Gallbladder Dyskinesia Is Associated With an Impaired Postprandial Fibroblast Growth Factor 19 Response in Critically Ill Patients

HEPATOLOGY

HEPATOLOGY, VOL. 70, NO. 1, 2019

Gallbladder Dyskinesia Is Associated With an Impaired Postprandial Fibroblast Growth Factor 19 Response in Critically Ill Patients

Kiran V.K. Koelfat ,[1] Mark P. Plummer,[2] Frank G. Schaap ,[1,3] Martin Lenicek,[4] Peter L.M. Jansen,[1] Adam M. Deane,[2] and Steven W.M. Olde Damink[1,3]

Critical illness is associated with a disturbed regulation of gastrointestinal hormones resulting in functional and metabolic anomalies. Fibroblast growth factor 19 (FGF19) is an ileum-derived metabolic hormone induced by bile salts upon gallbladder emptying after enteral nutrient stimulation. Our aim was to study the nutrient-stimulated FGF19 response in 24 patients admitted to the intensive care unit (ICU) compared with 12 healthy controls. All subjects received intraduodenal high-lipid nutrient infusion for 120 minutes. Blood was collected every 30 minutes until 1 hour after infusion, and gallbladder emptying was studied by ultrasound. Serum levels of bile salts and FGF19 were assessed. ICU patients had significantly higher fasting bile salt serum levels compared with controls, whereas FGF19 serum levels were similar. In both groups, nutrient infusion elicited substantial bile salt elevations ($P < 0.001$), peaking at 90 minutes, albeit with a significantly lower peak in the ICU patients ($P = 0.029$). In controls, FGF19 was significantly elevated relative to baseline from 120 minutes onward ($P < 0.001$). In ICU patients, the FGF19 response was blunted, as reflected by significantly lower FGF19 elevations at 120, 150, and 180 minutes ($P < 0.05$) and significantly lower area under the curve (AUC) values compared with controls ($P < 0.001$). Gallbladder dysmotility was associated with the impaired FGF19 response in critical illness. The gallbladder ejection fraction correlated positively with FGF19 AUC values ($\rho = +0.34$, $P = 0.045$). In 10 of 24 ICU patients, gallbladder emptying was disturbed. These patients had significantly lower FGF19 AUC values ($P < 0.001$). Gallbladder emptying and the FGF19 response were respectively disturbed or absent in patients receiving norepinephrine. *Conclusion:* The nutrient-stimulated FGF19 response is impaired in ICU patients, which is mechanistically linked to gallbladder dysmotility in critical illness. This may contribute to disturbed liver metabolism in these patients and has potential as a nutritional biomarker. (Hepatology 2019;70:308-318).

Critical illness is associated with disturbances in production and secretion of gastrointestinal (GI) hormones, and this may contribute to GI, immune, and metabolic abnormalities.[1] Bile salts are endocrine signaling molecules that undergo enterohepatic circulation. By interacting with bile salt–sensing receptors, bile salts regulate the production of a number of GI hormones, including fibroblast growth factor 19 (FGF19).[2] Gallbladder motility is often impaired in critical illness, which could result in reduced entry of bile into the small intestine as well as impaired enterohepatic bile salt signaling.[3] Thus, gallbladder dysfunction could contribute to disturbances in bile salt–regulated GI hormones.

Abbreviations: AUC, area under the curve; C4, 7-alpha-hydroxy-4-cholesten-3-one; FGF19, fibroblast growth factor 19; FXR, farnesoid X receptor; GBEF, gallbladder ejection fraction; GI, gastrointestinal; ICU, intensive care unit.

Received September 16, 2018; accepted March 14, 2019.
Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.30629/suppinfo.
Supported by a grant (to K.V.K.K.) from the Netherlands Organization for Scientific Research (NWO 022.003.011).

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.30629

Potential conflict of interest: Nothing to report.

FGF19 (termed Fgf15 in rodents) is an enterokine produced in response to bile salt reabsorption in the terminal ileum.[4,5] Meal-induced elevation of FGF19 is governed by the bile salt–activated transcription factor farnesoid X receptor (FXR).[4,5] FGF19 primarily targets the liver, which expresses both components (FGFR4 and β-klotho) required for FGF19 signaling.[6-8] (Pre)clinical studies revealed a myriad of functions of FGF19/Fgf15 including regulation of major metabolic pathways (e.g., energy, carbohydrate and lipid metabolism, bile salt and protein synthesis) and functional processes such as gallbladder relaxation, skeletal muscle mass homeostasis, and GI motility.[5,9-13] Considering the beneficial metabolic effects, FGF19 administration is evaluated as treatment of chronic liver diseases.[14-17] Reduced circulating FGF19 has been observed in nonalcoholic fatty liver disease, intestinal failure–associated liver disease (IFALD), and bile salt diarrhea.[18-20] In IFALD, impaired FGF19-mediated repression of hepatic bile salt synthesis appeared to be the pathological mechanism leading to overproduction of bile salts and was associated with hepatotoxicity.[19] Of note, critical illness has been associated with elevated bile salts and hyperbilirubinemia, indicating dysregulated bile salt homeostasis and secretory dysfunction.[21,22]

In healthy subjects, systemic FGF19 levels peak 3 to 4 hours postprandially, following earlier elevation of bile salts at 1.5 to 2 hours after a meal.[23,24] Thus far, fasting and nutrient-stimulated FGF19 levels have not been reported in critically ill patients. We hypothesized that patients with critical illness and associated gallbladder dysmotility have an abrogated postprandial course of FGF19. The aim of this study was to compare the bile salt and the enteral FGF19 response after intraduodenal lipid infusion in patients admitted to the intensive care unit (ICU) and in healthy controls.

Materials and Methods

SUBJECTS AND DATA COLLECTION

We analyzed clinical data and stored plasma samples of patients who were initially enrolled in a prospective observational comparison study to quantify gallbladder dysfunction during critical illness.[3] Description of the original study has been detailed elsewhere.[3] In short, 24 mechanically ventilated critically ill patients capable of receiving enteral nutrition were studied, as were a control group of 12 age- and sex-matched healthy subjects without known diseases of the hepatobiliary or GI tract.[3] None of the patients had confirmed intestinal inflammation or ischemia of the ileal region. Study approval was provided by the Human Research Ethics Committee of the Royal Adelaide Hospital and performed according to Australian National Health and Medical Research Centre guidelines for the conduct of research on unconscious patients. Informed consent was obtained from the patients' next of kin. All healthy participants provided written, informed consent.[3]

PROTOCOL

Briefly, both groups were fed intraduodenally through a nasogastric tube after 8 hours of overnight fasting.[3] In both healthy controls and critically

ARTICLE INFORMATION:

From the [1]Department of Surgery, Maastricht University Medical Center and NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University, Maastricht, the Netherlands; [2]Intensive Care Unit, Royal Melbourne Hospital, University of Melbourne, Parkville, Australia; [3]Department of General, Visceral and Transplantation Surgery, RWTH University Hospital Aachen, Aachen, Germany; [4]Department of Medical Biochemistry and Laboratory Diagnostics, 1st Faculty of Medicine, Charles University, Prague, Czech Republic.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Kiran V.K. Koelfat, M.D., M.Sc.
Department of Surgery, Maastricht University Medical Center and NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University
Universiteitssingel 50 6229 ER
P.O. Box 616
6200 MD Maastricht, the Netherlands
E-mail: k.koelfat@maastrichtuniversity.nl

ill patients, a small intestinal feeding catheter was inserted using an electromagnetic guidance technique.[3] All subjects received a high-fat meal (120 mL; 20% Intralipid from Baxter Healthcare, Deerfield, IL) infused postpylorically at a rate of 2 kcal/minute (60 mL•hour^{-1}) during a 2-hour interval. Both healthy controls and critically ill patients were investigated while lying in a supine position, and the head of the bed was placed at an angle of 30 degrees.[3] Blood was collected before the nutrient infusion (–30, 0 minutes), at 30-minute intervals during nutrient infusion (30, 60, 90, 120 minutes), and at two additional 30-minute intervals after termination of the infusion (150 and 180 minutes). Blood was taken from an arterial line *in situ* for ICU patients and from an intravenous cannula placed in a peripheral vein for controls. For baseline values, the average between –30 and 0 minutes was used. Gallbladder volume (mL) at 30-minute intervals from –30 to 180 minutes was previously assessed by three-dimensional ultrasound.[3] Gallbladder ejection fraction (GBEF) was defined as the percentage volume change between start (0 minutes) and completion of lipid infusion (120 minutes). Normal GBEF was considered to be >35%.[25] To determine presence of biliary sludge, static images of the gallbladder at –30 minutes were used.[3]

ANALYTICAL PROCEDURES

Blood samples (ethylene diamine tetraacetic acid anticoagulated) were originally collected on ice, immediately centrifuged, and stored as plasma at –70°C, before dry-ice shipping to Maastricht University for additional analyses. Total bile salts were determined using an enzymatic cycling method according to the manufacturer's protocol (Diazyme Laboratories, CA). FGF19 was assayed by sandwich enzyme-linked immunosorbent assay as described.[26] Levels of 7-alpha-hydroxy-4-cholesten-3-one (C4), a systemic marker of bile salt synthesis, were determined as described.[27] If C4 levels were not detectable, values were set to the lower limit of detection, i.e., 0.1 ng/mL.

STATISTICS

Data are expressed as median [interquartile range] or mean ± SEM when appropriate, and displayed as box and whisker plots with the 10th and 90th percentile, unless indicated otherwise. Significant differences between critically ill patients and healthy volunteers were evaluated with the Mann-Whitney U test. Correlations were evaluated by the Spearman's correlation test. Of two healthy subjects, plasma samples representing single time points (60 and 180 minutes, respectively) were not available (2.3% missing data). To avoid subject deletion, data of these subjects were included in the statistical analyses using multiple imputation method to estimate missing values. A Friedman repeated measures on ranks or two-way analysis of variance was used to evaluate postprandial courses, with Dunn's multiple comparison test to compare differences between baseline and subsequent individual time points. Area under the curve (AUC) values were calculated using the trapezoidal rule at 0 to 180 minutes (total AUC), and 120 to 180 minutes ("postprandial phase"). *P* values below 0.05 were considered statistically significant. For visual purposes, graphs are depicted as mean ± SEM. Statistical analyses were performed using GraphPad Prism 6.0 (GraphPad Software Inc., CA) and SPSS 22.0 (IBM SPSS Inc, Chicago, IL).

Results

SUBJECTS

Detailed clinical characteristics of the included patients have been reported,[3] and parameters relevant for the present study are summarized in Table 1. Clinical data and plasma samples of 24 patients (mean age 54 ± 16 years, 25% female) admitted to the ICU and 12 healthy volunteers (mean age 55 ± 20 years, 33% female) were analyzed and compared. None of the healthy volunteers had prior GI diseases or biliary sludge.

FASTED LEVELS OF BILE SALTS ARE ELEVATED IN CRITICALLY ILL PATIENTS

The bile salt-FXR-FGF19 regulatory axis is activated after nutrients enter the small intestine and elicit gallbladder contraction. First, we studied fasted plasma levels of bile salts, FGF19, and C4 in controls and ICU patients. At baseline, bile salt levels were significantly higher in ICU patients (1.6 [0.9-2.4] vs. 3.1 [1.6-9.1] μmol/L, $P = 0.024$) (Table 2). Baseline levels of FGF19 and C4 were comparable in both groups ($P = 0.221$ and $P = 0.151$, respectively) (Table 2). Similar to findings

TABLE 1. Patient Characteristics

Item	ICU Patients (n = 24)	Healthy Controls (n = 12)	*P* Value
Age (years)	54 ± 16	55 ± 20	0.946
Sex			
Female, n (%)	6 (25)	4 (33)	0.650
Body mass index (kg/m^2)	29 ± 6	24 ± 4	**0.010**
Mean blood glucose (mmol/L)	7.1 ± 2.1	5.5 ± 0.5	**0.001**
Gallbladder wall thickness (cm)	0.41 [0.37-0.55]	0.25 [0.20-0.27]	**0.000**
GBEF (%)	51 [9-83]	78 [2-83]	**0.001**
Admission diagnosis, n (%)			
Respiratory	15 (63)	—	—
Neurological	3 (13)	—	—
Sepsis	3 (13)	—	—
After operation	2 (8)	—	—
Trauma	1 (4)	—	—
APACHE II score	17 ± 6	—	—
SOFA score	8 ± 4	—	—
Day of ICU admission	5 ± 4	—	—
Vasopressor support, n (%)	9 (38)	—	—
Acute renal failure, n (%)*	7 (29)	—	—
Antibiotic support, n (%)	19 (79)	—	—
Hospital mortality, n (%)	9 (38)	—	—
Liver tests (median [IQR])			
ALT (IU/L)	34 [23-47]	—	—
AST (IU/L)	31 [35-72]	—	—
GGT (IU/L)	79 [35-186]	—	—
ALP (IU/L)	97 [65-150]	—	—
Total bilirubin (μmol/L)	7 [4-17]	—	—
Biliary sludge (severity score, n)			
0	7	12	—
1	7	—	—
2	8	—	—
3	1	—	—
4	1	—	—

*Acute renal failure was diagnosed as per Risk, Injury, Failure, Loss, and End-Stage Kidney Disease criteria. Data are mean ± SD or median + IQR. Table is adapted from Plummer et al. 2016.[3]
The significance (alpha) level is 0.05.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; APACHE, Acute Physiology and Chronic Health Evaluation; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; IQR, interquartile range; SOFA, sequential organ failure assessment.

TABLE 2. Fasted Bile Salts, FGF19, and C4 Levels in Healthy Controls and Critically Ill Patients

Item	Healthy Controls (n = 12), Median [IQR]	ICU Patients (n = 24), Median [IQR]	*P* Value
Bile salts (μmol/L)	1.6 [0.9-2.4]	3.1 [1.6-9.1]	**0.024**
FGF19 (ng/mL)	0.10 [0.07-0.14]	0.08 [0.06-0.13]	0.221
C4 (ng/mL)	18.8 [10.3-31.5]	5.0 [1.0-28.0]	0.151

The significance (alpha) level is 0.05.
Abbreviation: IQR, interquartile range.

reported by Vanwijngaerden et al., fasting bile salt levels were strongly correlated with total bilirubin levels (ρ = +0.64, P < 0.001) in our study.[21]

LIPID INFUSION–INDUCED FGF19 RESPONSE IS IMPAIRED IN CRITICAL ILLNESS

Next, we studied the bile salt and FGF19 responses in both healthy controls and critically ill patients. Note that the FGF19 response refers

to lipid infusion–induced changes in circulating FGF19 levels, rather than the hepatic response to this hormone. Excursions of bile salts and FGF19 following lipid infusion are illustrated in Fig. 1 (see Supporting Figs. S1 and S2 for the individual responses). Note that previously reported gallbladder volumes were included in the graphs to illustrate changes in gallbladder volume relative to bile salt and FGF19 excursions.[3] The bile salt and FGF19 responses were significant over time in both groups ($P < 0.001$) (Fig. 2A,B). As expected, intraduodenal lipid infusion resulted in elevation of systemic bile salts. In healthy controls, bile salt levels were elevated relative to baseline from 60 minutes onward (P values between < 0.001 and 0.049) (Fig. 2A). Similar to controls, bile salt levels in ICU patients were elevated compared with baseline values from 90 minutes onward (P values between < 0.001 and 0.045) (Fig. 2A). Median bile salt levels at 90 minutes were higher in healthy controls (17.8 [9.4-25.6] vs. 7.5 [2.1-18.4] μmol/L, $P = 0.028$) (Fig. 2A). Furthermore, the total AUC of bile salts was higher in controls (1,659 [1,117-2,109] vs. 421 [261-1,354] AUC, $P = 0.008$) (Fig. 2D).

FG19 levels in controls were elevated at 150 minutes ($P = 0.003$) and 180 minutes ($P = 0.004$) compared with baseline values (Fig. 2B). In ICU patients, FGF19 levels were elevated relative to baseline only at 180 minutes ($P = 0.013$) (Fig. 2B). FGF19 levels were markedly lower in ICU patients compared with controls at 120 minutes ($P = 0.031$), 150 minutes ($P < 0.001$), and 180 minutes ($P < 0.001$) (Fig. 2B). The total AUC was notably lower in ICU patients compared with controls ($P = 0.001$) (Fig. 2E).

FGF19 signaling results in repression of bile salt synthesis, as reflected by lowering of systemic C4 levels 1-2 hours after postprandial peaking of FGF19.[24] In the studied time frame, intraduodenal lipid infusion did not affect C4 levels in controls ($P = 0.289$) or ICU patients ($P = 0.202$) (Fig. 2C).

ABSENT OR IMPAIRED GALLBLADDER EMPTYING IS ASSOCIATED WITH AN IMPAIRED FGF19 RESPONSE AFTER NUTRIENT STIMULATION

Because gallbladder contraction is followed by entry of bile salts into the duodenum, subsequent reabsorption of bile salts in the ileum causes activation of ileal FXR and stimulation of FGF19 synthesis. Thus, FGF19 synthesis follows gallbladder contraction. Note that bile salts levels at 90 minutes correlated positively with FGF19 levels at 180 minutes ($\rho = +0.38$, $P = 0.023$) (data not shown). Our results showed that GBEF is positively correlated ($\rho = +0.34$, $P = 0.045$) with the AUC of FGF19 in all subjects (Fig. 3A). Additionally, residual gallbladder volume after 30 minutes (when the largest absolute volume change has occurred) was negatively correlated with the AUC of FGF19 across all subjects ($\rho = -0.56$, $P < 0.001$) (Fig. 3B).

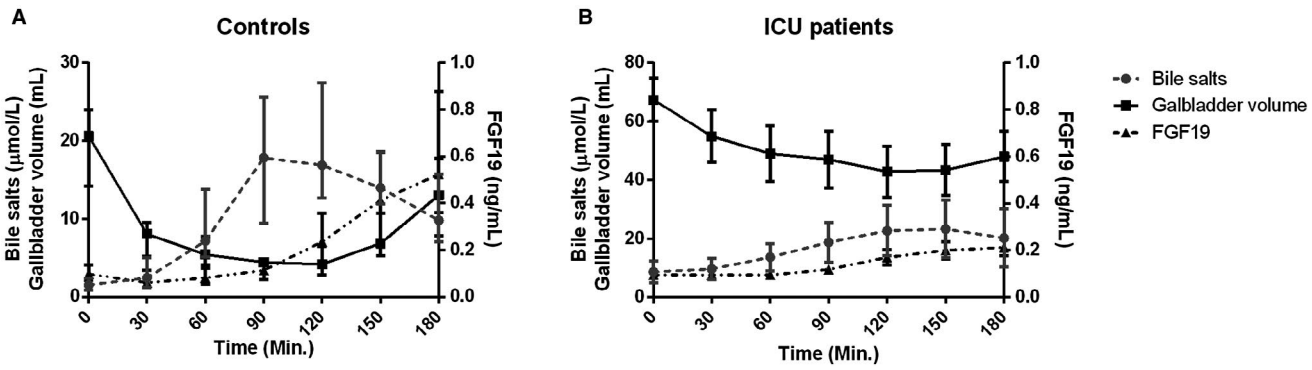

FIG. 1. Gallbladder volume, bile salt, and FGF19 dynamics after nutrient infusion. Gallbladder volume (black line), bile salts (gray striped line), and FGF19 (black intermittent line) dynamics during lipid infusion (0 to 120 minutes) and "postprandially" (120 to 180 minutes) in healthy controls and ICU patients. Data points are depicted as median + range (A) or mean ± SEM (B). Note that to illustrate the bile salt and FGF19 excursions, previously published gallbladder volume are depicted in this graph.[3]

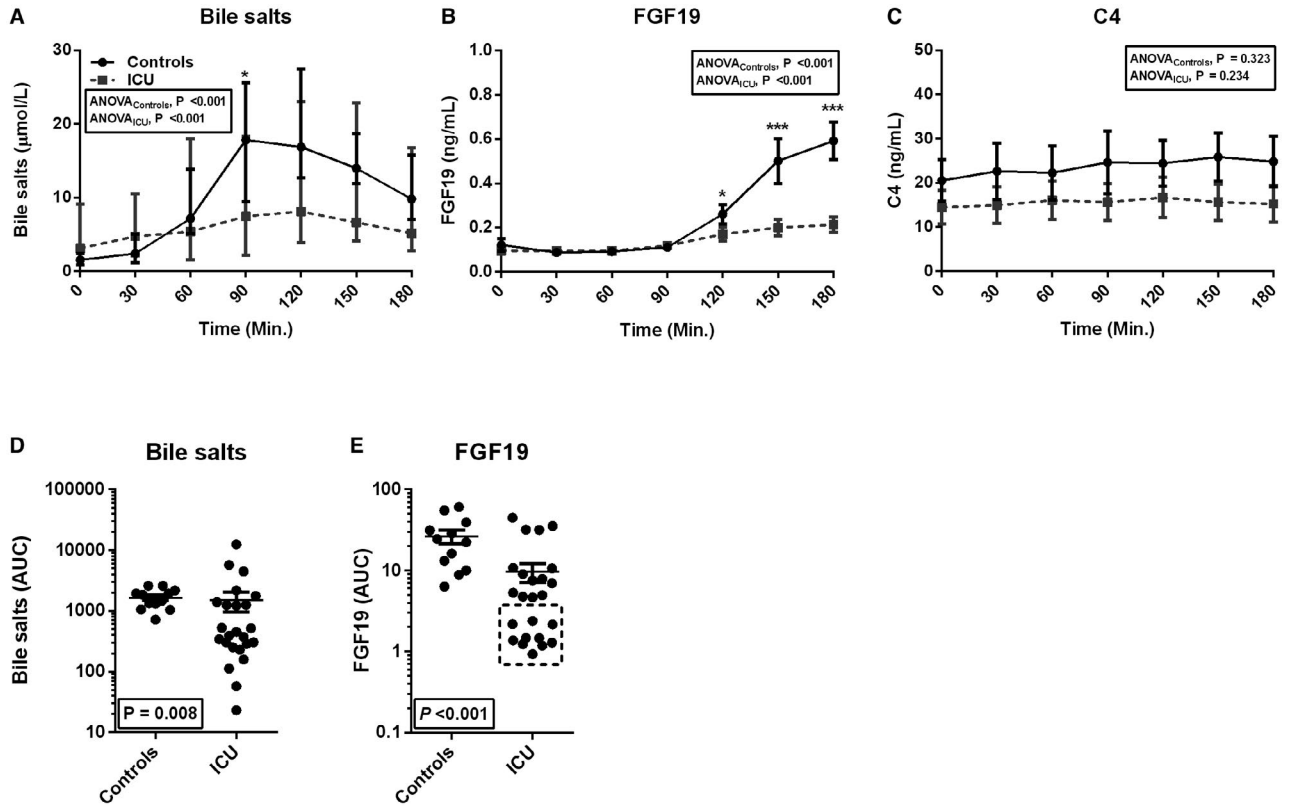

FIG. 2. Plasma excursions after nutrient infusion in healthy controls and ICU patients. (A) Bile salt, (B) FGF19, and (C) C4 responses. Total AUC values of (D) bile salts and (E) FGF19 in controls and ICU patients. Note that the lined rectangle in Fig. 2E resembles FGF19 nonresponders. Data points are depicted as median + range (A) or mean ± SEM (B-E). Significant differences between controls and ICU patients at the various time points are reflected by asterisks: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. Box-whisker plots with 10th to 90th percentile.

Thus, patients with absent or impaired GBEF (i.e., below < 35%; n = 10) had lower FGF19 AUC values than patients with GBEF ≥35% (n = 14; $P = 0.096$) (Fig. 3C). Note that all healthy subjects had GBEF values >71%. Moreover, gallbladder wall thickness, which is increased in patients with gallbladder dysmotility, was negatively correlated with the AUC of FGF19 ($\rho = -0.39$, $P = 0.019$) (Fig. 3D). Considering the temporal phases (i.e., prandial vs. postprandial) of the nutrient-stimulated FGF19 response, it is noteworthy to mention that correlations between FGF19 AUC and GBEF, gallbladder volume at 30 minutes, and gallbladder wall thickness were stronger when using (postprandial) FGF19 $AUC_{120\text{-}180\ minute}$ values (Supporting Fig. S3A-C). The marked difference in FGF19 excursion between ICU patients with absent or impaired gallbladder emptying and controls was maintained when using postprandial $AUC_{120\text{-}180\ minute}$ values ($P < 0.001$) (Supporting Fig. S3D). Biliary sludge, observed only in the patient population, was negatively correlated with FGF19 AUC values ($\rho = -0.34$, $P = 0.042$) and postprandial FGF19 AUC values ($\rho = -0.63$, $P < 0.001$) (Supporting Fig. S3E,F).

In this study, we demonstrate that gallbladder dysmotility is associated with an impaired FGF19 response. We further investigated whether the postprandial bile salt response was also affected. First, the GBEF was not related to bile salt AUC values ($\rho = +0.28$, $P = 0.095$) (Fig. 3E). However, the residual gallbladder volume at 30 minutes was negatively correlated with bile salt AUC values ($\rho = -0.41$, $P = 0.013$) (Fig. 3F). Furthermore, ICU patients with absent or impaired gallbladder emptying had lower bile salt AUC values compared with controls ($P = 0.020$) (Fig. 3G). Finally, gallbladder wall thickness was negatively correlated with bile salt AUC values ($\rho = -0.41$, $P = 0.014$) (Fig. 3H).

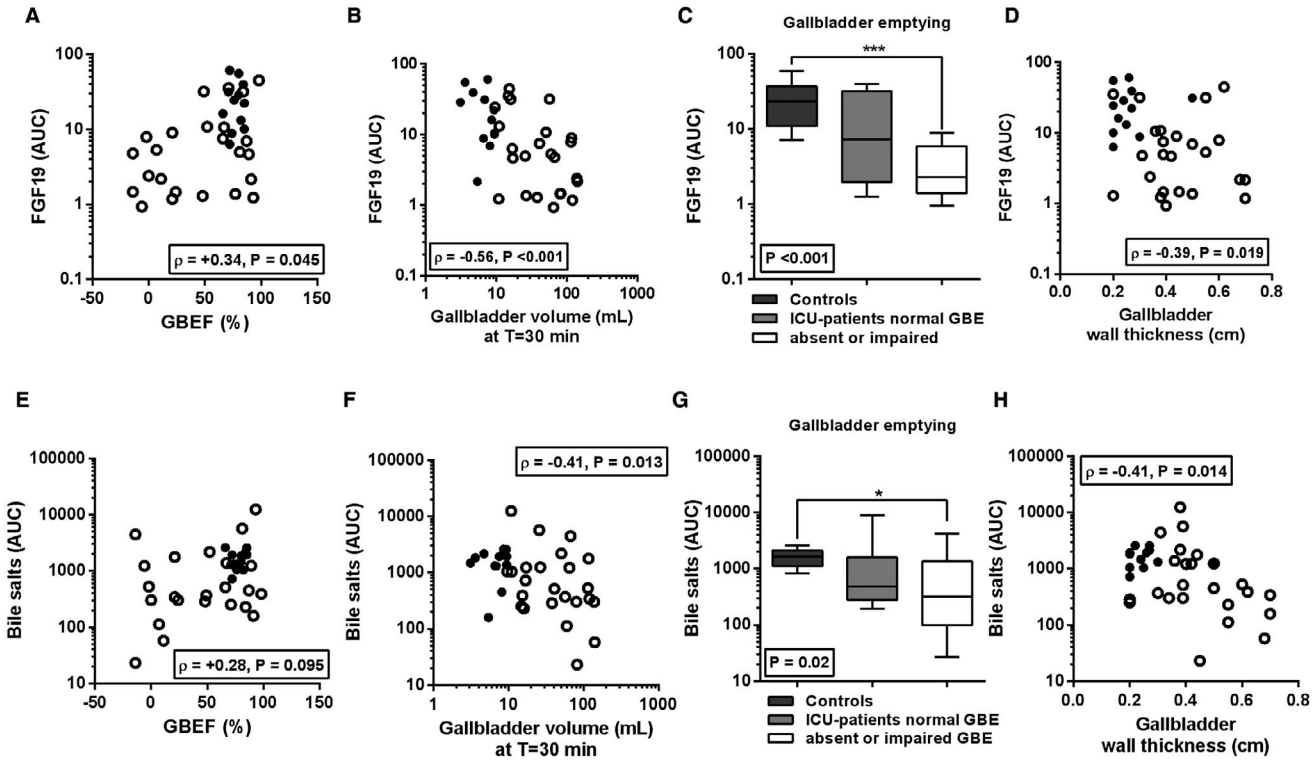

FIG. 3. Gallbladder dysmotility is associated with an impaired nutrient-stimulated FGF19 response in ICU patients. (A, E) Correlation between GBEF (%), (B, F) gallbladder volume (mL), and (D, H) gallbladder wall thickness (mm) with FGF19 or bile salt AUC values. Open circles depict data points from ICU patients, and filled circles represent data from healthy controls. (C, G) FGF19 AUC values were compared between ICU patients with normal gallbladder emptying, absent or impaired emptying, and controls.

As can be appreciated from the individual response curves (Supporting Fig. S2) and AUC values (Fig. 2D,E), FGF19 levels are virtually unaffected by lipid infusion in a number of ICU patients. Careful examination of clinical data revealed that patients receiving norepinephrine for hemodynamic support had no appreciable FGF19 response following lipid infusion (P_{time} = 0.911; Fig. 4A). In this patient subgroup, the median FGF19 levels were 0.076 ng/mL and 0.095 ng/mL at baseline and 180 minutes, respectively (Fig. 4A). In contrast, patients not receiving norepinephrine had a significant FGF19 response (P < 0.001), and levels were significantly elevated relative to baseline at 150 minutes (P = 0.007) and 180 minutes (P < 0.001) (Fig. 4A). Finally, patients receiving norepinephrine (n = 9) had lower GBEFs (11% [–4 to 45] vs. 71% [48-87], P = 0.029) and lower AUC of FGF19 (P = 0.012) compared with patients without administration of norepinephrine (Fig. 4C,D), further delineating the association between gallbladder contraction and the nutrient-stimulated FGF19 response.

Lipid infusion elicited significant bile salt responses in both patients receiving norepinephrine and patients not receiving norepinephrine (P < 0.001) (Fig. 4B). In contrast to the FGF19 response, ICU patients receiving norepinephrine had a comparable bile salt response as patients not receiving norepinephrine (bile salt AUC value, P = 0.433) (Fig. 4E).

Discussion

Critically ill patients may encounter disturbed production of bile salt–regulated enterokines. In this context, we studied intestinal FGF19 production following postpyloric nutrient infusion in critically ill patients and compared this with responses in age- and sex-matched healthy controls. The major finding of this study is that the nutrient-stimulated response of FGF19 is impaired

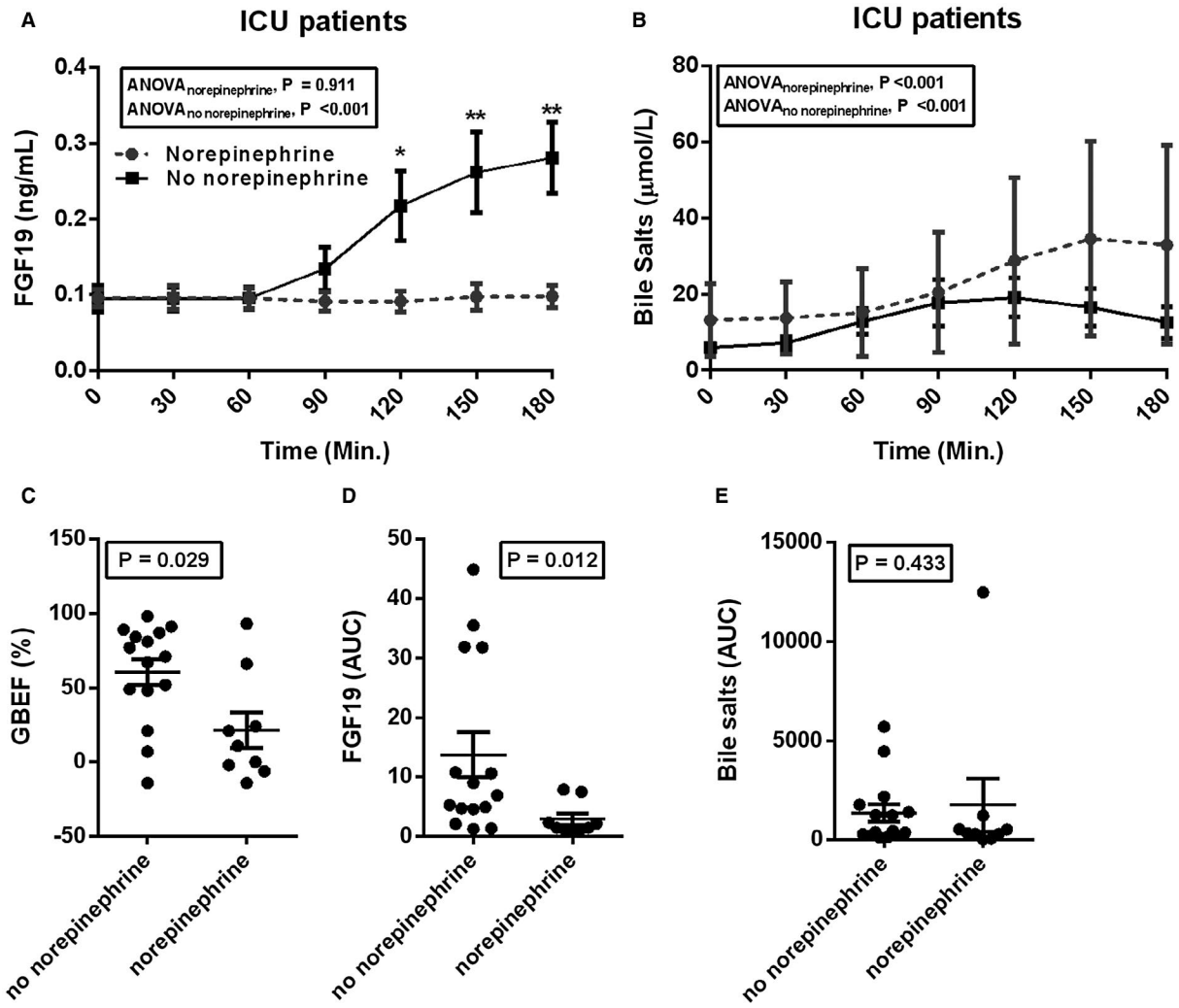

FIG. 4. ICU patients receiving norepinephrine are associated with a virtually blunted FGF19 response. (A) Plasma course of FGF19 and (B) bile salts in ICU patients with and without norepinephrine administration and comparison of (C) GBEF and (D) FGF19 and (E) bile salt AUC values between ICU patients receiving and not receiving norepinephrine. Significant differences between ICU patients with and without norepinephrine at the various time points are reflected by asterisks: *, $P < 0.05$; **, $P < 0.01$.

in critically ill patients. Moreover, our data support that gallbladder dysmotility is related and may be the cause of the impaired postprandial FGF19 response observed in these patients. A schematic overview of our major finding is depicted in Fig. 5.

A previous study in humans demonstrated that low circulating FGF19 in the fasted state is associated with bile salt toxicity–related liver injury.[19] Data from the present study show that fasted serum levels of FGF19 in healthy controls and ICU patients are comparable, although ICU patients show elevated fasted bile salt levels. Similar to previous reports in critically ill patients, fasted bile salt levels were higher in ICU patients in the present cohort.[21,22] Elevated bile salt levels in ICU patients may be explained by altered hepatic expression of bile salt transporters.[21]

Enteric passage of the bile salt pool is critical for activation of intestinal FXR and subsequent production of FGF19 in the ileum. Thus far, few studies addressed the nutrient-stimulated FGF19 response in humans.[23,24] Similar to our earlier observations, bile salt concentrations peaked at 2 hours and FGF19 levels >3 hours after meal ingestion.[23] In addition, we observed that gallbladder emptying preceded the bile salt/FGF19 response, indicating that enteral delivery of biliary bile salts induces FGF19 production. ICU

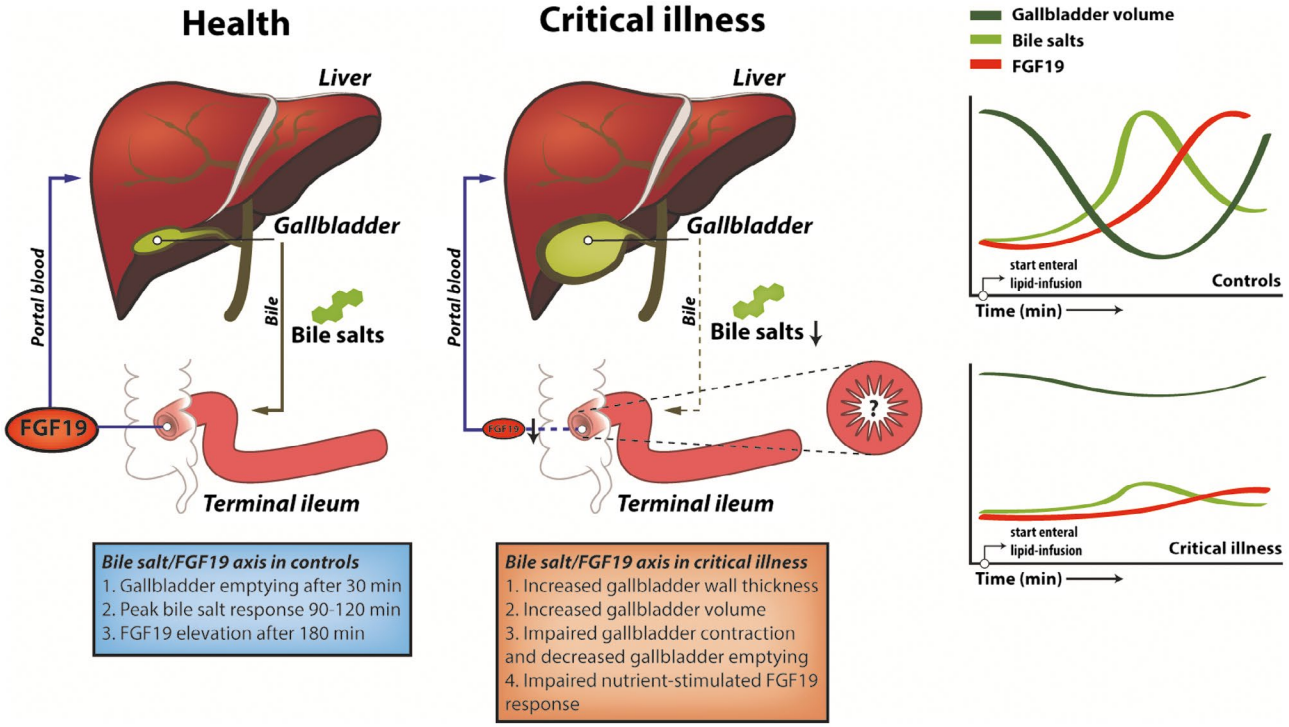

FIG. 5. Schematic overview of the postprandial bile salt/FGF19 axis in healthy participants and critically ill patients.

patients with absent or low gallbladder emptying had low FGF19 AUC values in the postprandial phase.

Remarkably, the (post)prandial FGF19 response was virtually absent in ICU patients. The abrogated FGF19 response in ICU patients occurred despite an apparently normal postprandial plasma bile salt response, although the AUC was lower compared with healthy controls. The lack of correlation between the bile salt and FGF19 response could be explained by gallbladder dysmotility. Despite an apparently normal plasma bile salt response, gallbladder dysmotility could contribute to reduced enteral bile salt delivery. Note that gallbladder volume at 30 minutes correlated negatively with the postprandial response of FGF19, indicating that high residual gallbladder volume (due to impaired emptying) is associated with low induction of FGF19 levels in the postprandial phase. Catecholamines inhibit contraction of the gallbladder by activating adrenoreceptors located in the biliary epithelium; therefore, administration of norepinephrine could contribute to impaired gallbladder emptying. Indeed, patients receiving norepinephrine had a virtually absent postprandial FGF19 response, and postprandial AUC values were significantly lower compared with ICU patients not treated with norepinephrine. However, given the systemic effects of norepinephrine and the more severe clinical condition of ICU patients receiving this drug, these results need to be interpreted with caution. Nonetheless, gallbladder dysmotility could be a contributing factor to the impaired postprandial FGF19 response. Note that additional analysis indicated that systemic inflammation, here assessed by circulating levels of IL6 at baseline, had no effect on FGF19 levels at baseline or the FGF19 response following lipid infusion (Supporting Fig. S4). This suggests that the intestinal FXR/FGF19 axis is not negatively affected by an acute phase response.

What other etiological factors, apart from gallbladder dysmotility, could underlie the divergent FGF19 response in ICU patients compared with healthy controls? Intestinal dysmotility is a frequent problem observed in ICU patients.[28] Hence, diminished propagation of anterograde pressure waves in the biliary-digestive system could lead to slow delivery of bile salts to the distal part of the small intestine.[29] Furthermore, intestinal inflammation is related to low ileal *FXR* expression and therefore low FGF19

production, in pediatric intestinal failure.[30] In critical illness, bile salt malabsorption likely caused by inflammation of the intestinal epithelium could contribute to reduced FXR activation. Although intestinal inflammation was not assessed in our study, baseline values of plasma FGF19 in controls and ICU patients showed no dissimilarities. Finally, it could also be theorized that increased critical illness–associated cell death at the intestinal level could contribute to an impaired FXR function or nuclear localization.[31] Further studies are needed to investigate the etiology underlying the impaired postprandial FGF19 response.

A key function of FGF19 is regulation of hepatic bile salt synthesis, which can be assessed by measuring systemic levels of an intermediate in the bile salt synthetic pathway, *viz*., C4. Changes in C4 levels can be used as a functional readout of FGF19 activity.[32] Note that FGF19 and C4 levels at baseline correlated across all subjects in the present study ($\rho = -0.58$, $P = 0.001$) (data not shown). Postprandial changes in C4 were not apparent in either group, which is likely related to the relatively short time frame of the intervention. In an earlier study, we demonstrated that C4 levels started to decline approximately 1 hour after the postprandial FGF19 peak.[21] It is conceivable that FGF19 levels have not yet peaked at 3 hours after start of the lipid infusion. Baseline C4 levels were comparable in ICU patients and healthy controls (Table 1). At present, it is unclear whether the postprandial FGF19 response in ICU patients is only delayed or is impaired in the long run. Similar C4 levels at baseline suggest that homeostatic control of bile salt synthesis is maintained. Studies delineating the metabolic consequences of low postprandial levels of FGF19 ICU patients are needed.

Findings from this study offer research perspectives in care of critically ill patients. Enteral feeding of critically ill patients is still a matter of debate in terms of feeding pattern (bolus vs. continuous feeding), parenteral supply, and timing of feeding.[33] Because FXR responds to nutrients in the intestinal lumen and exerts its metabolic actions in the liver through FGF19, postprandial FGF19 could be used as an enteral nutrient tolerance marker. In fact, FGF19 is produced in the most distal part of the small intestine, whereas other intestinal hormones (e.g., PYY and GLP1/2) are secreted by entero-endocrine L cells located more proximally in the small intestine. Therefore, considering that the digestive motility of the duodenum is often normal in ICU patients, assessment of postprandial FGF19 levels may be a better alternative to evaluate functional nutritional handling.

In conclusion, we observed an impaired postprandial response of FGF19 in critically ill patients. Findings demonstrate that gallbladder dysmotility is associated with the abrogated response. Further studies are needed to study the exact etiology and long-term metabolic consequences of reduced postprandial levels of FGF19 in critical illness. Finally, use of postprandial FGF19 as a marker for enteral nutritional handling warrants further research.

REFERENCES

1) Deane A, Chapman MJ, Fraser RJ, Horowitz M. Bench-to-bedside review: the gut as an endocrine organ in the critically ill. Crit Care 2010;14:228.
2) Schaap FG, Trauner M, Jansen PL. Bile acid receptors as targets for drug development. Nat Rev Gastroenterol Hepatol 2014;11:55-67.
3) Plummer MP, Kar P, Cousins CE, Hausken T, Lange K, Chapman MJ, et al. Critical illness is associated with impaired gallbladder emptying as assessed by 3D ultrasound. Crit Care Med 2016;44:e790-e796.
4) **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.
5) Inagaki T, Choi M, Moschetta A, Peng L, 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.
6) Song KH, Li T, Owsley E, Strom S, Chiang JY. Bile acids activate fibroblast growth factor 19 signaling in human hepatocytes to inhibit cholesterol 7alpha-hydroxylase gene expression. Hepatology 2009;49:297-305.
7) Morton GJ, Matsen ME, Bracy DP, Meek TH, Nguyen HT, Stefanovski D, et al. FGF19 action in the brain induces insulin-independent glucose lowering. J Clin Invest 2013;123:4799-4808.
8) Lin BC, Wang M, Blackmore C, Desnoyers LR. Liver-specific activities of FGF19 require Klotho beta. J Biol Chem 2007;282:27277-27284.
9) Potthoff MJ, Boney-Montoya J, Choi M, He T, Sunny NE, Satapati S, et al. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1alpha pathway. Cell Metab 2011;13:729-738.
10) Kir S, Beddow SA, Samuel VT, Miller P, Previs SF, Suino-Powell K, et al. FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. Science 2011;331:1621-1624.
11) Choi M, Moschetta A, Bookout AL, Peng L, Umetani M, Holmstrom SR, et al. Identification of a hormonal basis for gallbladder filling. Nat Med 2006;12:1253-1255.
12) Benoit B, Meugnier E, Castelli M, Chanon S, Vieille-Marchiset A, Durand C, et al. Fibroblast growth factor 19 regulates skeletal muscle mass and ameliorates muscle wasting in mice. Nat Med 2017;23:990-996.

13) Oduyebo I, Camilleri M, Nelson AD, Khemani D, Nord SL, Busciglio I, et al. Effects of NGM282, an FGF19 variant, on colonic transit and bowel function in functional constipation: a randomized phase 2 trial. Am J Gastroenterol 2018;113:725-734.
14) Luo J, Ko B, Elliott M, Zhou M, Linhout DA, Phung V, et al. A nontumorigenic variant of FGF19 treats cholestatic liver diseases. Sci Transl Med 2014;6:247ra100.
15) 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.
16) 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.
17) 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.
18) Wojcik M, Janus D, Dolezal-Oltarzewska K, Kalicka-Kasperczyk A, Poplawska K, Drozdz D, et al. A decrease in fasting FGF19 levels is associated with the development of non-alcoholic fatty liver disease in obese adolescents. J Pediatr Endocrinol Metab 2012;25:1089-1093.
19) Mutanen A, Lohi J, Heikkila 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.
20) Walters JR, Tasleem AM, Omer OS, Brydon WG, Dew T, le Roux CW. A new mechanism for bile acid diarrhea: defective feedback inhibition of bile acid biosynthesis. Clin Gastroenterol Hepatol 2009;7:1189-1194.
21) Vanwijngaerden YM, Wauters J, Langouche L, Vander Perre S, Liddle C, Coulter S, et al. Critical illness evokes elevated circulating bile acids related to altered hepatic transporter and nuclear receptor expression. Hepatology 2011;54:1741-1752.
22) **Horvatits T**, **Drolz A**, Rutter K, Roedl K, Langouche L, Van den Berghe G, et al. Circulating bile acids predict outcome in critically ill patients. Ann Intensive Care 2017;7:48.
23) **Schreuder TC**, **Marsman HA**, Lenicek M, van Werven JR, Nederveen AJ, Jansen PL, et al. The hepatic response to FGF19 is impaired in patients with nonalcoholic fatty liver disease and insulin resistance. Am J Physiol Gastrointest Liver Physiol 2010;298:G440-G445.
24) Lundasen T, Galman C, Angelin B, Rudling M. Circulating intestinal fibroblast growth factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. J Intern Med 2006;260:530-536.
25) Fink-Bennett D, DeRidder P, Kolozsi WZ, Gordon R, Jaros R. Cholecystokinin cholescintigraphy: detection of abnormal gallbladder motor function in patients with chronic acalculous gallbladder disease. J Nucl Med 1991;32:1695-1699.
26) Schaap FG, van der Gaag NA, Gouma DJ, Jansen PL. 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.
27) Lenicek M, Vecka M, Zizalova K, Vitek L. Comparison of simple extraction procedures in liquid chromatography-mass spectrometry based determination of serum 7alpha-hydroxy-4-cholesten-3-one, a surrogate marker of bile acid synthesis. J Chromatogr B Analyt Technol Biomed Life Sci 2016;1033-1034:317-320.
28) Fruhwald S, Holzer P, Metzler H. Intestinal motility disturbances in intensive care patients pathogenesis and clinical impact. Intensive Care Med 2007;33:36-44.
29) Chapman M, Fraser R, Vozzo R, Bryant L, Tam W, Nguyen N, et al. Antro-pyloro-duodenal motor responses to gastric and duodenal nutrient in critically ill patients. Gut 2005;54:1384-1390.
30) Xiao YT, Cao Y, Zhou KJ, Lu LN, Cai W. Altered systemic bile acid homeostasis contributes to liver disease in pediatric patients with intestinal failure. Sci Rep 2016;6:39264.
31) Yasuhara S, Asai A, Sahani ND, Martyn JA. Mitochondria, endoplasmic reticulum, and alternative pathways of cell death in critical illness. Crit Care Med 2007;35(Suppl. 9):S488-S495.
32) Walters JR, Johnston IM, Nolan JD, Vassie C, Pruzanski ME, Shapiro DA. The response of patients with bile acid diarrhoea to the farnesoid X receptor agonist obeticholic acid. Aliment Pharmacol Ther 2015;41:54-64.
33) Marik PE. Feeding critically ill patients the right 'whey': thinking outside of the box. A personal view. Ann Intensive Care 2015;5:51.

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.30629/suppinfo.