

RESEARCH

Open Access



Immunosuppressive effects of circulating bile acids in human endotoxemia and septic shock: patients with liver failure are at risk

Julia Leonhardt^{1,2*}, Mirrin J. Dorresteijn^{3,4}, Sophie Neugebauer⁵, Diana Mihaylov⁵, Julia Kunze⁵, Ignacio Rubio^{1,2}, Frank-Stephan Hohberger⁶, Silke Leonhardt⁷, Michael Kiehntopf^{2,5}, Klaus Stahl⁸, Christian Bode⁹, Sascha David^{10,11}, Frank A. D. T. G. Wagener¹², Peter Pickkers³ and Michael Bauer^{1,2}

Abstract

Background Sepsis-induced immunosuppression is a frequent cause of opportunistic infections and death in critically ill patients. A better understanding of the underlying mechanisms is needed to develop targeted therapies. Circulating bile acids with immunosuppressive effects were recently identified in critically ill patients. These bile acids activate the monocyte G-protein coupled receptor TGR5, thereby inducing profound innate immune dysfunction. Whether these mechanisms contribute to immunosuppression and disease severity in sepsis is unknown. The aim of this study was to determine if immunosuppressive bile acids are present in endotoxemia and septic shock and, if so, which patients are particularly at risk.

Methods To induce experimental endotoxemia in humans, ten healthy volunteers received 2 ng/kg *E. coli* lipopolysaccharide (LPS). Circulating bile acids were profiled before and after LPS administration. Furthermore, 48 patients with early (shock onset within < 24 h) and severe septic shock (norepinephrine dose > 0.4 µg/kg/min) and 48 healthy age- and sex-matched controls were analyzed for circulating bile acids. To screen for immunosuppressive effects of circulating bile acids, the capability to induce TGR5 activation was computed for each individual bile acid profile by a recently published formula.

Results Although experimental endotoxemia as well as septic shock led to significant increases in total bile acids compared to controls, this increase was mild in most cases. By contrast, there was a marked and significant increase in circulating bile acids in septic shock patients with severe liver failure compared to healthy controls (61.8 µmol/L vs. 2.8 µmol/L, $p=0.0016$). Circulating bile acids in these patients were capable to induce immunosuppression, as indicated by a significant increase in TGR5 activation by circulating bile acids (20.4% in severe liver failure vs. 2.8% in healthy controls, $p=0.0139$).

Conclusions Circulating bile acids capable of inducing immunosuppression are present in septic shock patients with severe liver failure. Future studies should examine whether modulation of bile acid metabolism can improve the clinical course and outcome of sepsis in these patients.

Keywords Cholestasis, TGR5, GPBAR1, Lipopolysaccharide, LPS, Monocytes, Endotoxin tolerance, Endotoxemia, Immunosuppression

*Correspondence:

Julia Leonhardt

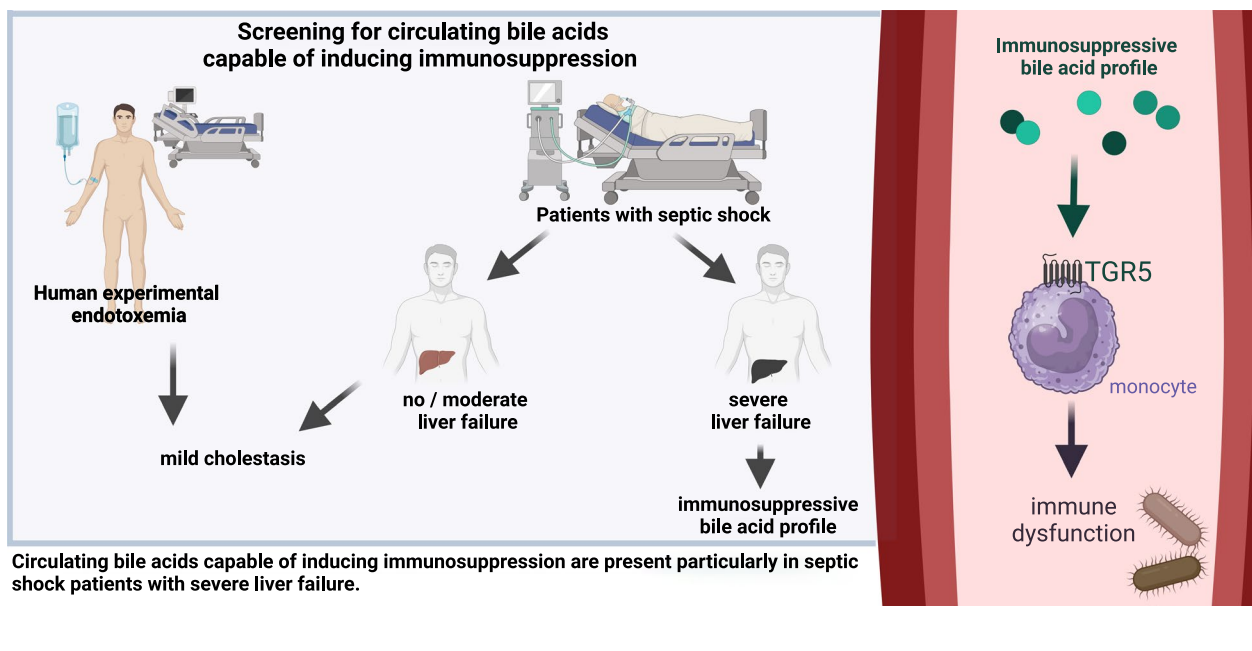
julia.leonhardt@med.uni-jena.de

Full list of author information is available at the end of the article



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Graphical abstract



Background

For many years, bile acids secreted by the liver were thought to function only as emulsifiers of dietary fats. However, the discovery of bile acid receptors has revolutionized our understanding of the physiology of bile acids [1, 2]. Increasing evidence suggests that bile acids also function as signaling molecules or can even be understood as steroid hormones. Bile acid receptors such as the Farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor 1 (GPBAR1, also known as TGR5) regulate metabolism and immunity, respectively [1–4].

The immunomodulatory TGR5 is expressed by monocytes and macrophages [1, 5]. These innate immune cells lose their ability to phagocytose and produce pro-inflammatory cytokines when TGR5 is activated [1, 6, 7]. Apart from monocytes, other leukocytes show little to no expression of bile acid receptors [5, 8]. Activation of TGR5 in monocytes is dependent on the exact bile acid composition and quantity. Generally speaking, secondary bile acids are more potent TGR5 agonists than primary bile acids [1, 9]. For example, tauro lithocholic acid (a secondary bile acid) is 20- to 200 times more potent than cholic acid (a primary bile acid) [1, 7].

Physiological bile acid compositions that activate TGR5 are found in the intestine but not in the blood. In cholestasis, however, the bile acid flow is disrupted

leading to the retention and spill-over of bile acids into the blood. A recent study by us showed that increased circulating bile acids in liver failure patients activate TGR5, leading to significant immune dysfunction. The study detected circulating immunosuppressive bile acids in patients with acute-on-chronic liver failure, acute liver failure and liver graft failure [7]. However, increased circulating bile acids are frequently detected in critically ill patients, particularly in those with septic shock [10, 11]. Whether the bile acid compositions that appear in patients with septic shock can activate TGR5 and thus contribute to sepsis-induced immunosuppression has yet to be evaluated.

In animal models, endotoxins (such as bacterial lipopolysaccharide) and the subsequent release of inflammatory cytokines (particularly IL-6 and TNF α) lead to the disruption of hepatocellular bile acid excretion and increased circulating bile acids [12–14]. Likewise, it is assumed that endotoxemia, and subsequent cytokine storm, are the drivers of increased circulating bile acids in human sepsis.

This study was therefore designed to determine whether the composition of circulating bile acids in human endotoxemia and septic shock activates the immunosuppressive receptor TGR5 and, if so, which patients are at risk.

Patients and methods

Sepsis patients and healthy controls

To study the effect of endotoxemia on bile acid profiles, ten healthy male non-smoking volunteers were recruited and experimental human endotoxemia was conducted. Briefly, subjects were admitted to the Research Intensive Care Unit of the Radboud University Medical Centre, Nijmegen, the Netherlands. Cannulation of the brachial artery and the antecubital vein was performed for invasive blood pressure monitoring and intravenous fluid or drug administration, respectively. After fluid loading (1.5 L crystalloid intravenously), continuous infusion of 150 mL/h crystalloid was initiated with continuous monitoring of vital signs, including body temperature, which was measured using an infrared tympanic thermometer. Purified lipopolysaccharide (LPS, US Standard Reference Endotoxin *E. coli* O:113, obtained from the Pharmaceutical Development Section of the National Institutes of Health (Bethesda, MD, USA)) was administered at a dose of 2 ng/kg body weight. At the indicated times, peripheral blood samples were collected for analysis of bile acid profiles, cytokines, C-reactive protein (CRP) and total bilirubin. Circulating cytokines were measured using a multiplex assay according to the manufacturer's instructions (Bio-Plex, Bio-Rad Laboratories, Hercules, CA, USA).

To study the effect of bile acid profiles in septic shock, serum samples from 48 patients enrolled in two related clinical trials investigating the therapeutic effect of total plasma exchange were collected [15, 16]. To exclude treatment effects, all blood samples for our study were taken prior to total plasma exchange. All laboratory (including total serum bilirubin) and clinical data (including scores) were obtained at the time of blood collection and before plasma exchange. Inclusion criteria for patients with septic shock were: (1) age ≥ 18 years, (2) sepsis according to the SEPSIS-3 definition [17], (3) profound systemic hypotension requiring ≥ 0.4 $\mu\text{g/kg/min}$ norepinephrine despite adequate intravenous fluid resuscitation of at least 30 mL crystalloids per kg bodyweight, and iv) onset of vasopressor use < 24 h prior to screening. Exclusion criteria were pregnancy and breast feeding. Liver failure in patients with septic shock was assessed according to the individual hepatic Sequential Organ Failure Assessment (SOFA) sub-score using serum bilirubin. A hepatic SOFA score of ≥ 3 was defined as liver failure as described before [18, 19].

Furthermore, serum samples from 48 age- and sex-matched healthy volunteers with no signs of infection within the last 14 days and no past medical history of cholestasis or liver diseases were used as controls.

The study and transfer of samples between study centers were approved by the local ethical committees of

all study centers (Hannover Medical School: No. 2786-2015 and No. 8852_MPG_23b_2020, University Hospital Bonn: No. 024/20, Radboud University Medical Center: 2009/047 and NL27052.091.09, University Hospital Jena: 2022-2571-Material and 2022-2606-Material). Informed consent was obtained from all volunteers and patients or their legal representatives before inclusion in the study.

Bile acids

Individual bile acid profiles were assessed using an LC-MS/MS in-house assay. Bile acid standards were purchased from VWR International GmbH (Darmstadt, Germany), TCI Deutschland GmbH (Eschborn, Germany) and Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany) and had a purity of at least of 91%. HPLC-grade methanol, ethanol, ammonium acetate and formic acid were obtained from Carl Roth (Karlsruhe, Germany), Merck KGaA (Darmstadt, Germany) and Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). 270 μL of 85% aqueous methanol was added to 30 μL sample in a Thomson Single Step[®] Filter Vial (PES membrane 0.2 μM , Thomson Instrument Company, California). This solution was mixed for 20 s, centrifuged at $200\times g$ for 1 min, filtered and placed in the autosampler. An Agilent 1200 high performance liquid chromatography system (Agilent Technologies GmbH, Germany) with a CTC-PAL autosampler coupled to an API 4000 Triple Quadrupole mass spectrometer with electrospray ionization source (AB Sciex, Germany) was used for quantification. All chromatographic separations were performed with a reverse-phase Agilent Zorbax Eclipse XDB-C18 (3.5 μm , 100 \times 3 mm) analytical column equipped with a guard column (C18, 4 \times 3 mm; Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of water (A) and methanol (B), both containing 0.012% formic acid and 5 mM ammonium acetate, at a total flow rate of 300 $\mu\text{L/min}$. Total bile acids were calculated as the sum of individual bile acids.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.4.1 (La Jolla, CA, USA) or SPSS (IBM, Chicago, IL, USA). Continuous variables are represented as median and 25–75% interquartile range (IQR). The Wilcoxon signed rank test (nonparametric) or two-tailed, paired Student's t-test (parametric data) was used for comparisons between two matched groups. Kruskal–Wallis one-way analysis of variance on ranks with Dunn's post-hoc test was used to compare the effects of multiple groups. Comparisons between multiple time points were made with one-way repeated measures analysis of variance with Geisser-Greenhouse correction and Dunnett's post-hoc test. Correlations were calculated using Spearman

coefficients. For all comparisons, $p < 0.05$ was considered significant. The graphical abstract and figures were created with [BioRender.com](https://www.biorender.com).

Results

Experimental endotoxemia induces sepsis-like symptoms and an increase in markers of inflammation and cholestatic liver dysfunction

To study the effects of endotoxemia on bile acids, ten healthy volunteers were admitted to our research

intensive care unit and received *E. coli* lipopolysaccharide (LPS) intravenously (Fig. 1A). Baseline characteristics (age and sex) of healthy volunteers can be found in Table 1. Administration of intravenous LPS resulted in sepsis-like symptoms (e.g., shivering and muscle pain) in all subjects. Furthermore, we observed a significant change in systemic hemodynamics (Table 2), C-reactive protein (CRP, Fig. 1B) and circulating cytokines (Fig. 1C).

In sepsis and critical illness, circulating bilirubin and bile acids are well established markers of cholestatic

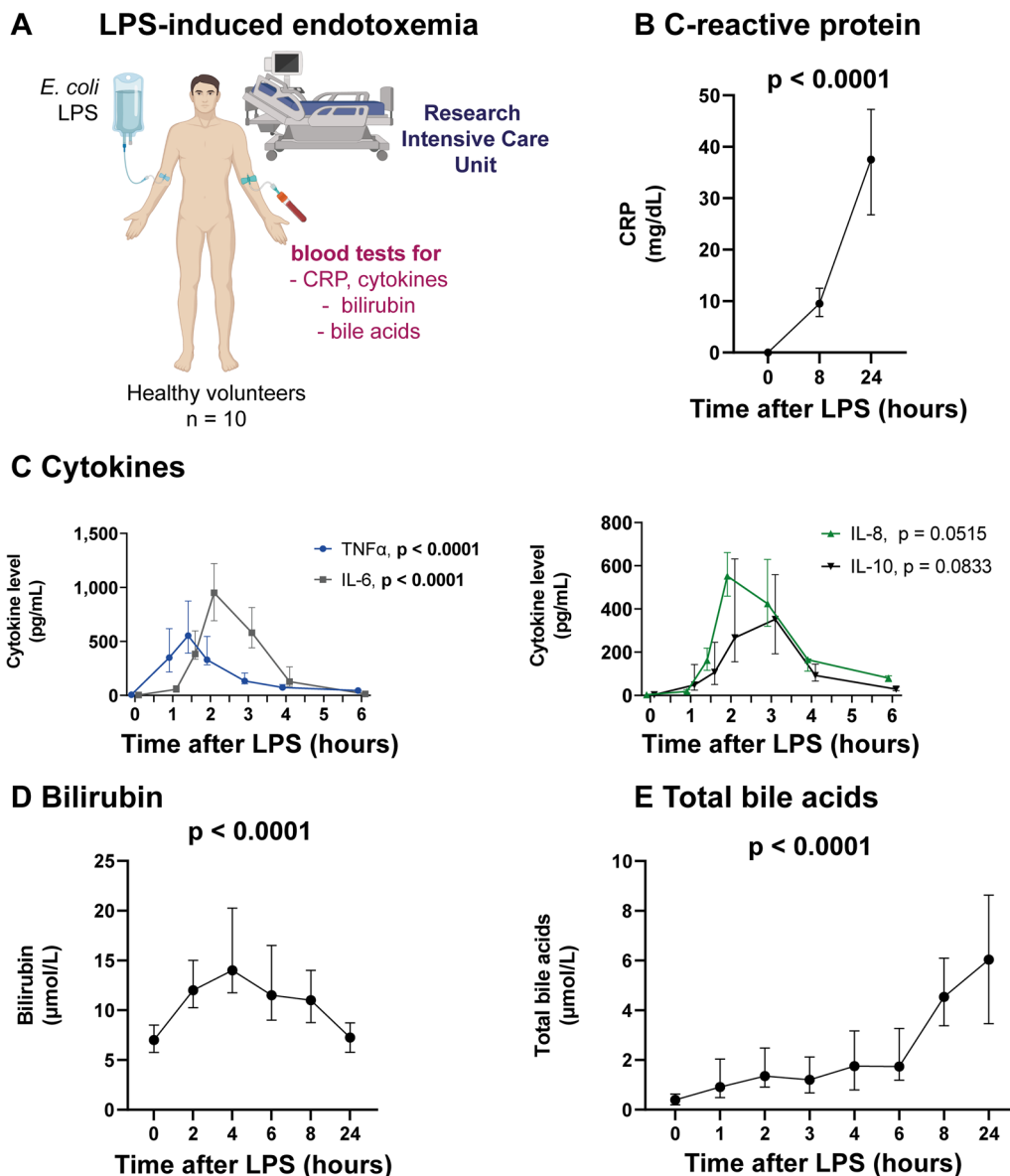


Fig. 1 Experimental endotoxemia induces sepsis-like symptoms and an increase in markers of inflammation and cholestatic liver dysfunction. **A–E** Ten healthy volunteers received 2 ng *E. coli* LPS per kg body weight. **A** The figure was created with BioRender.com. **B–E** C-reactive protein, cytokines, bilirubin and total bile acids were assessed. Graphs show median and IQR. One-way repeated measures analysis of variance. *CRP* C-reactive protein, *LPS* lipopolysaccharide

Table 1 Characteristics of healthy volunteers with LPS-induced endotoxemia, patients with septic shock and age- and sex-matched healthy controls

	Healthy volunteers with LPS-induced endotoxemia	Septic shock	Healthy controls	Septic shock versus healthy controls (<i>p</i> value)
<i>n</i> =	10	48	48	
Male (<i>n</i> , %)	10 (100)	30 (63)	30 (63)	1.0
Age (years)	22.5 (21.5–25.3)	53.5 (47.0–61.5)	53.5 (45.0–62.5)	1.0
<i>Site of infection (n, %)</i>				
Pneumonia		26 (54)		
Endocarditis		2 (4)		
Soft tissue		4 (8)		
Abdomen		14 (29)		
Other sepsis		2 (4)		
<i>Identified pathogen (n, %)</i>				
Gram-positive bacteria		10 (21)		
Gram-negative bacteria		15 (31)		
Fungal		5 (10)		
Viral		3 (6)		
Mixed		6 (13)		
Non-identified		9 (19)		
SOFA score		17 (14–19)		
NE dose (µg/kg/min)		0.66 (0.46–0.93)		
Lactate (mmol/L)		4.0 (2.7–7.0)		
Bilirubin (µmol/L)		29.0 (13.5–51.8)		
CRP (mg/L)		248 (131–343)		
PCT (µg/L)		19.7 (6.4–53.4)		
WBC (10 ³ /µL)		13.5 (1.7–21.4)		

Continuous variables are expressed as median and IQR

CRP C-reactive protein, LPS lipopolysaccharide, NE norepinephrine, PCT procalcitonin, SOFA Sequential Organ Failure Assessment, WBC white blood cell count. Wilcoxon signed-rank test

Table 2 Changes in vital signs in healthy volunteers receiving LPS

	Before LPS	4 h after LPS	<i>p</i> value
Heart rate (bpm)	69 (63–83)	92 (83–102)	0.0006
MAP (mmHg)	89 (87–101)	77 (74–83)	0.0002
Temperature (°C)	37.0 (36.5–37.2)	37.9 (37.8–38.5)	0.0018

Significant *p* values are in bold

Data are expressed as median and IQR

Bpm beats per minute, *MAP* mean arterial pressure

Two-tailed, paired Student's *t* test

liver dysfunction [20, 21]. Healthy volunteers developed a mild increase in these markers after onset of experimental endotoxemia. Bilirubin levels peaked after 4 h (Fig. 1D, 14.0 µmol/L vs. 7.0 µmol/L at

baseline, *p* = 0.0003). Total bile acids rose steadily over time (Fig. 1E). The most significant increase in total bile acid levels was found 8 h after LPS infusion (4.5 µmol/L vs. 0.4 µmol/L before LPS infusion).

Experimental endotoxemia induces changes in bile acid profiles

We further analyzed the increase in total bile acids by assessing the individual bile acid profiles of our healthy volunteers with experimental endotoxemia. While unconjugated bile acids remained unchanged, most conjugated bile acids were significantly increased (Fig. 2B, C). Conjugated primary bile acids (i.e., GCA, TCA, GCDCA and TCDCA) were the predominant circulating bile acids, accounting for 71% of total bile acids (3.2 µmol/L of 4.5 µmol/L total bile acids 8 h after LPS infusion, Fig. 2C).

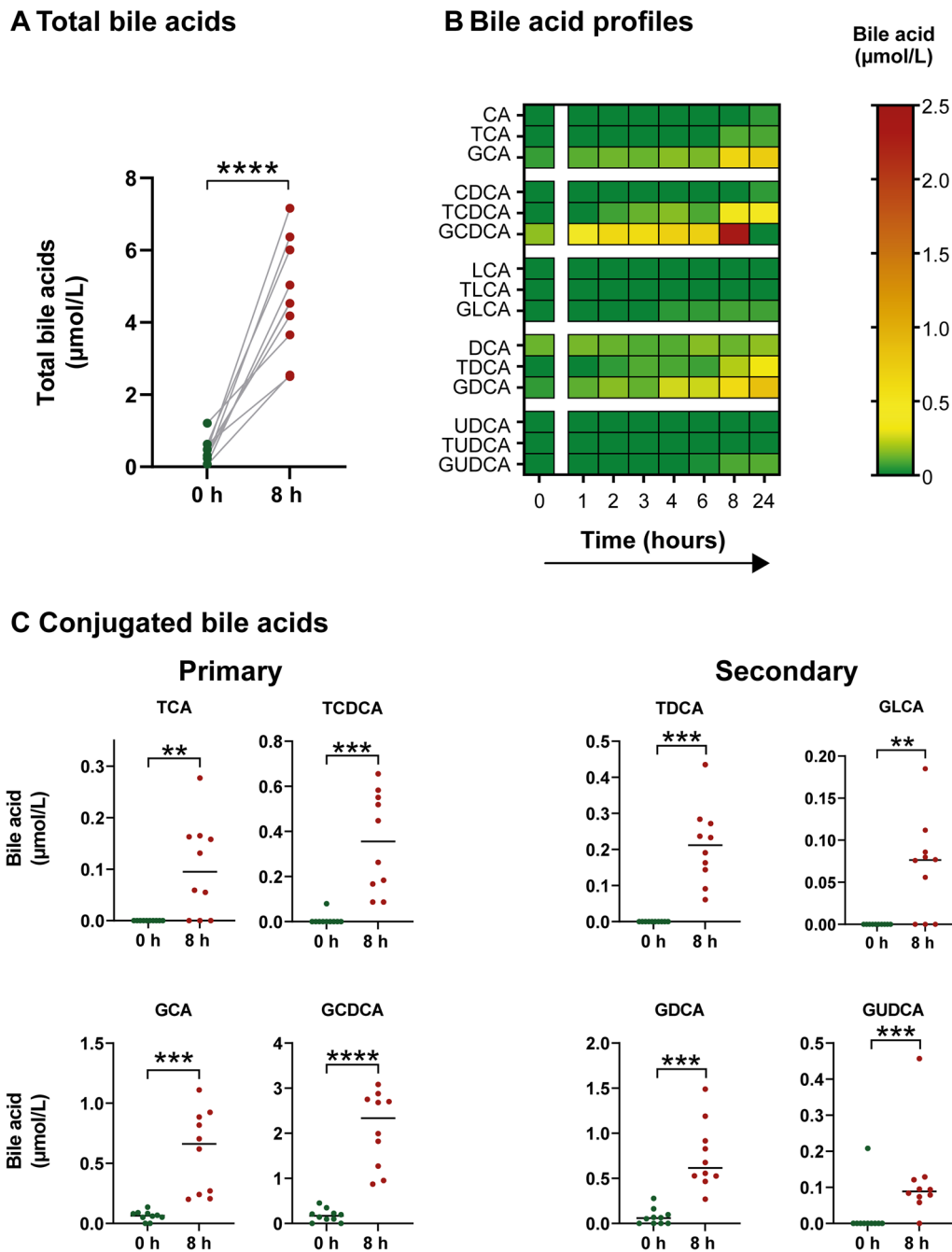


Fig. 2 Experimental endotoxemia induces changes in bile acid profiles. **A–C** Total bile acids and bile acid profiles of ten healthy volunteers were assessed before and after IV administration of 2 ng *E. coli* LPS per kg body weight. **A** Total bile acids before and 8 h after LPS infusion. **B** Heat map of all measured bile acids at different time points. **C** Graphs of all bile acids with significant differences before and 8 h after LPS infusion. Each dot represents the value for a single subject at a given time point. The horizontal line represents the median. Two-tailed, paired Student's *t* test. ***p* < .01, ****p* < .001, *****p* < .0001. CA cholic acid; CDCA chenodeoxycholic acid; DCA deoxycholic acid; GCA glycocholic acid; GCDCA glycochenodeoxycholic acid; GDCA glycodeoxycholic acid; GLCA glycolithocholic acid; GUDCA glycoursoxycholic acid; LCA lithocholic acid; TCA taurocholic acid; TCDCA taurochenodeoxycholic acid; TDCA taurodeoxycholic acid; TLCA tauroolithocholic acid; TUDCA tauroursoxycholic acid; UDCA ursodeoxycholic acid

Experimental endotoxemia increases immunosuppressive bile acids

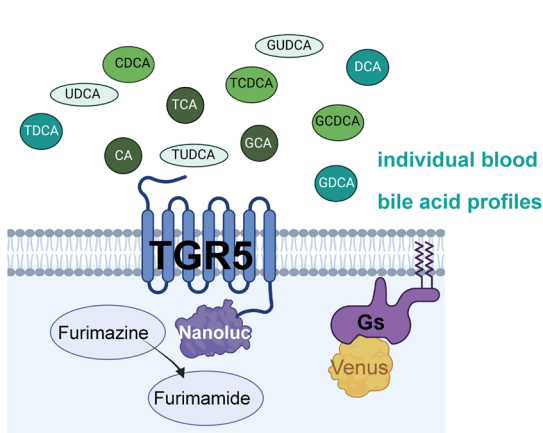
To investigate, whether the complex compositions of circulating bile acids found in experimental endotoxemia could indeed activate TGR5, we calculated TGR5 activity using the NanoBRET-based formula described previously (Fig. 3A) [7]. The formula takes into account the concentration of each individual bile acid and its respective potency to activate TGR5. A bile acid profile significantly activating TGR5 was found in experimental endotoxemia (Fig. 3B). However, in line with the weak increases in bilirubin and total bile acids that we observed, TGR5 activation was mild: 4.5% 8 h after vs. 0.5% before LPS infusion ($p=0.0003$).

Circulating bile acids are significantly increased in septic shock

We further assessed whether the effects observed in experimental endotoxemia would also be detectable in critically ill patients with sepsis. Septic patients admitted to our intensive care units were screened for early (shock onset within <24 h) and severe septic shock (norepinephrine dose >0.4 µg/kg/min). Forty-eight patients were identified, and a group of 48 age- and sex-matched controls was selected accordingly (Fig. 4A, Table 1).

Patients with septic shock showed significant differences in bile acid quantity and composition compared to healthy controls (Table 3 and Fig. 4). Their total bile acid

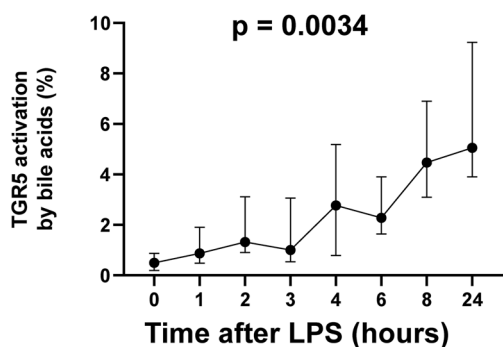
A NanoBRET-based formula used to calculate TGR5 activation



$$T = \sum_{b=1}^{15} (c_b \times P_b \times k)$$

- T Calculated TGR5 activity (%)
- b Respective bile acid in mixture
- c_b Concentration of respective bile acid (µmol/L)
- P_b TGR5 potency of respective bile acid
- k 0.085 (% x L/µmol)

B TGR5 activation by circulating bile acids (time course)



C TGR5 activation by circulating bile acids (8 hours)

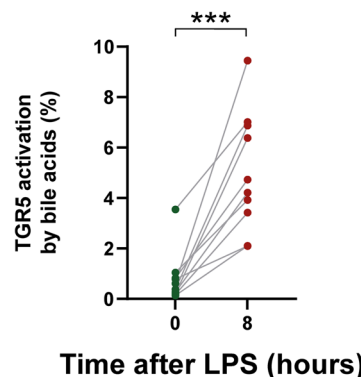
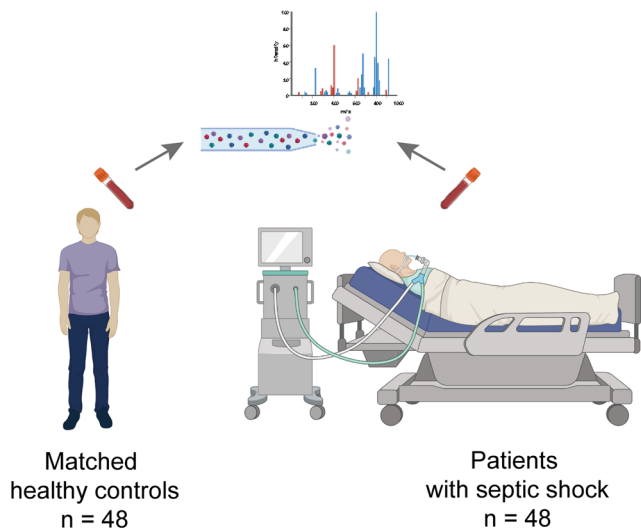
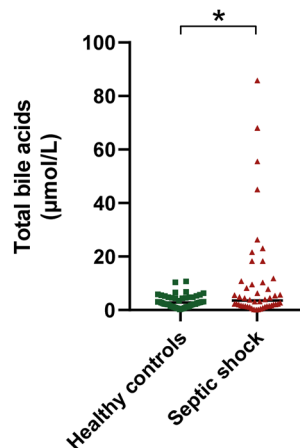


Fig. 3 Experimental endotoxemia increases immunosuppressive bile acids. **A–C** Bile acid profiles of ten healthy volunteers were assessed before and after IV administration of 2 ng *E. coli* LPS per kg body weight. TGR5 activation by the individual bile acid profile was calculated as described previously (7). **A** NanoBRET-based formula used to calculate TGR5 activity. The figure was created with BioRender.com. **B** TGR5 activation induced by circulating bile acids 8 h after LPS infusion. Graph shows mean and IQR. One-way repeated measures analysis of variance. **C** TGR5 activation induced by circulating bile acids 8 h after LPS administration. Each dot represents the value for a single subject at a given time point. Two-tailed, paired Student’s *t* test. *** $p < .001$. LPS lipopolysaccharide

A Collection of blood for bile acid profiles



B Total bile acids



C Conjugated primary bile acids

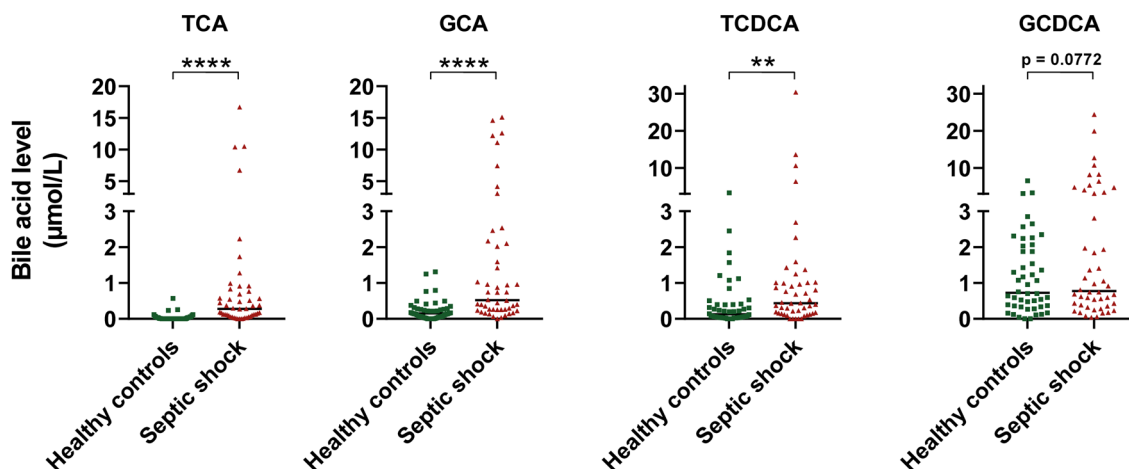


Fig. 4 Bile acid profiles of healthy controls and patients with septic shock. **A**, Bile acid profiles of 48 patients with septic shock and 48 age- and sex-matched controls were measured by mass spectrometry. The figure was created with BioRender.com. **B**, Total bile acids were significantly increased in patients with septic shock. **C**, The increase of total bile acids was due to a significant increase in primary conjugated bile acids. Each dot represents the value for a single subject at a given time point. The horizontal line represents the median. Wilcoxon signed-rank test. * $p < .05$, ** $p < .01$, **** $p < .0001$. GCA glycocholic acid, GCDCA glycochenodeoxycholic acid, TCA taurocholic acid, TCDCA taurochenodeoxycholic acid

level was significantly higher than that of healthy controls (Fig. 4B, 3.51 $\mu\text{mol/L}$ vs. 2.78 $\mu\text{mol/L}$, $p = 0.0139$). This increase was due to significantly elevated conjugated primary bile acids (e.g., taurocholic acid, TCA and glycocholic acid, GCA, Fig. 4C). By contrast, secondary bile acids, which were present in low quantities in healthy controls, were even lower in patients with septic shock (Table 3).

Patients with severe liver failure show a marked increase in circulating bile acids

Most patients with septic shock had shown a mild increase in circulating bile acids similar to that of human subjects with experimental endotoxemia. However, the patient population was heterogeneous. Grubbs test detected four outliers with markedly increased circulating bile acids (Fig. 5A). In septic shock patients, increased

Table 3 Detailed bile acid profiles of healthy controls and patients with septic shock

	Septic shock	Healthy controls	Septic shock versus healthy controls (<i>p</i> -value)
Total bile acids (μmol/L)	3.51 (1.56–10.14)	2.78 (1.38–4.81)	0.0139
<i>Primary bile acids (μmol/L)</i>			
CA	0.02 (0.00–0.05)	0.03 (0.01–0.11)	0.5535
TCA	0.28 (0.07–0.75)	0.00 (0.00–0.04)	<0.0001
GCA	0.52 (0.26–2.08)	0.16 (0.06–0.29)	<0.0001
CDCA	0.01 (0.00–0.11)	0.11 (0.00–0.24)	0.3412
TCDCa	0.43 (0.15–1.01)	0.13 (0.04–0.40)	0.0067
GCDCA	0.78 (0.36–3.32)	0.73 (0.34–1.99)	0.0772
<i>Secondary bile acids (μmol/L)</i>			
LCA	0.00 (0.00–0.00)	0.03 (0.00–0.04)	<0.0001
TLCA	0.00 (0.00–0.00)	0.00 (0.00–0.00)	0.0625
GLCA	0.00 (0.00–0.00)	0.01 (0.00–0.03)	<0.0001
DCA	0.00 (0.00–0.40)	0.20 (0.04–0.37)	<0.0001
TDCA	0.00 (0.00–0.09)	0.06 (0.00–0.16)	0.3721
GDCA	0.06 (0.00–0.22)	0.22 (0.09–0.53)	0.0008
UDCA	0.00 (0.00–0.04)	0.04 (0.02–0.13)	0.0103
TUDCA	0.00 (0.00–0.01)	0.00 (0.00–0.00)	0.0484
GUDCA	0.07 (0.02–0.36)	0.06 (0.01–0.14)	0.0486

Significant *p* values are in bold

Data are expressed as median and IQR

CA cholic acid, CDCA chenodeoxycholic acid, DCA deoxycholic acid, GCA glycocholic acid, GCDCA glycochenodeoxycholic acid, GDCA glycodeoxycholic acid, GLCA glycolithocholic acid, GUDCA glyoursodeoxycholic acid, LCA lithocholic acid, TCA taurocholic acid, TCDCa taurochenodeoxycholic acid, TDCA taurodeoxycholic acid, TLCA tauroolithocholic acid, TUDCA taoursodeoxycholic acid, UDCA ursodeoxycholic acid. Wilcoxon signed-rank test

levels of circulating bile acids may not only reflect cholestatic liver dysfunction due to endotoxemia and cytokine storm, but also liver failure. Bilirubin is the best-established laboratory parameter for the detection and scoring of liver failure in septic patients [20, 22]. In line with this, total bile acids closely correlated with bilirubin levels in our patients (Fig. 5B). The Sequential Organ Failure Assessment (SOFA) score uses serum bilirubin levels to indicate and discriminate liver failure in sepsis. We therefore grouped our patients according to the presence and severity of liver failure using the hepatic SOFA sub-score (Fig. 5C). Four patients were classified as having severe liver failure. Strikingly, these four patients were identical with the outliers in Fig. 5A. In line with this, patients with severe liver failure showed significantly increased circulating bile acids (Fig. 5D).

The patients' past medical history was also examined. All patients with severe liver failure had advanced pre-existing liver diseases before developing septic shock: two had liver cirrhosis, one had hepatic graft-versus-host

disease (GvHD), and one had acute alcoholic steatohepatitis. By contrast, none of the patients with moderate liver failure and only 6 of the 42 patients (14.3%) without liver failure had a past medical history of liver disease, including liver cirrhosis (*n* = 4), autoimmune hepatitis (*n* = 1), and secondary sclerosing cholangitis (*n* = 1).

Patients with severe liver failure have circulating immunosuppressive bile acids

Although the quantity of total bile acids in septic patients generally was significantly increased, the levels of highly immunosuppressive secondary bile acids were decreased (Table 3). Consequently, the capability of circulating bile acids to induce TGR5 activation was comparable between septic shock patients without liver failure and healthy controls (Fig. 6). By contrast, patients with severe liver failure showed a bile acid profile inducing significant TGR5 activation: 20.4% vs. 1.7% compared to patients without liver failure (*p* = 0.0006) and 20.4% versus 2.8% compared to healthy controls (*p* = 0.0139).

Discussion

This study shows that septic shock patients develop cholestasis with significantly increased bile acids. Two main causes of cholestasis in septic shock patients were analyzed in this study: endotoxemia and liver failure. Endotoxemia with subsequent cytokine storm and systemic inflammation led to a significant but mild increase in circulating bile acids. A similar pattern was found in most patients with septic shock. By contrast, septic shock patients with liver failure showed markedly increased bile acid levels, which were capable of inducing significant TGR5 activation.

The accumulation of circulating bile acids is an early and critical event in sepsis. In animal models, increased levels of circulating bile acids have been detected as early as 1 h after induction of experimental sepsis [23]. In line with this, our data show a significant increase in circulating bile acids within 8 h after LPS infusion in experimental endotoxemia. A significant and early increase in circulating bile acids (within 24 h of onset of vasopressor use) was also observed in our septic shock patients.

Conjugated primary bile acids (GCA, TCA, GCDCA and TCDCa) were the main drivers of bile acid accumulation in experimental endotoxemia and septic shock patients. These findings are consistent with those of previous studies investigating circulating bile acids in sepsis, acute respiratory distress syndrome (ARDS) or critically ill patients [10, 11, 24, 25]. Endotoxemia and subsequent cytokine storm have been reported to disrupt hepatocellular bile acid export. In particular, a marked downregulation of the canalicular bile salt export pump (BSEP) and of the multidrug resistance-associated protein 2 (MRP2)

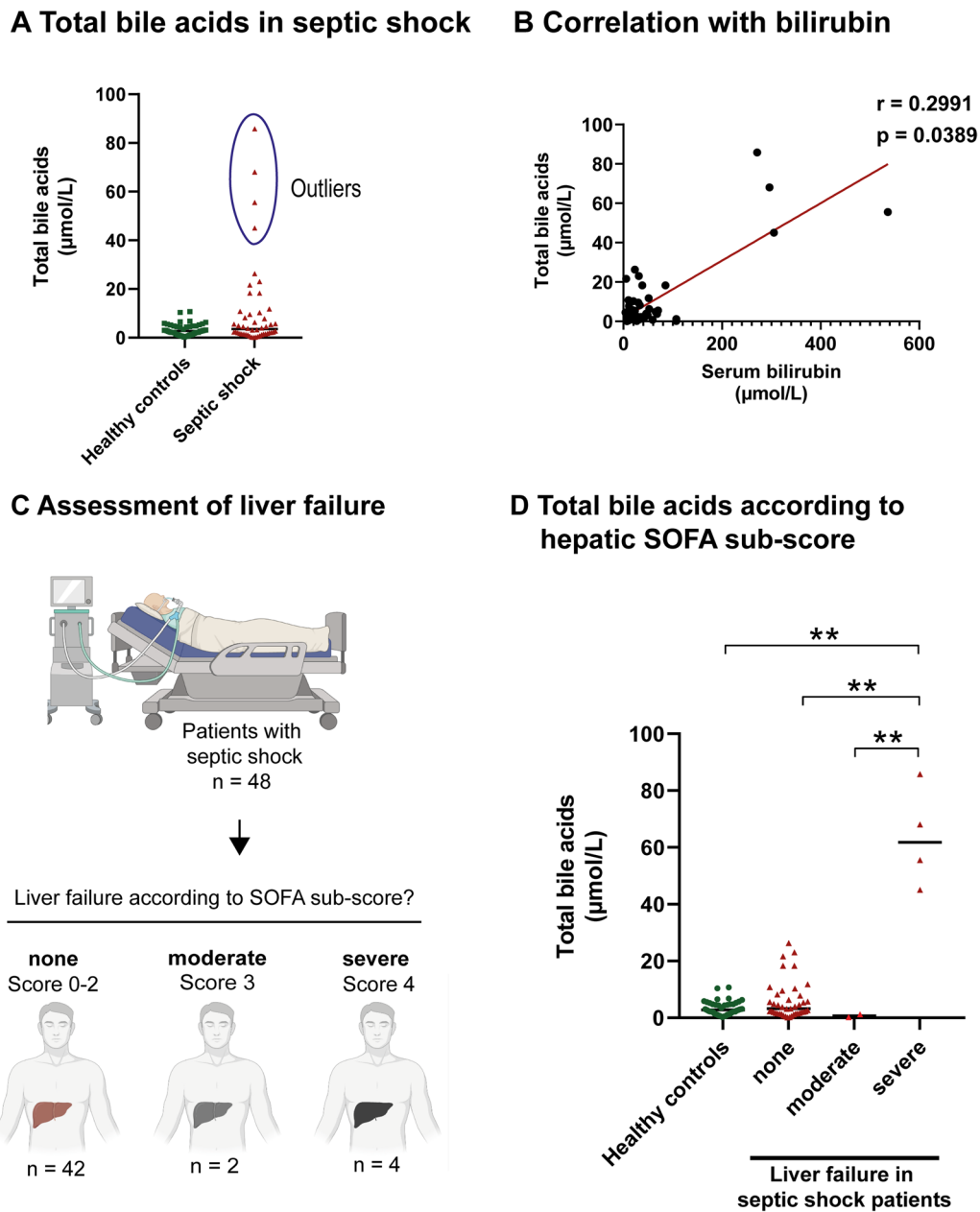


Fig. 5 Total bile acids in septic patients with severe liver failure. **A** Total bile acids in 48 patients with septic shock versus 48 age- and sex-matched controls. Grubbs test identified four outliers in the septic shock group. Each dot represents the value of a single subject, the horizontal line represents the median. **B** Total bile acid levels correlated significantly with serum bilirubin. Values for Spearman’s rank correlation coefficient (*r*) are given. **C** Patients with septic shock were grouped according to the presence and severity of liver failure, as indicated by the hepatic SOFA sub-score. The figure was created with BioRender.com. **D** Total bile acids in patients with and without liver failure. Each dot represents the value of a single subject, the horizontal line represents the median, Kruskal–Wallis one-way analysis of variance. *******p* < .01. *SOFA* Sequential Organ Failure Assessment

has been observed [11–14, 26–29]. However, despite the strong disturbance of bile acid export, hepatocytes are still able to synthesize and conjugate bile acids with glycine and taurine [11]. Consequently, conjugated bile acids that are either enterohepatically recirculated or de novo

synthesized, accumulate in hepatocytes and spill over into the blood.

Interestingly, our data show that the conjugated secondary bile acids (taurine- and glycine-conjugated DCA, LCA and UDCA) were significantly increased in

TGR5 activation by circulating bile acids

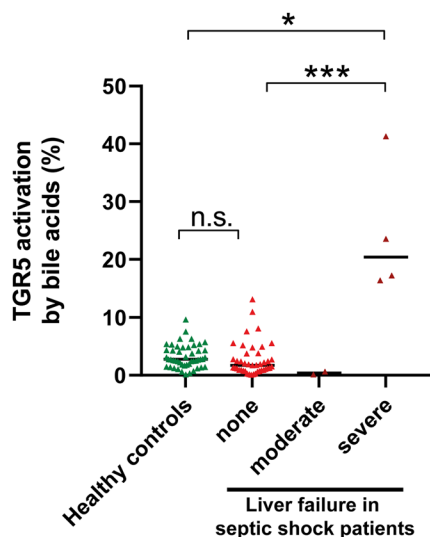


Fig. 6 Patients with severe liver failure have TGR5-activating bile acid profiles. Patients with severe liver failure showed significant extrapolated TGR5 activation by circulating bile acids. Each dot represents the value of a single subject. The horizontal line represents the median, Kruskal–Wallis one-way analysis of variance. *n.s.* not significant, * $p < .05$, *** $p < .001$

experimental endotoxemia but not in septic shock. Secondary bile acids originate from primary bile acids that are excreted into the intestine and transformed by the gut microbiota. Reabsorption and recycling by the enterohepatic circulation raise secondary bile acids to detectable levels in the blood. The disruption of canalicular bile acid export by endotoxemia may consequently lead to increased levels of circulating secondary bile acids. However, unlike healthy volunteers, patients with septic shock were treated with antibiotics. Antibiotic treatments have been shown to reduce gut microbiota and, thus, decrease circulating secondary bile acids by up to 1000-fold [30–32]. Therefore, the marked reduction in secondary bile acids caused by antibiotics could have outweighed the effects of endotoxemia in our patients.

Although TGR5-activating circulating bile acids were found in experimental endotoxemia, this effect was mild (4.5% receptor activation), though significant. Strikingly, TGR5-activating circulating bile acids were absent in septic shock patients without liver failure due to the lack of highly immunosuppressive secondary bile acids. By contrast, circulating bile acids in patients with severe liver failure were capable of activating TGR5 significantly and relevantly (20.4% receptor activation). TGR5 activation has been reported to induce monocyte dysfunction, as characterized by a decrease in the release of tumor necrosis factor α (TNF α), IL-1 β and IL-6 upon LPS stimulation and an unaltered release of anti-inflammatory

IL-10 [1, 7]. This monocyte dysfunction is associated with increased mortality [7].

Although advanced intensive care medicine and early goal-directed therapies have improved survival rates from the primary septic hyper-inflammatory phase, sepsis is still the leading cause of in-hospital death in Western societies [33–35]. The high mortality of sepsis is at least partly due to a secondary anti-inflammatory phase, which is characterized by increased susceptibility to opportunistic infections [36]. This sepsis-induced immunosuppression is typically characterized by dysfunctional monocytes, which show a decreased release of pro-inflammatory cytokines such as tumor necrosis factor α (TNF α), IL-1 β and IL-6 upon stimulation with LPS and an enhanced secretion of anti-inflammatory IL-10 [37, 38]. Strikingly, immunosuppressive bile acids induce an identical immune phenotype in monocytes [1, 7]. Several attempts have been made to boost septic patients' innate immune system with cytokines (e.g., IFN- γ), growth factors (e.g., GM-CSF) or pathogen-associated molecular patterns (β -glucan) [39–42]. However, a specific treatment that prevents septic immunosuppression is still lacking. Therefore, the identification of mechanisms leading to the impairment of monocyte function in sepsis is of paramount importance.

Circulating bile acids closely correlate with mortality in patients with sepsis and critical illness [10, 43, 44]. Indeed, circulating bile acids are better predictors of sepsis-related mortality than liver function parameters, such as bilirubin [10, 43]. Therefore, several authors speculated that bile acids play an active role in the pathogenesis of sepsis [10, 29, 45]. Moreover, studies have shown that HMG-CoA reductase inhibitors (drugs that significantly inhibit cholesterol and, subsequently, bile acid synthesis) reduce mortality in sepsis and infection-related ARDS [46–48]. However, the results of the available studies and meta-analyses of the effects of statins on sepsis are inconsistent [49, 50]. The beneficial effect of statins may at least partially be due to a reduction of circulating immunosuppressive bile acids in a subset of septic patients.

Our study shows for the first time that bile acids capable of inducing immunosuppression are present in a subset of septic patients. Our findings suggest that marked TGR5 activation is restricted to septic patients with liver failure. These patients may benefit from therapeutic approaches reducing circulating bile acids. As mentioned above, HMG-CoA reductase inhibitors such as simvastatin can efficiently reduce bile acid synthesis [51, 52]. Furthermore, the enterohepatic circulation of bile acids can be inhibited by bile acid sequestrants, such as cholestyramine and colestevlam [53–55]. Direct elimination of circulating bile acids can be achieved by albumin dialysis or total plasma exchange [56, 57]. Apart from simply

removing bile acids from the circulation, future therapies might directly target the sepsis-induced disruption of the excretory liver function. Accordingly, we recently showed that liver-specific inhibition of phosphatidylinositol-3-kinase (PI3K) restored both the canalicular architecture of hepatocytes and biliary excretion [58]. However, these therapies may only be appropriate as part of an individualized approach for patients with circulating immunosuppressive bile acids rather than as a “one size fits all” concept for septic patients.

Our study has several limitations. First and foremost, the sample size is too small to draw conclusions about the consequences of circulating immunosuppressive bile acids in patients. Therefore, our results on the occurrence of circulating immunosuppressive bile acids in septic shock were intended to be hypothesis-generating and a useful aid for designing a larger ongoing follow-up study. The follow-up study will thus be appropriate for investigating the consequences of circulating immunosuppressive bile acids, such as mortality or secondary infections, and for evaluating potential therapeutic options. Furthermore, the present study used the SOFA score to screen septic shock patients for the presence of liver failure, as described before [18, 19]. This simple and pragmatic score defines liver failure by serum bilirubin [59] and does not distinguish different forms or causes of liver failure. Moreover, it does not discriminate between patients with and without pre-existing liver diseases. However, the SOFA score is the best-established and best-evaluated method of screening for organ failure in septic patients [17, 22]. In our study, it sufficiently identified all patients at risk for circulating immunosuppressive bile acids.

Conclusions

Septic shock patients with severe liver failure develop cholestasis with a massive increase in circulating bile acids. These circulating bile acids are capable of activating the immunosuppressive bile acid receptor TGR5. Future studies should evaluate the potential of HMG-CoA reductase inhibitors, albumin dialysis and other therapies to reduce immunosuppressive bile acids and their effects on sepsis outcome.

Abbreviations

Bpm	Beats per minute
MAP	Mean arterial pressure
BSEP	Bile salt export pump
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CRP	C-reactive protein
DCA	Deoxycholic acid
GCA	Glycocholic acid
GCDCa	Glycochenodeoxycholic acid

GDCA	Glycodeoxycholic acid
GLCA	Glycolithocholic acid
GPBAR1	G protein-coupled bile acid receptor 1 (aka TGR5)
GUdCA	Glyoursodeoxycholic acid
HMG-CoA	3-Hydroxy-3-methyl-glutaryl-coenzyme A
IL	Interleukin
LCA	Lithocholic acid
LPS	Lipopolysaccharide
MRP2	Multidrug resistance associated protein 2
NanoBRET	Bioluminescence resonance energy transfer using nanoluciferase
NE	Norepinephrine
PCT	Procalcitonin
SOFA	Sequential Organ Failure Assessment
TCA	Taurocholic acid
TCDCa	Taurochenodeoxycholic acid
TDCA	Taurodeoxycholic acid
TGR5	The G protein-coupled bile acid receptor TGR5 (aka GPBAR1)
TLCA	Tauroolithocholic acid
TNF α	Tumor necrosis factor α
TUDCA	Tauroursodeoxycholic acid
UDCA	Ursodeoxycholic acid
WBC	White blood cell count

Acknowledgements

We thank all patients and healthy volunteers who participated in this study and gratefully acknowledge Lisa Wedekind (Institute of Medical Statistics, Computer and Data Sciences, University Hospital Jena), for statistical support.

Author contributions

MB and JL had the original idea for this study. KS, CB, SD, FSH and JL recruited septic shock patients and the respective healthy controls and collected clinical data. MJD, FADTGW and PP recruited healthy volunteers, induced experimental endotoxemia and collected data. SN, DM, JK and MK determined bile acid profiles in blood samples from healthy volunteers, septic shock patients and healthy controls. JL performed the statistical analyses and generated the figures for this publication. JL, MJD, IR, SL, KS, CB, SD, PP and MB interpreted the data. JL and MB wrote the manuscript. All authors read and approved the final manuscript.

Funding

Open Access funding enabled and organized by Projekt DEAL. This study was supported by the German Federal Ministry of Education and Research (BMBF), Photonics Research Germany funding program (FKZ: 13N15716) and is integrated into the Leibniz Center for Photonics in Infection Research (LPI). The LPI was initiated by Leibniz Institute of Photonic Technology (IPHT), Leibniz Institute for Natural Product Research and Infection Biology, University Hospital Jena and Friedrich Schiller University Jena. The LPI is part of the BMBF national roadmap for research infrastructures. Laboratory experiments were supported by Levi-Montalcini Biomedical Sciences Award from the European Society of Intensive Medicine (ESICM) awarded to JL. MJD is the recipient of a ZonMW AGIKO grant (No. 92003507). MB was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) under Germany's Excellence Strategy (EXC 2051: Balance of the Microverse, project number 390713860).

Availability of data and materials

The datasets used and analyzed are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study and transfer of samples between study centers were approved by all local ethical committees (Hannover Medical School: No. 2786-2015 and No. 8852_MPG_23b_2020, University Hospital Bonn: No. 024/20, Radboud University Medical Center: 2009/047 and NL27052.091.09, University Hospital Jena: 2022-2571-Material and 2022-2606-Material). Informed consent was obtained from all volunteers and patients or their legal representatives before inclusion in the study. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Anesthesiology and Intensive Care Medicine, Jena University Hospital, Member of the Leibniz Center for Photonics in Infection Research (LPI), Jena, Germany. ²Center for Sepsis Control and Care (CSCC), Jena University Hospital-Friedrich Schiller University, Jena, Germany. ³Department of Intensive Care Medicine, Radboud University Medical Center, Nijmegen, the Netherlands. ⁴Department of Intensive Care Medicine, Alrijne Hospital, Leiderdorp, the Netherlands. ⁵Institute of Clinical Chemistry and Laboratory Diagnostics and Integrated Biobank Jena, Jena University Hospital, Member of the Leibniz Center for Photonics in Infection Research (LPI), Jena, Germany. ⁶Department of Oral and Maxillofacial Surgery and Plastic Surgery, Jena University Hospital, Jena, Germany. ⁷Department of Hepatology and Gastroenterology, Charité-Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Humboldt-Universität Zu Berlin, Campus Virchow Klinikum, Berlin, Germany. ⁸Department of Gastroenterology, Hepatology and Endocrinology, Hannover Medical School, Hannover, Germany. ⁹Department of Anesthesiology and Intensive Care Medicine, University Hospital Bonn, Bonn, Germany. ¹⁰Institute of Intensive Care Medicine, University Hospital Zurich, Zurich, Switzerland. ¹¹Department of Nephrology, Hannover Medical School, Hannover, Germany. ¹²Department of Dentistry-Orthodontics and Craniofacial Biology, Research Institute for Medical Innovation, Radboud University Medical Center, Nijmegen, The Netherlands.

Received: 16 May 2023 Accepted: 18 August 2023

Published online: 27 September 2023

References

- Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. *J Biol Chem*. 2003;278(11):9435–40.
- Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, et al. Identification of a nuclear receptor for bile acids. *Science*. 1999;284(5418):1362–5.
- Perino A, Demagny H, Velazquez-Villegas L, Schoonjans K. Molecular physiology of bile acid signaling in health, disease, and aging. *Physiol Rev*. 2021;101(2):683–731.
- Bertolini A, Fiorotto R, Strazzabosco M. Bile acids and their receptors: modulators and therapeutic targets in liver inflammation. *Semin Immunopathol*. 2022;44(4):547–64.
- Lewis ND, Patnaude LA, Pelletier J, Souza DJ, Lukas SM, King FJ, et al. A GPBAR1 (TGR5) small molecule agonist shows specific inhibitory effects on myeloid cell activation in vitro and reduces experimental autoimmune encephalitis (EAE) in vivo. *PLoS ONE*. 2014;9(6): e100883.
- Haselow K, Bode JG, Wammers M, Ehlting C, Keitel V, Kleinbrecht L, et al. Bile acids PKA-dependently induce a switch of the IL-10/IL-12 ratio and reduce proinflammatory capability of human macrophages. *J Leukoc Biol*. 2013;94(6):1253–64.
- Leonhardt J, Haider RS, Sponholz C, Leonhardt S, Drube J, Spengler K, et al. Circulating bile acids in liver failure activate TGR5 and induce monocyte dysfunction. *Cell Mol Gastroenterol Hepatol*. 2021;12(1):25–40.
- Gu F, Zhu S, Tang Y, Liu X, Jia M, Malmuthuge N, et al. Gut microbiome is linked to functions of peripheral immune cells in transition cows during excessive lipolysis. *Microbiome*. 2023;11(1):40.
- Maruyama T, Miyamoto Y, Nakamura T, Tamai Y, Okada H, Sugiyama E, et al. Identification of membrane-type receptor for bile acids (M-BAR). *Biochem Biophys Res Commun*. 2002;298(5):714–9.
- Horvatis T, Drolz A, Rutter K, Roedel K, Langouche L, Van den Berghe G, et al. Circulating bile acids predict outcome in critically ill patients. *Ann Intensive Care*. 2017;7(1):48.
- Harnisch LO, Mihaylov D, Bein T, Apfelbacher C, Kiehnopf M, Bauer M, et al. Determination of individual bile acids in acute respiratory distress syndrome reveals a specific pattern of primary and secondary bile acids and a shift to the acidic pathway as an adaptive response to the critical condition. *Clin Chem Lab Med*. 2022;60(6):891–900.
- Hartmann G, Cheung AK, Piquette-Miller M. Inflammatory cytokines, but not bile acids, regulate expression of murine hepatic anion transporters in endotoxemia. *J Pharmacol Exp Ther*. 2002;303(1):273–81.
- Andrejko KM, Raj NR, Kim PK, Cereda M, Deutschman CS. IL-6 modulates sepsis-induced decreases in transcription of hepatic organic anion and bile acid transporters. *Shock*. 2008;29(4):490–6.
- Ashino T, Nakamura Y, Ohtaki H, Iwakura Y, Numazawa S. Interleukin-6 regulates the expression of hepatic canalicular efflux drug transporters after cecal ligation and puncture-induced sepsis: a comparison with lipopolysaccharide treatment. *Toxicol Lett*. 2023;374:40–7.
- Stahl K, Wand P, Seeliger B, Wendel-Garcia PD, Schmidt JJ, Schmidt BMW, et al. Clinical and biochemical endpoints and predictors of response to plasma exchange in septic shock: results from a randomized controlled trial. *Crit Care*. 2022;26(1):134.
- Knaup H, Stahl K, Schmidt BMW, Idowu TO, Busch M, Wiesner O, et al. Early therapeutic plasma exchange in septic shock: a prospective open-label nonrandomized pilot study focusing on safety, hemodynamics, vascular barrier function, and biologic markers. *Crit Care*. 2018;22(1):285.
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for sepsis and septic shock (sepsis-3). *JAMA*. 2016;315(8):801–10.
- Vincent JL, Angus DC, Artigas A, Kalil A, Basson BR, Jamal HH, et al. Effects of drotrecogin alfa (activated) on organ dysfunction in the PROWESS trial. *Crit Care Med*. 2003;31(3):834–40.
- Brun-Buisson C, Meshaka P, Pinton P, Vallet B, Group ES. EPISEPSIS: a reappraisal of the epidemiology and outcome of severe sepsis in French intensive care units. *Intensive Care Med*. 2004;30(4):580–8.
- Perez Ruiz de Garibay A, Kortgen A, Leonhardt J, Zipprich A, Bauer M. Critical care hepatology: definitions, incidence, prognosis and role of liver failure in critically ill patients. *Crit Care*. 2022;26(1):289.
- Jenniskens M, Langouche L, Vanwijngaerden YM, Mesotten D, Van den Berghe G. Cholestatic liver (dys)function during sepsis and other critical illnesses. *Intensive Care Med*. 2016;42(1):16–27.
- Moreno R, Rhodes A, Piquilloud L, Hernandez G, Takala J, Gershengorn HB, et al. The sequential organ failure assessment (SOFA) Score: has the time come for an update? *Crit Care*. 2023;27(1):15.
- Hao H, Cao L, Jiang C, Che Y, Zhang S, Takahashi S, et al. Farnesoid X receptor regulation of the NLRP3 inflammasome underlies cholestasis-associated sepsis. *Cell Metab*. 2017;25(4):856–67.
- 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(5):1741–52.
- Vanwijngaerden YM, Langouche L, Brunner R, Debaveye Y, Gielen M, Casaer M, et al. Withholding parenteral nutrition during critical illness increases plasma bilirubin but lowers the incidence of biliary sludge. *Hepatology*. 2014;60(1):202–10.
- Bolder U, Schmidt A, Landmann L, Kidder V, Tange S, Jauch KW. Heat stress prevents impairment of bile acid transport in endotoxemic rats by a posttranscriptional mechanism. *Gastroenterology*. 2002;122(4):963–73.
- Trauner M, Arrese M, Soroka CJ, Ananthanarayanan M, Koeppel TA, Schlosser SF, et al. The rat canalicular conjugate export pump (Mrp2) is down-regulated in intrahepatic and obstructive cholestasis. *Gastroenterology*. 1997;113(1):255–64.
- Kubitz R, Wettstein M, Warskulat U, Haussinger D. Regulation of the multidrug resistance protein 2 in the rat liver by lipopolysaccharide and dexamethasone. *Gastroenterology*. 1999;116(2):401–10.
- Jenniskens M, Langouche L, Van den Berghe G. Cholestatic alterations in the critically ill: some new light on an old problem. *Chest*. 2018;153(3):733–43.
- Vrieze A, Out C, Fuentes S, Jonker L, Reuling I, Kootte RS, et al. Impact of oral vancomycin on gut microbiota, bile acid metabolism, and insulin sensitivity. *J Hepatol*. 2014;60(4):824–31.
- Hagan T, Cortese M, Roupheal N, Boudreau C, Linde C, Maddur MS, et al. Antibiotics-driven gut microbiome perturbation alters immunity to vaccines in humans. *Cell*. 2019;178(6):1313–28.
- Shen R, Ke L, Li Q, Dang X, Shen S, Shen J, et al. Abnormal bile acid-microbiota crosstalk promotes the development of hepatocellular carcinoma. *Hepatal Int*. 2022;16(2):396–411.

33. Liu V, Escobar GJ, Greene JD, Soule J, Whippy A, Angus DC, et al. Hospital deaths in patients with sepsis from 2 independent cohorts. *JAMA*. 2014;312(1):90–2.
34. Vincent JL, Marshall JC, Namendys-Silva SA, Francois B, Martin-Loeches I, Lipman J, et al. Assessment of the worldwide burden of critical illness: the intensive care over nations (ICON) audit. *Lancet Respir Med*. 2014;2(5):380–6.
35. Rhee C, Jones TM, Hamad Y, Pande A, Varon J, O'Brien C, Anderson DJ, Warren DK, Dantes RB, Epstein L, Klompas M. Prevalence, underlying causes, and preventability of sepsis-associated mortality in US acute care hospitals. *JAMA Network Open*. 2019;2(2):e187571. <https://doi.org/10.1001/jamanetworkopen.2018.7571>
36. Hotchkiss RS, Monneret G, Payen D. Sepsis-induced immunosuppression: from cellular dysfunctions to immunotherapy. *Nat Rev Immunol*. 2013;13(12):862–74.
37. Poehlmann H, Scheffold JC, Zuckermann-Becker H, Volk HD, Meisel C. Phenotype changes and impaired function of dendritic cell subsets in patients with sepsis: a prospective observational analysis. *Crit Care*. 2009;13(4):R119.
38. Munoz C, Carlet J, Fitting C, Misset B, Bleriot JP, Cavaillon JM. Dysregulation of in vitro cytokine production by monocytes during sepsis. *J Clin Investig*. 1991;88(5):1747–54.
39. Docke WD, Randow F, Syrbe U, Krausch D, Asadullah K, Reinke P, et al. Monocyte deactivation in septic patients: restoration by IFN-gamma treatment. *Nat Med*. 1997;3(6):678–81.
40. Meisel C, Scheffold JC, Pschowski R, Baumann T, Hetzger K, Gregor J, et al. Granulocyte-macrophage colony-stimulating factor to reverse sepsis-associated immunosuppression: a double-blind, randomized, placebo-controlled multicenter trial. *Am J Respir Crit Care Med*. 2009;180(7):640–8.
41. Hall MW, Knatz NL, Vetterly C, Tomarello S, Wewers MD, Volk HD, et al. Immunoparalysis and nosocomial infection in children with multiple organ dysfunction syndrome. *Intensive Care Med*. 2011;37(3):525–32.
42. Novakovic B, Habibi E, Wang SY, Arts RJ, Davar R, Megchelenbrink W, et al. beta-Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell*. 2016;167(5):1354–68.
43. Recknagel P, Gonnert FA, Westermann M, Lambeck S, Lupp A, Rudiger A, et al. Liver dysfunction and phosphatidylinositol-3-kinase signalling in early sepsis: experimental studies in rodent models of peritonitis. *PLoS Med*. 2012;9(11): e1001338.
44. Seymour CW, Yende S, Scott MJ, Pribis J, Mohny RP, Bell LN, et al. Metabolomics in pneumonia and sepsis: an analysis of the GenIMS cohort study. *Intensive Care Med*. 2013;39(8):1423–34.
45. Bauer M. The liver-gut-axis: initiator and responder to sepsis. *Curr Opin Crit Care*. 2022;28(2):216–20.
46. Morel J, Hargreaves I, Brealey D, Neergheen V, Backman JT, Lindig S, et al. Simvastatin pre-treatment improves survival and mitochondrial function in a 3-day fluid-resuscitated rat model of sepsis. *Clin Sci (Lond)*. 2017;131(8):747–58.
47. Ouellette DR, Moscoso EE, Corrales JP, Peters M. Sepsis outcomes in patients receiving statins prior to hospitalization for sepsis: comparison of in-hospital mortality rates between patients who received atorvastatin and those who received simvastatin. *Ann Intensive Care*. 2015;5:9.
48. Boyle AJ, Ferris P, Bradbury I, Conlon J, Shankar-Hari M, Rogers AJ, et al. Baseline plasma IL-18 may predict simvastatin treatment response in patients with ARDS: a secondary analysis of the HARP-2 randomised clinical trial. *Crit Care*. 2022;26(1):164.
49. Chen M, Ji M, Si X. The effects of statin therapy on mortality in patients with sepsis: a meta-analysis of randomized trials. *Medicine (Baltimore)*. 2018;97(31): e11578.
50. Ghayda RA, Han CH, Lee KH, Kim JS, Kim SE, Hong SH, et al. The effect of statins on mortality among patients with infection: umbrella review of meta-analyses. *Eur Rev Med Pharmacol Sci*. 2021;25(6):2685–95.
51. Bertolotti M, Zambianchi L, Carulli L, Simonini MS, Del Puppo M, Kienle MG, et al. Influence of newly synthesized cholesterol on bile acid synthesis during chronic inhibition of bile acid absorption. *Hepatology*. 2003;38(4):939–46.
52. Loria P, Bertolotti M, Cassinadri MT, Dilengite MA, Bozzoli M, Carubbi F, et al. Short-term effects of simvastatin on bile acid synthesis and bile lipid secretion in human subjects. *Hepatology*. 1994;19(4):882–8.
53. Murphy GM, Ross A, Billing BH. Serum bile acids in primary biliary cirrhosis. *Gut*. 1972;13(3):201–6.
54. Kondrackiene J, Beuers U, Kupcinskis L. Efficacy and safety of ursodeoxycholic acid versus cholestyramine in intrahepatic cholestasis of pregnancy. *Gastroenterology*. 2005;129(3):894–901. <https://doi.org/10.1053/j.gastro.2005.06.019>.
55. Jonsson I, Bojsen-Møller KN, Kristiansen VB, Veedfald S, Sewer Albrechtsen NJ, Clausen TR, Kuhre RE, Rehfeld JF, Holst JJ, Madsbad S, Svane MS. Effects of manipulating circulating bile acid concentrations on postprandial GLP-1 secretion and glucose metabolism after Roux-en-Y gastric bypass. *Front Endocrinol*. 2021;12:681116. <https://doi.org/10.3389/fendo.2021.681116>.
56. Sponholz C, Matthes K, Rupp D, Backaus W, Klammt S, Karailieva D, et al. Molecular adsorbent recirculating system and single-pass albumin dialysis in liver failure: a prospective, randomised crossover study. *Crit Care*. 2016;20:2.
57. Ovadia C, Lovgren-Sandblom A, Edwards LA, Langedijk J, Geenes V, Chambers J, et al. Therapeutic plasma exchange as a novel treatment for severe intrahepatic cholestasis of pregnancy: case series and mechanism of action. *J Clin Apher*. 2018;33(6):638–44.
58. Press AT, Babic P, Hoffmann B, Muller T, Foo W, Hauswald W, et al. Targeted delivery of a phosphoinositide 3-kinase gamma inhibitor to restore organ function in sepsis. *EMBO Mol Med*. 2021;13(10): e14436.
59. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure On behalf of the Working Group on sepsis-related problems of the European society of intensive care medicine. *Intensive Care Med*. 1996;22(7):707–10.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.