were at relatively low levels and decreased non-significantly during CR ($P = .052$) (**Figure 4F**). We also tested systemic levels of pro-inflammatory 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 non-inflammatory state of the patients before and during CR.

Improvement of the nutritional status by chyme reinfusion

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 [1 to 9] days after start of CR. Most patients ($n = 8$) had poor nutritional status before CR, but this improved during CR (**Supplementary Table S5**).

The C4 response after 7 days dependent of the FGF19 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) (**Figure 1** and **Figure 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 onwards. First, the reciprocal interaction between $\Delta_{\text{FGF19}} * \Delta_{\text{C4}}$ significantly predicted CIT values over time (estimate: $-.54$, 95% CI: $[-.91 \text{ to } -.18]$, $P = .007$). The interaction plot ($R^2 = +0.67$, $P = .007$) is depicted in **Figure 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: $[-40.2 \text{ to } -3.8]$, $P = .022$) and tended to predict GGT (estimate: -26.8 , 95% CI: $[-58.3 \text{ to } 47.6]$, $P = .095$) (data not shown).

DISCUSSION

CR re-establishes small intestinal continuity and hence functionally restores the enterohepatic circulation of bile salts. In this prospective study, the key 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 IF patients with a TDE. Our findings support a role for recovery of homeostatic control of bile salt synthesis by CR in patients with IF with a TDE. The pathophysiology and mechanistic findings were schematically summarized in **Figure 6**.

To our knowledge the bile salt-FGF19 axis was not previously investigated in IF patients 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 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 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 upregulated mRNA expression of the bile salt sensor FXR, the bile salt transporters *OSTα/β*, and 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 pro-inflammatory cytokine (*IL6*) and positively related to intestinal epithelial-specific genes (*VIL1*, *SI*, *CUBN* and *OCT*) (data not shown). Mechanistically, this may be attributed to normalized trans-epithelial bile salt flux facilitated by CR, hence intestinal FXR activation. Bile salts play a direct role in preserving the intestinal barrier function and protection 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 choleric effect and eventually elevated jejunal output.³⁶ Since 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 brake⁶, 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 remained to have a high fecal output (>2 liters per day) after 49 days of CR treatment. This patient had persistently the lowest FGF19 and highest C4 level during the course of CR. Intriguingly, this patient underwent a partial gastrectomy prior 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 pre- and post-intervention study without any control group without CR treatment. Importantly, CR is recommended, whenever feasible, by ASPEN guidelines and ESPEN recommendations. 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. Secondly, 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 biopsies 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 persevering 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 IF patients without an accessible distal small bowel.

In conclusion, CR improves intestinal and liver function in IF patients with a 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 is associated with improved gut-liver health. CR needs to be considered as first-line treatment in IF patients with a TDE. Further, novel therapeutic approaches with FGF19 analogues might be promising in the management of IF and related complications in case a distal intestine is not accessible.

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FIGURE LEGENDS

FIGURE 1. Dysregulated bile salt synthesis in IF patients with a temporary double enterostomy prior to CR.

(A) Baseline values of TBS, FGF19 and C4 of IF patients with a 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 IF patients with a TDE (blue circles) and controls (black circles). (C) Correlation between jejunal efflux adjusted by weight, and FGF19 or C4. Data is depicted as mean \pm 95% CI. Differences were evaluated by Mann-Whitney U test. Correlations were evaluated with Spearman's (ρ) correlation coefficient. *P* values are depicted.

FIGURE 2. CR restores homeostatic control of bile salt synthesis within 7 days.

Twelve patients with a temporary double enterostomy on PN underwent CR for up to 7 weeks. Blood was sampled on 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 is depicted as mean \pm 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* < .05), **(*P* < .01) and *** (*P* < .001).

FIGURE 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- 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 is depicted as mean \pm 95% CI. Trends in time were evaluated by ANOVA with repeated measures. *P* values are depicted. Asterisks indicate significance levels: *(*P* < .05) and **(*P* < .01).

FIGURE 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 μ mol/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*, *TNF α* , *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 is depicted as mean \pm 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.

FIGURE 5. The C4 response, dependent on the FGF19 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. (A) Interaction plot depicting the association between the interaction of $\Delta_{\text{FGF19}} * \Delta_{\text{C4}}$ after 7 days and predicted CIT values over time (from 7 days onwards). The Δ_{FGF19} (ng/mL) is depicted on the x-axis and the Δ_{C4} (ng/mL) is shown at the corresponding dots. The y-axis shows the predicted CIT values ($\mu\text{mol/L}$). The regression line is shown with 95% confidence interval. R-squared and *P* values are depicted.

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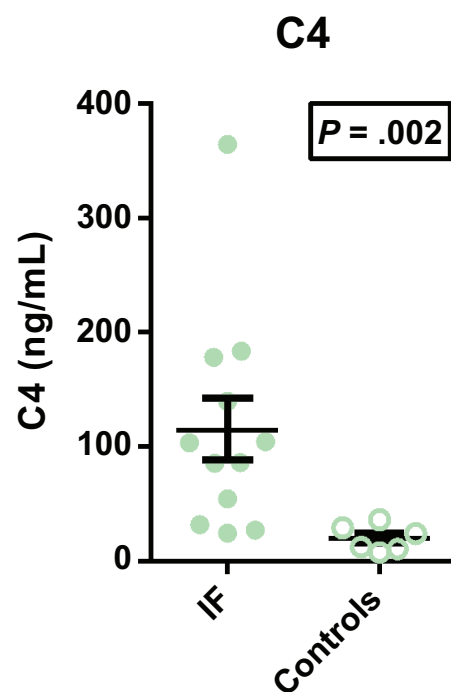
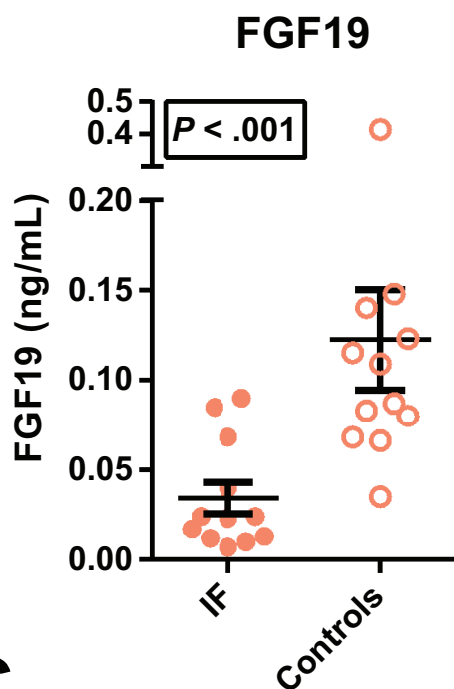
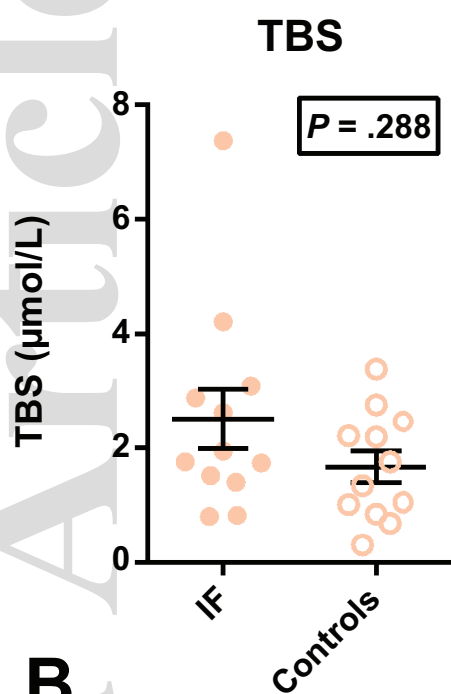
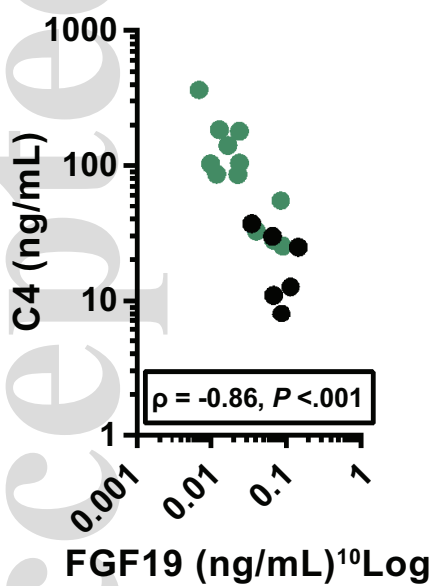
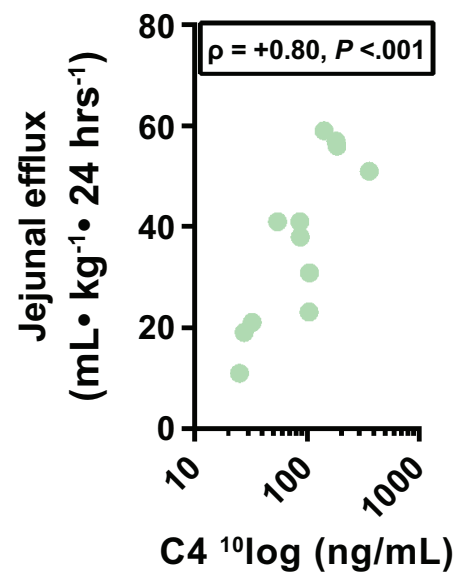
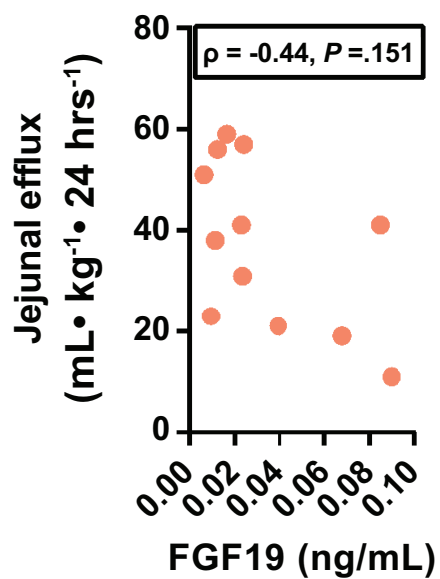
TABLES

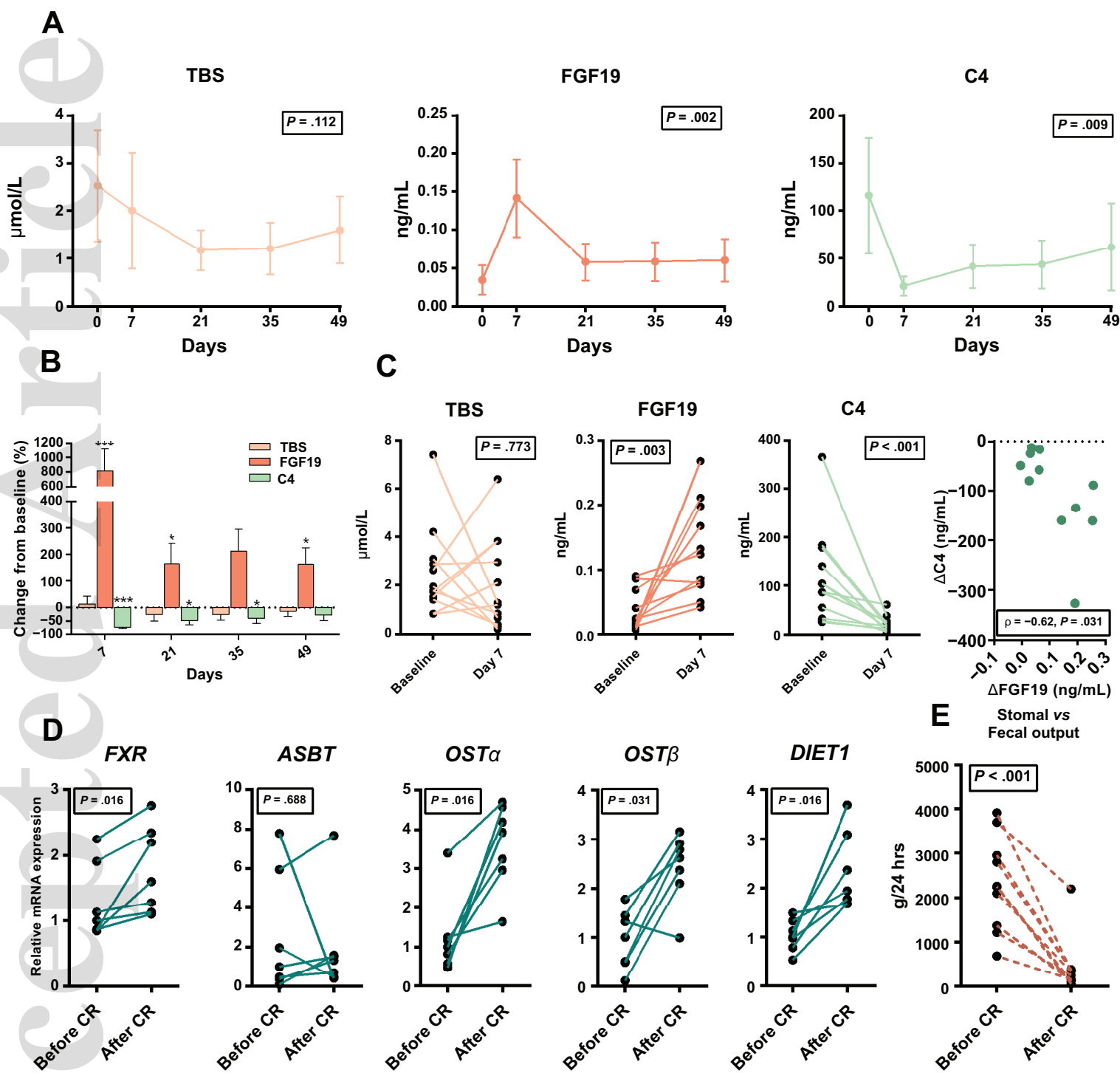
TABLE 1. Patient demographics and surgical characteristics at baseline.

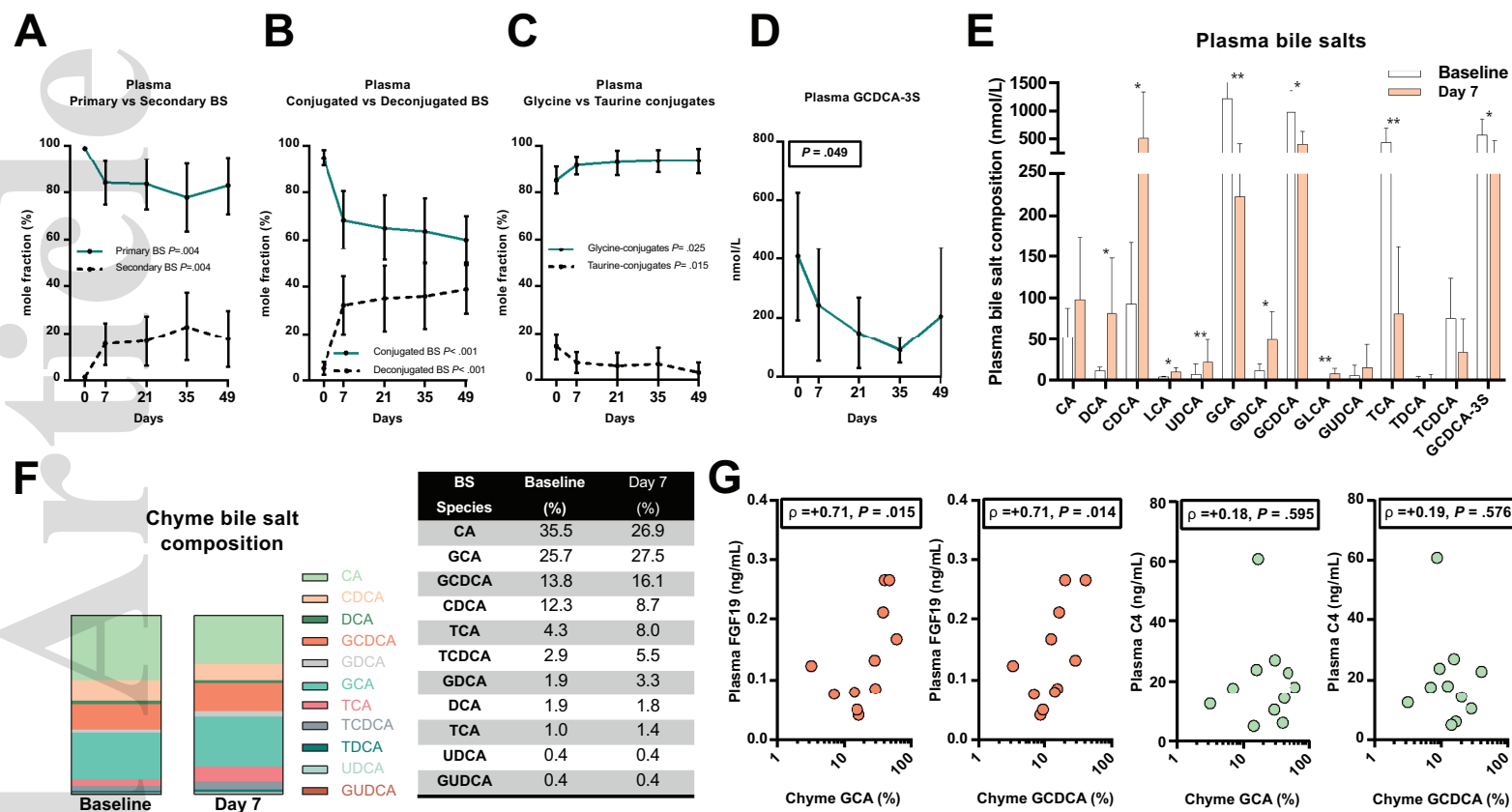
Variable	Study cohort
Patients, n*	12
Age, yrs	67.7 ± 14.6
Male/female	7/5
Underlying etiology, n (%)	
Mechanical occlusion	3 (25)
Ischemia	4 (33)
Peritonitis	5 (42)
PN dependence, n (%)	10 (83)
Jejunal efflux (mL/24 hrs)	2245 [1366 to 2913]
Jejunal efflux (mL•weight⁻¹•24 hrs⁻¹)	39 [22 to 52]
Hospital admission time before referral (days)	28 [18 to 59]
Plasma citrulline ≤ 20 μmol/L, n (%)	7 (58)
SB resection ≥ 30 cm, n (%)	5 (42)
Resection SB length if resection (n = 6), cm	105 [45 to 135]
Upstream SB length (n = 9), cm	120 [100 to 200]
Upstream SB length ≤ 100 cm, n (%)	4 [44]
Downstream SB length (n=11), cm	160 [120 to 225]
Theoretical total SB length** (n = 8), cm	315 [268 to 353]
Downstream SB anatomy, n (%)	
Ileo-colon-rectum	8 (67)
Ileo-colon-stoma	4 (33)
Serum liver tests (IU/L)	
ALP	216 [163 to 291]
GGT	176 [73 to 252]
ALT	66 [45 to 109]
AST	37 [29 to 57]
Total bilirubin, μmol/L	6.2 [3.7 to 9.3]
Serum CRP (mg/L)	6.3 [2.1 to 36.0]

* If data are missing, numbers in parentheses indicate the number of assessments for a particular factor.

Data are depicted as percentages (%), mean \pm SD or median [IQR]. Abbreviations: PN, parenteral nutrition; SB, small bowel; ALP, alkaline phosphatase; GGT, γ -glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. ** Theoretical total SB length = upstream + downstream SB length

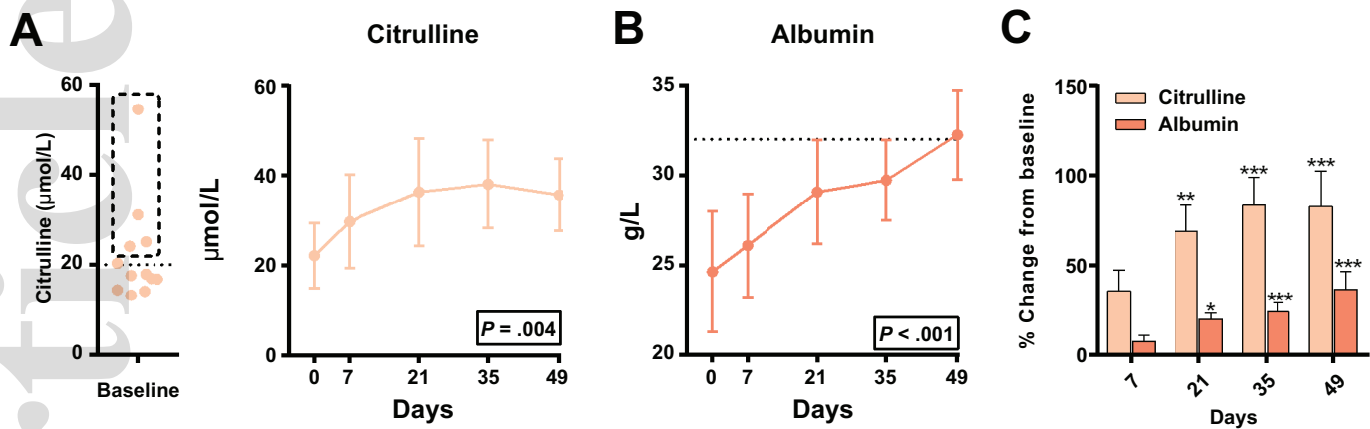
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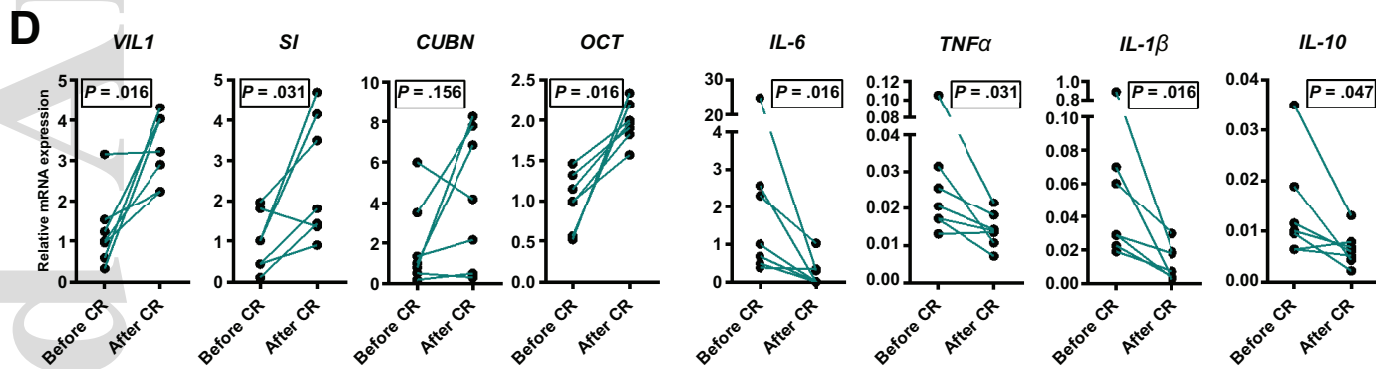


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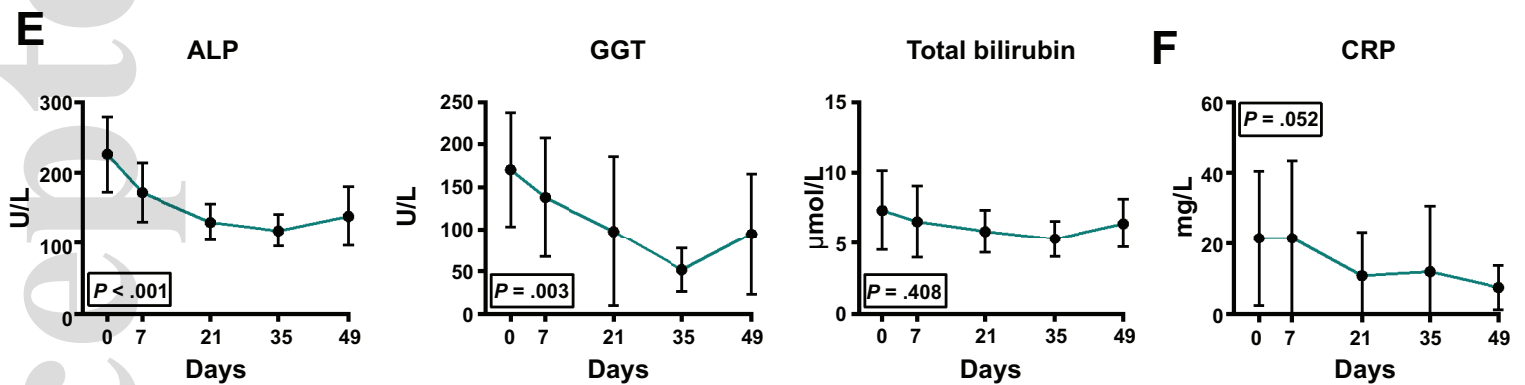
Intestinal function and nutritional status



Intestinal epithelial-specific genes and local inflammation

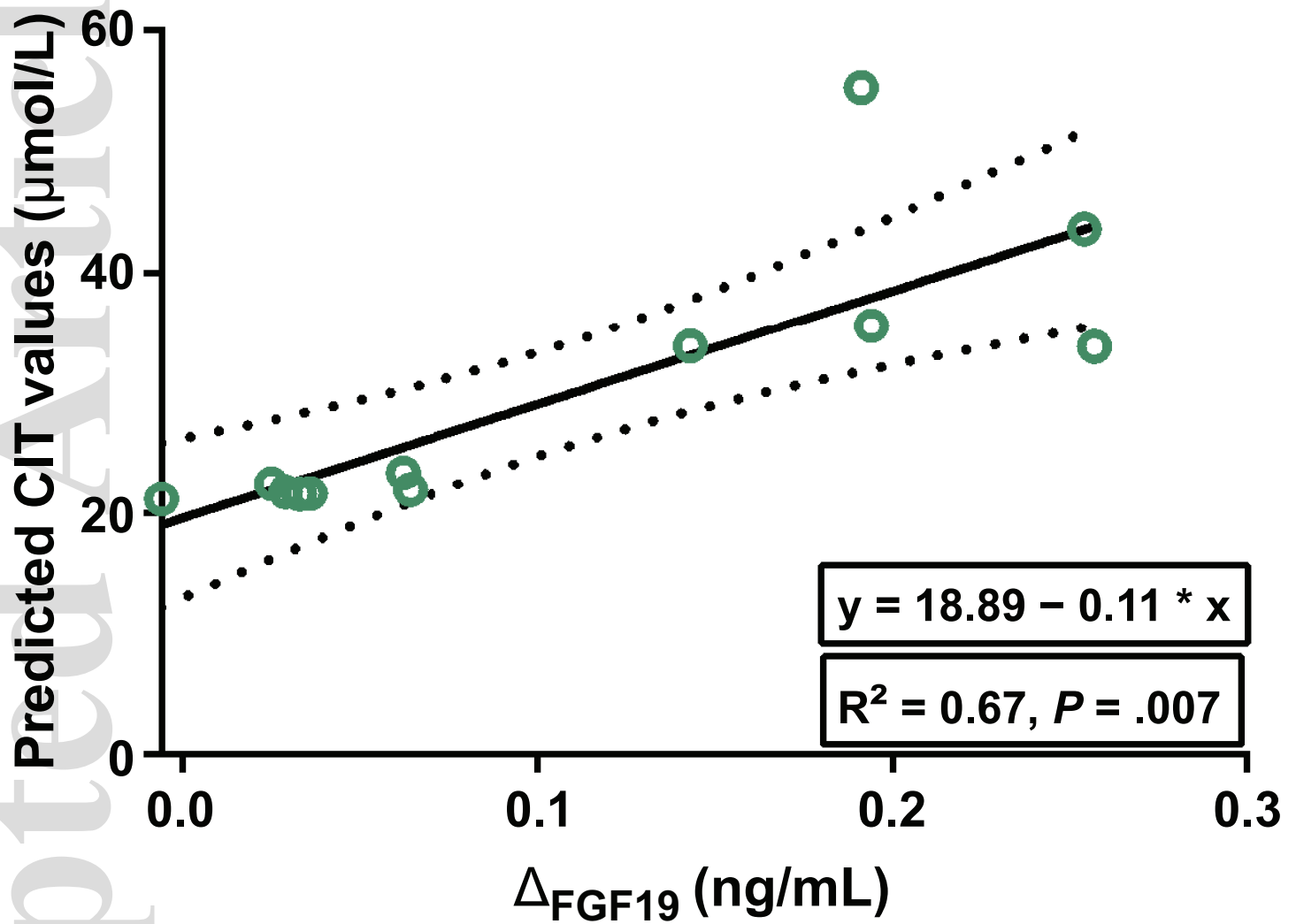


Liver injury and systemic inflammation

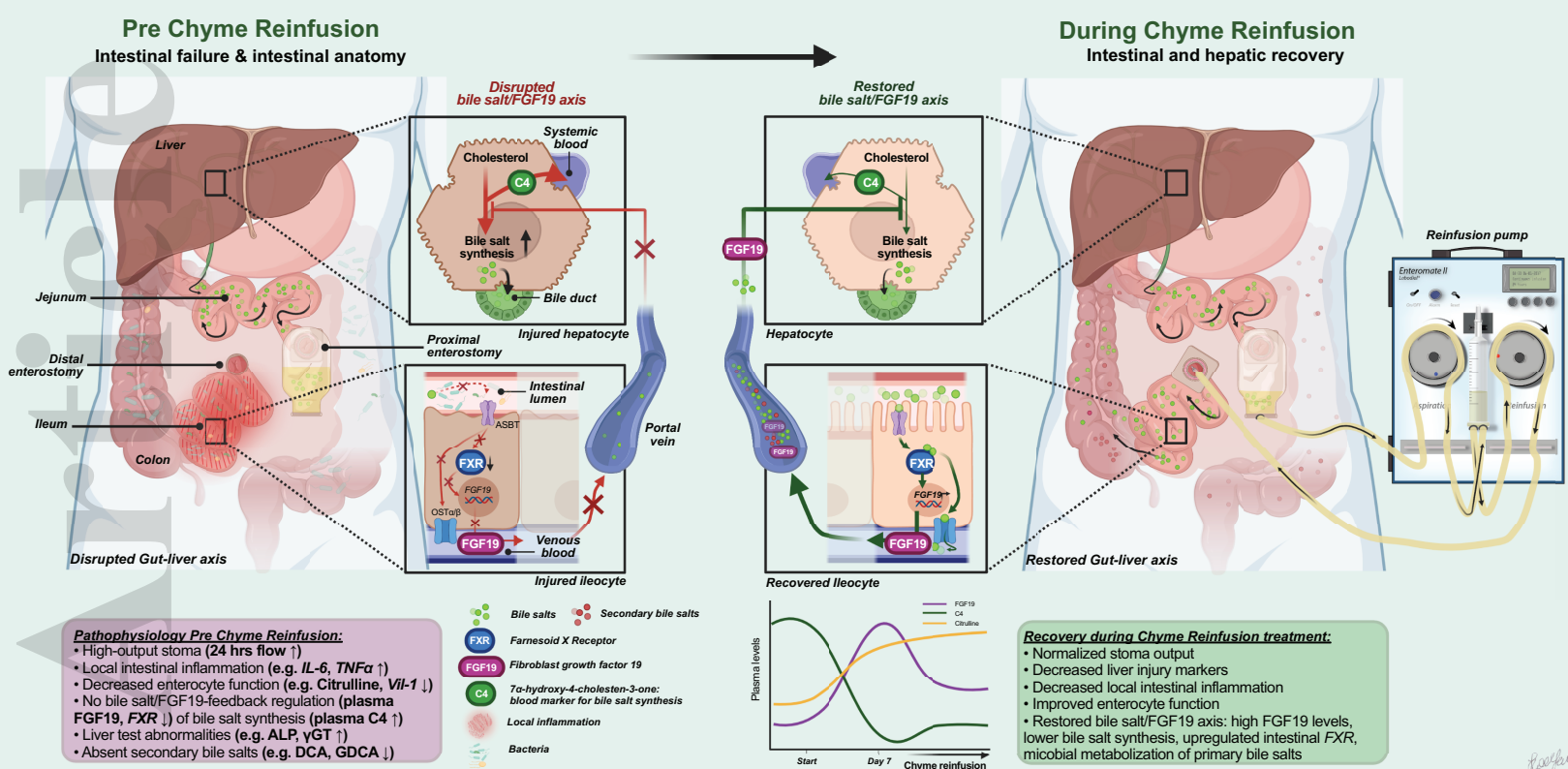


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Interaction of $\Delta_{\text{FGF19}} * \Delta_{\text{C4}}$



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