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Circulating levels of butyrate are inversely related to portal hypertension, endotoxemia, and systemic inflammation in patients with cirrhosis

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ABSTRACT: Short-chain fatty acids (SCFAs) are gut microbiota–derived products that participate in maintaining the gut barrier integrity and host's immune response. We hypothesize that reduced SCFA levels are associated with systemic inflammation, endotoxemia, and more severe hemodynamic alterations in cirrhosis. Patients with cirrhosis referred for a hepatic venous pressure gradient (HVPG) measurement ($n = 62$) or a transjugular intrahepatic portosystemic shunt placement ($n = 12$) were included. SCFAs were measured in portal (when available), hepatic, and peripheral blood samples by GC-MS. Serum endotoxins, proinflammatory cytokines, and NO levels were quantified. SCFA levels were significantly higher in portal *vs.* hepatic and peripheral blood. There were inverse relationships between SCFAs and the severity of disease. SCFAs (mainly butyric acid) inversely correlated with the model for end-stage liver disease score and were further reduced in patients with history of ascites, hepatic encephalopathy, and spontaneous bacterial peritonitis. There was an inverse relationship between butyric acid and HVPG values. SCFAs were directly related with systemic vascular resistance and inversely with cardiac index. Butyric acid inversely correlated with inflammatory markers and serum endotoxin. A global reduction in the blood levels of SCFA in patients with cirrhosis is associated with a more advanced liver disease, suggesting its contribution to disease progression.—Juanola, O., Ferrusquía-Acosta, J., García-Villalba, R., Zapater, P., Magaz, M., Marín, A., Olivas, P., Baiges, A., Bellot, P., Turon, F., Hernández-Gea, V., González-Navajas, J. M., Tomás-Barberán, F. A., García-Pagán, J. C., Francés, R. Circulating levels of butyrate are inversely related to portal hypertension, endotoxemia, and systemic inflammation in patients with cirrhosis. FASEB J. 33, 000–000 (2019). www.fasebj.org

KEY WORDS: SCFAs · liver · hemodynamics · cytokines

Portal hypertension is a landmark associated with increased mortality and morbidity in patients with cirrhosis. It is primarily caused by an increased intrahepatic vascular resistance, and it may trigger serious complications, such as the development of varices with significant risk for gastrointestinal bleeding, ascites, or hepatic encephalopathy (1).

Short-chain fatty acids (SCFAs) are small products derived from the gut microbiota and the fermentation of dietary fibers. These products are present in the bloodstream and help us cope with several nutritional needs that we are unable to perform (2, 3). In addition, SCFAs participate in maintaining the gut barrier integrity, contribute to epithelial cell reposition, and facilitate the host's immune system maturation (4, 5). The interaction between SCFAs and the host's immune response has been documented to include the regulation of macrophage activity (6), the skewing of naive T cells to regulatory T (T_{reg}) cells (7), and the expansion of IL-10–producing T_{reg} cells

ABBREVIATIONS: BT, bacterial translocation; FHVP, free hepatic venous pressure; HVPG, hepatic venous pressure gradient; GPR43, G-protein–coupled receptor 43; HE, hepatic encephalopathy; MELD, model for end-stage liver disease; NO_x, NO levels; SBP, spontaneous bacterial peritonitis; SCFA, short-chain fatty acid; SVR, systemic vascular resistance; TIPS, transjugular intrahepatic portosystemic shunt; T_{reg}, regulatory T; UE, endotoxin unit

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through the specific GPCR, G-protein–coupled receptor 43 (GPR43) (8), a key receptor mediating the host's inflammatory responses to SCFAs (9). On the other hand, SCFAs have also been linked to blood pressure modulation through a complex network of receptors that play opposed effects on the vascular tone, contributing firstly to prevent dramatic changes in blood pressure and, secondly, to facilitate nutrient absorption from the gut (10). Overall, SCFAs are thought to have a beneficial effect in liver homeostasis and may contribute to prevent the progression of disease.

Bacterial translocation (BT) is enhanced in patients with cirrhosis because of an intestinal dysbiotic state resulting from a pathologic crosstalk within the gut-liver axis. Intestinal microbiota are known to shift during liver-damage progression. Phyla such as *Firmicutes* are replaced by others, such as *Proteobacteria*, in cirrhosis (11, 12). These changes have been associated with liver-disease progression and its evolution from compensated to decompensated stages. In addition, portal hypertension is worsened by inflammation and the immune interactions established in the liver sinusoid in response to intestinal BT (13).

Main SCFA-producing bacterial phyla, such as *Firmicutes* and *Bacteroidetes* (14, 15), are significantly reduced in advanced stages of cirrhosis. Therefore, the anti-inflammatory features that SCFAs play may be compromised in cirrhosis, aggravating the inflammatory outlook and the gut permeability in these patients. This likely contributes to the translocation of bacterial products and the development of bacteria-derived complications in patients, worsening their hemodynamic status.

The aim of our study was to evaluate in patients with cirrhosis at different stages of the disease the potential relationship between SCFA concentrations in different vascular territories with systemic inflammation, endotoxemia, and the severity of the hemodynamic alterations.

MATERIALS AND METHODS

Study cohort

We retrospectively analyzed data of all consecutive patients with cirrhosis of any etiology who underwent an HVPG measurement for clinical purposes and those who were electively treated with a transjugular intrahepatic portosystemic shunt (TIPS) from January 2016 to February 2018. Patients who were not in stable conditions and those treated with an emergent TIPS were not included. In general, all patients with cirrhosis who are referred to our unit for a hepatic venous pressure gradient (HVPG) measurement or for a TIPS placement are asked for permission to obtain blood samples for research purposes. Blood obtained from peripheral and hepatic veins during the HVPG measurement and blood obtained from the portal vein when a TIPS was performed were collected into a citrate-containing tube (0.129 M, 3.8%, Vacutainer System; Becton Dickinson, San Diego, CA, USA). The samples were centrifuged, and aliquots of the platelet-poor plasma were frozen at −80°C until assayed. Written informed consent was obtained from all subjects, and all the procedures followed were in accordance with the World Medical Association's Declaration of Helsinki.

Cardio-pulmonary pressures, cardiac output, and HVPG measurements

Hemodynamic studies were performed after overnight fasting under light sedation with intravenous midazolam. Under local anesthesia, a venous introducer was placed in the right internal jugular vein using the Seldinger technique. Under fluoroscopy, a Swan-Ganz catheter (Edwards Lifesciences, Irvine, CA, USA) was advanced into the pulmonary artery for measurement of cardio-pulmonary pressures and cardiac output by the thermal dilution method. Systemic and pulmonary resistances were derived from these values. After that, HVPG measurement was performed. A 7F balloon-tipped catheter (Edwards Lifesciences) was guided into the main right or middle hepatic vein for measurements of wedged hepatic venous pressure and free hepatic venous pressure (FHVP). The adequate occlusion of the hepatic vein was checked by manual injection of a small amount of radiologic contrast. FHVP was measured in the right or middle hepatic vein close to the inferior vena cava. The HVPG results from the difference between wedged hepatic venous pressure and FHVP. All measurements were taken in triplicate, and the mean was used to calculate the HVPG. In patients who underwent a TIPS, a portal blood sample was obtained immediately after the puncture of the portal vein and before angioplasty and stent placement.

Analysis of SCFAs in plasma

Plasma samples (200 μl) were acidified with 20 μl of 5% *o*-phosphoric acid (final concentration 0.5%) and after vortexing were extracted with 200 μl of methyl tert butyl ether. Samples were homogenized with a vortex for about 2 min and centrifuged for 10 min at 17,000 *g* at 4°C. Organic phase was collected, and 50 μl was transferred to an insert in a vial for the injection in the GC-MS. Five microliters of 10 mM of 4-methyl valeric acid (final concentration, 500 μM) was added as internal standard. The rest of the sample was stored at −20°C. The GC-MS system consisted of an Agilent 7890A (Agilent Technologies, Santa Clara, CA, USA) equipped with an automatic liquid sampler (MPS2; Gerstel, Mülheim, Germany) and coupled with an Agilent 5975C mass selective detector. Acquisition was done using Chemstation software (Hewlett-Packard, Palo Alto, CA, USA). The gas chromatograph was fitted with a high-polarity, polyethylene glycol, fused silica capillary column DB-WAXetr (30 m, 0.25-mm id, 0.25-μm film thickness; Agilent Technologies), and helium was used as the carrier gas at 1 ml/min. Injection was made in splitless mode with an injection volume of 1 μl and an injector temperature of 250°C. A glass liner with a glass wool plug at the lower end of the liner was used to avoid the contamination of the gas chromatrograph column with nonvolatile material. Every 10 plasma samples injected, a blank sample with hexane was inserted to check for memory effects. The column temperature was initially 90°C, then increased to 150°C at 15°C/min, to 170°C at 5°C/min, and finally to 250°C at 20°C/min and kept at this temperature for 2 min (total time 14 min). Solvent delay was 3.5 min. The detector was operated in electron impact ionization mode (electron energy 70 eV), scanning the 30–250 m/z range. The temperatures of the ion source, quadrupole, and interface were 230, 150, and 280°C, respectively. Identification of the SCFAs was based on the retention time of standard compounds and with the assistance of the National Institute of Standards and Technology (NIST) 08 Mass Spectral Library (*http://nistmassspeclibrary.com/*) and the Wiley Registry of Mass Spectral Data, 7th Ed.. (*https://www.wiley.com/*)A characteristic single ion was selected for the quantification of each compound: acetic acid 60, propionic acid 74, isobutyric acid 88, butyric acid 73, isovaleric acid 87, and valeric acid 73.

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Serum endotoxin levels

A quantitative chromogenic limulus amoebocyte lysate test (Lonza, Basel, Switzerland) was used to evaluate serum endotoxin levels. Because of LPS ubiquity, samples and reagents were handled in an airflow chamber and processed with pyrogen-free material tested by manufacturers. *Escherichia coli*–lyophilized endotoxin [22 endotoxin units (UE)/ml] provided by the kit was used to set standard endotoxin concentrations ranging from 5.0–0.1 UE/ml. To verify the lack of product inhibition by plasma protein, a dilution and heating inactivation protocol was followed prior to endotoxin measurement. A pooled *E. coli* endotoxin spike solution (0.4 UE/ml) was prepared with serum samples. Dilutions ranging from 1/2–1/20 were performed over spiked and unspiked serum samples. All test samples were then incubated at 60°C during 30 min (16) The test was performed after this period. The noninhibitory dilution was established when the difference between spiked and unspiked endotoxin values was equal to the known concentration of the spike ± 25%, as detailed by the manufacturer. Final sample dilutions used were 1/10 (spike recovery after correction of dilution: 0.34 UE/ml). All samples were tested in triplicate and read in a Thermomax microplate reader (Molecular Devices, San Jose, CA, USA).

Serum cytokines and NO levels

ELISAs for the quantitative measurement of TNF-α and IL-6 levels were performed in the sera of all blood samples obtained from included patients using Human Quantikine kits (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions. The sum of the NO metabolites nitrite (NO_2^-) and nitrate (NO_3^-) is widely used as an index of NO generation and is expressed as NO levels (NO_x) (17). NO_x in serum samples were calculated by measuring conversion of NO_3^- to NO_2^- by the enzyme nitrate reductase through an ELISA (R&D Systems) based on the Griess reaction. All samples were tested in triplicate using a Thermomax microplate reader. Standard curves were generated for each plate, and the mean zero standard optical densities were subtracted from the rest of standards, controls, and samples to obtain corrected cytokines and NO_x concentrations.

Statistical analysis

Continuous variables are reported as means ± SD and categorical variables as frequency or percentages. Statistical differences between groups were analyzed using the Fisher test for categorical data and the Mann-Whitney *U* test for quantitative data. Bivariate correlations between continuous variables were calculated using the Spearman test. All tests were conducted using a 2-sided approach with a 5% significance level. Bonferroni correction was performed for multiple comparisons. All statistical analyses were performed using the R software (R Foundation for Statistical Computing, Vienna, Austria; *https://www.R-project.org/*).

RESULTS

Patient characteristics

Sixty-two patients with cirrhosis were included. **Table 1** describes the main clinical and analytical characteristics of patients. Patients were mainly men (71%), with a mean age of 59 yr old. Main etiology of cirrhosis was alcohol abuse (43.5%). Mean model for end-stage liver disease (MELD) score was 12 ± 4. Seventy-two percent of patients had esophageal varices, and 50% had previous episodes of ascites. Twelve patients (19.4%) underwent TIPS placement immediately after the collection of blood samples. Main etiology in this subgroup was also alcohol abuse (83.3%), and the mean MELD score was 13 ± 6. The main indications for TIPS were variceal bleeding and refractory ascites or hydrothorax in 6 and 6 patients respectively. Following TIPS, the portal pressure gradient reduced from 19.7 ± 5.1 to 9.4 ± 2.4 mmHg.

SCFAs and severity of liver disease

Table 2 summarizes mean SCFA levels in blood samples of patients with cirrhosis. As shown, mean SCFA levels are similar between hepatic and peripheral blood, except for acetic acid, which was significantly higher in hepatic blood. However, for all SCFAs but isovaleric acid, levels in portal blood are significantly higher compared with both hepatic and peripheral blood, supporting the gut bacteria main origin of SCFAs and that they are partially metabolized by the liver.

Butyric acid levels significantly and inversely correlated with MELD score in hepatic and peripheral blood (**Fig. 1*A, B***) as well as in portal blood. This inverse relationship was also observed for the remaining SCFAs. However, acetic acid at hepatic and isobutyric acid at peripheral blood were the only ones also reaching statistical significance (Supplemental Table S1).

As shown in Supplemental Table S2, patients with decompensated disease had lower levels of the different SCFAs, achieving statistical significance mainly for butyric acid, further reinforcing the negative relationship between butyric acid levels and the severity of the liver disease. Indeed, peripheral levels of butyric acid were significantly lower in patients with history of ascites and spontaneous bacterial peritonitis (SBP), and peripheral and hepatic levels of butyric acid were significantly lower in patients with previous episodes of hepatic encephalopathy. Other SCFAs, such as acetic or propionic acids, were also significantly lower in patients with previous episodes of variceal bleeding, hepatic encephalopathy, and SBP (Supplemental Table S2). Interestingly, the use of antibiotics, either norfloxacin or rifaximin ($n = 5$) or lactulose ($n = 9$), was not associated with differences in circulating SCFA levels (unpublished results).

SCFAs and hemodynamics

An inverse relationship was present between HVPG and all SCFA levels both at hepatic and peripheral levels (Supplemental Table S3). This trend became significant for peripheral and hepatic levels of butyric acid (**Fig. 2*A, B***, respectively) and for peripheral acetic acid ($r = -0.350$; $P = 0.023$). The inverse relationship between butyric acid and HVPG was even higher at portal blood ($r = -0.837$; $P = 0.001$). Although levels of butyric acid at portal and hepatic levels were further reduced in those patients with higher HVPG values (Fig. 2*C*), the reduction was much smaller in hepatic than in portal levels, suggesting an impaired SCFA liver metabolism in patients with more severe liver disease as estimated by HVPG values (Fig. 2*D*).

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TABLE 1. *Clinical and analytical characteristics of patients*

Parameter	All patients (n = 62) Mean ± SD	Patients later treated with TIPS (n = 12) Mean ± SD
Age (yr)	59 ± 11	56 ± 9
Gender, male (%)	44 (71.0)	7 (58.3)
Etiology of cirrhosis		
Alcohol abuse	27 (43.5)	10 (83.3)
HCV infection	18 (29.0)	
Alcohol + HCV	12 (19.4)	1 (8.3)
NASH	3 (4.8)	-
Others	2 (3.2)	1 (8.3)
Child-Pugh score (A/B/C)	35/18/9	3/7/2
MELD score (points)	12 ± 4	13 ± 6
Esophageal varices [n (%)]	45 (72.6)	10 (83.3)
Previous episodes of hepatic encephalopathy [n (%)]	14 (22.6)	5 (41.7)
Previous episodes of ascites [n (%)]	29 (46.8)	10 (83.3)
Previous episodes of SBP [n (%)]	5 (8.1)	2 (16.6)
Previous episodes of variceal bleeding [n (%)]	13 (20.9)	6 (50.0)
Use of β-blockers [n (%)]	10 (16.1)	3 (25.0)
Use of PPIs [n (%)]	15 (24.2)	5 (41.7)
Use of norfloxacin [n (%)]	3 (4.8)	
Use of rifaximin [n (%)]	2 (3.2)	1 (8.3)
Use of lactulose [n (%)]	10 (16.1)	3 (25.0)
Total bilirubin (mg/dl)	1.8 ± 1.9	2.1 ± 3.5
AST (U/I)	86 ± 69	35 ± 15
ALT (U/I)	76 ± 81	19 ± 14
Hemoglobin (g/100 ml)	12.4 ± 3.3	9.6 ± 2.2
INR	1.3 ± 0.2	1.4 ± 0.2
Creatinine (mg/dl)	0.85 ± 0.28	0.98 ± 0.42
Albumin (mg/dl)	3.5 ± 0.7	3.2 ± 0.6
Total white blood cells (×10E3/μl)	5.2 ± 1.9	6.5 ± 1.6
Platelets (×10E3/μl)	115 ± 67.3	156 ± 118
Wedged hepatic vein pressure (mmHg)	25 ± 6	23.5 ± 5
Free hepatic vein pressure (mmHg)	8.5 ± 4	7.5 ± 3
HVPG (mmHg)	16.5 ± 5	16 ± 4
Inferior vena cava pressure (mmHg)	7 ± 4	6 ± 3
MAP (mmHg)	84 ± 31	81 ± 15
Cardiac index (L/min/m^2)	3.4 ± 1.4	3.7 ± 1.1
Pulmonary capillary wedged pressure (mmHg)	9 ± 6	8 ± 4
Mean pulmonary artery pressure (mmHg)	16 ± 7	13 ± 5.5
Right atrial pressure (mmHg)	5 ± 3	4.5 ± 3
Heart rate (bpm)	65 ± 24	69 ± 14
SVR (dyn × s × cm − 5)	1046 ± 552	1035 ± 380
Pulmonary vascular resistance (dyn × s × cm − 5)	76 ± 50	69 ± 33

ALT, alanine transaminase; AST, aspartate transaminase; HCV, hepatitis C virus; INR, international normalized ratio; MAP, mean arterial pressure; NASH, nonalcoholic steatohepatitis; PPI, proton pump inhibitor.

We also investigated the relationship between SCFA levels and systemic hemodynamics. Levels of SCFA were directly related with systemic vascular resistance (SVR) and inversely with cardiac index (Supplemental Table S4). This relationship reached statistical significance for portal and hepatic levels of isobutyric acid, SVR, and cardiac index (**Fig. 3*A–D***). The reduction between the concentrations of isobutyric acid in portal *vs.* hepatic blood was smaller in patients with lower SVR values (Fig. 3*E*) and higher cardiac indexes (Fig. 3*F*), suggesting that SCFAs may be a factor contributing to the hyperdynamic state caused by disease progression.

SCFAs, endotoxemia, and inflammatory response

Serum endotoxin levels as well as proinflammatory cytokines and NO concentrations were significantly higher in

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TABLE 2. *SCFA levels in samples from different vascular territories in the overall series of patients and in the subgroup of patients who later underwent a TIPS*

Variable	All patients (n = 62)		Patients later treated with TIPS (n = 12)		
	Hepatic	Peripheral	Portal	Hepatic	Peripheral
Acetic acid (μM)	32.9 ± 9.9	23.2 ± 5.1	46.3 ± 9.3*	29.5 ± 6.8	24.2 ± 5.2
Propionic acid (μM)	10.1 ± 4.5	9.7 ± 3.9	30.7 ± 6.7*	10.7 ± 3.3	10.7 ± 3.5
Butyric acid (μM)	17.9 ± 5.1	16.4 ± 4.5	29 ± 4.5*	15.9 ± 1.7	18.2 ± 4.5
Isobutyric acid (μM)	4.6 ± 1.4	4.2 ± 1.4	16.4 ± 3.2*	3.9 ± 1.1	4.2 ± 1.5
Valeric acid (μM)	3.2 ± 1.6	3.8 ± 1.8	17.4 ± 7*	2.9 ± 0.7	3.2 ± 1
Isovaleric acid (μM)	8.5 ± 5.1	12.4 ± 1.5	15 ± 7.8	11.3 ± 5.1	12.6 ± 1.5

*$P < 0.01$ compared with levels in hepatic and peripheral blood.

portal compared with hepatic and peripheral blood (**Table 3**), without significant differences in the latter 2 territories. As expected, endotoxins, proinflammatory cytokines, and NO levels were significantly and directly correlated in all the studied vascular territories (Supplemental Table S5). A significant correlation between serum endotoxin levels and HVPG values was present both in peripheral (r = 0.472; P = 0.001) and hepatic blood (r = 0.475; P = 0.001) and was not observed with TNF-α, IL-6, or NO levels.

Interestingly, different SCFAs inversely correlated with distinct proinflammatory mediators at hepatic, peripheral, or portal levels (Supplemental Table S6). However, only butyric acid showed a consistent inverse significant correlation with all 3 TNF-α, IL-6, and NO levels and with serum endotoxin levels both at hepatic (**Fig. 4*A***) and peripheral levels (Fig. 4*B*) and at the portal level (Fig. 4*C*).

Inflammatory cytokines and NO levels were lower in patients receiving antibiotic treatment (n = 5) (Supplemental Table S7). However, the difference didn't reach statistical significance, and no correlation was observed between the use of antibiotics and changes in SCFA amounts, probably because of the reduced number of patients.

DISCUSSION

In the present study we show that, in patients with cirrhosis, decreased circulating levels of SCFAs, especially butyric acid, are associated with more advanced liver disease, as shown by the inverse relationship between SCFA levels and the severity of portal hypertension, endotoxemia, systemic inflammation, and the prevalence of decompensating events. These results bring the attention to these bacterial-derived metabolites in the host-microbiome interaction established in cirrhosis and provide new venues for exploring SCFA modulation as a potential tool to prevent bacterial product–related complications in these patients.

Portal hypertension is considered a key step toward acute decompensation in cirrhosis (18, 19) and a facilitating event for the recurrence of gut BT episodes. BT promotes the immune system exacerbation (20) and further increases the risk of progression to acute levels in chronic liver failure (21, 22). There is evidence on both quantitative and qualitative changes in gut microbiota composition during development of liver damage (23) and how dysbiosis impacts disease progression (24, 25). As a result, the host-microbiome interaction is also unstable, and the gut

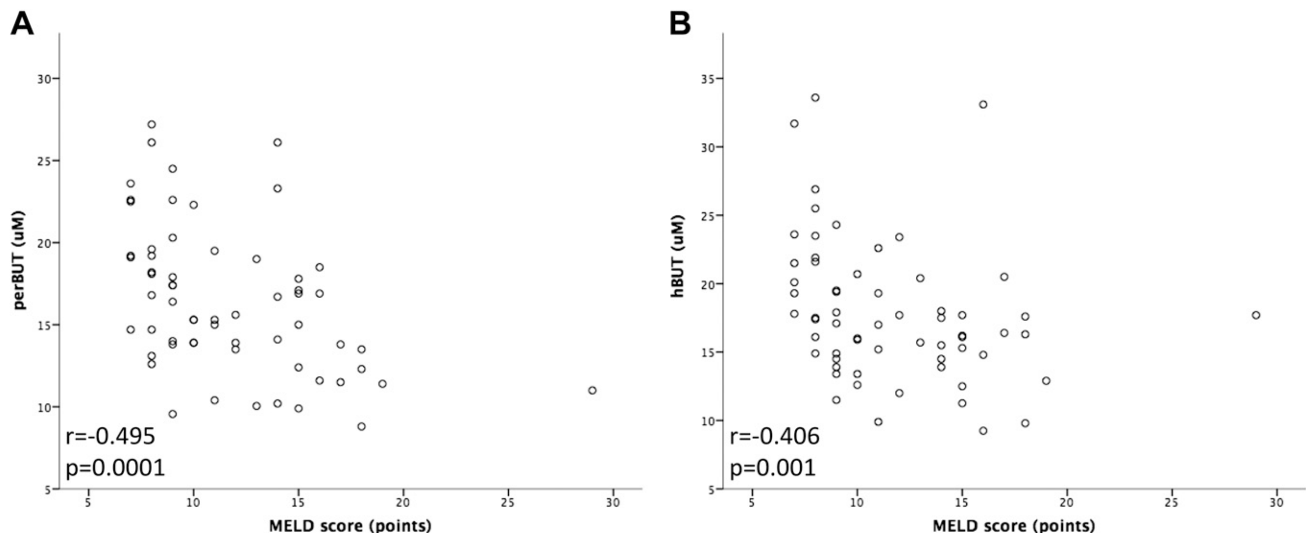

Figure 1. Correlation between hepatic (*A*) and peripheral (*B*) levels of butyric acid and MELD scores in the overall series of patients. hBUT, hepatic butyric acid level; perBUT, peripheral butyric acid level.

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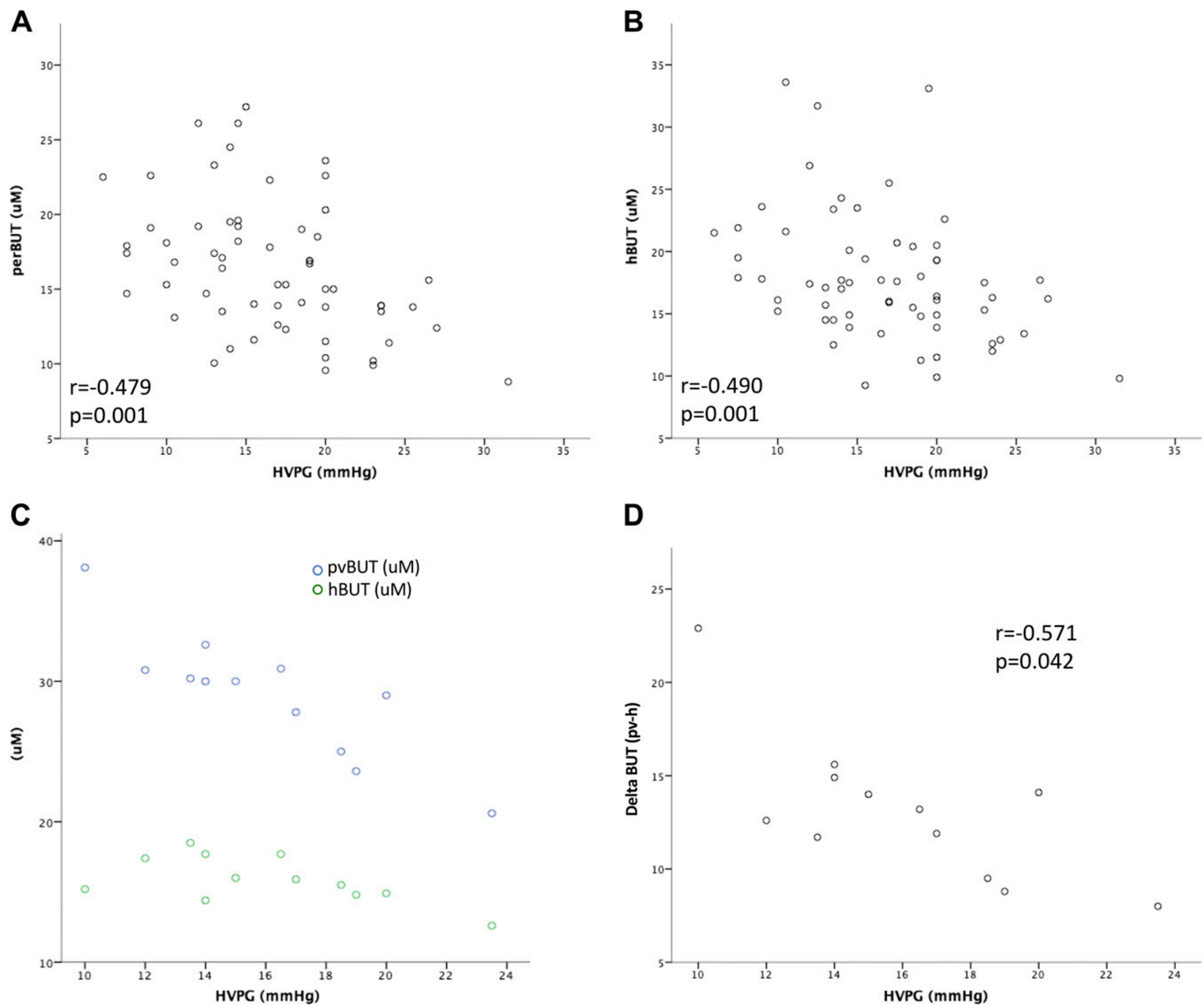

Figure 2. *A*, *B*) Correlation between hepatic (*A*) and peripheral (*B*) levels of butyric acid and HVPG values in the overall series of patients. *C*) Correlation between portal and hepatic levels of butyric acid and HVPG values in the subgroup of patients with TIPS. *D*) Correlation between the difference in portal minus hepatic butyric acid levels and HVPG values in the subgroup of patients with TIPS. BUT, butyric acid level; h, hepatic; perBUT, peripheral butyric acid level; pv, portal.

epithelial barrier is affected beyond disease-derived inflammation (26).

Indeed, our study suggests that reduction in SCFAs due to changes in the gut microbiota during the natural history of the disease may be an additional mechanism further facilitating disease progression. SCFAs are products derived from bacterial fermentation of polysaccharides that take part in the host-microbiome crosstalk, with a demonstrated beneficial role in intestinal homeostasis, lipid metabolism, mucin production, and expression of antimicrobial peptides, among others (27, 28). The gut microbiota catalog in cirrhosis shows a dysbiosis toward pathogenic, non–SCFA-producing bacteria compared with healthy individuals (23). In fact, we have previously shown in mice with induced cirrhosis an association between reduction of SCFA levels, an alteration of the gut barrier integrity, and the increase of BT rates. The loss in serum levels of SCFAs in these mice can be inhibited by the reposition of the T_{reg} cell tolerogenic activity, supporting an active role for these products in the interface between the immune system and gut microbiota (29).

SCFA levels in portal blood were significantly higher than in the hepatic and peripheral vein, providing evidence of the SCFA bacterial source and the participation of the liver in the metabolization of these products (30, 31). Importantly, more advanced stages of the liver disease correlated with lowest SCFA levels, the most likely explanation for this being a decrease in SCFA-producing gut microbiota during cirrhosis progression as has been shown in experimental models (11, 32). In fact, the reduced capacity of fecal microbiota from cirrhotic patients to ferment nondigestible carbohydrates into SCFAs compared with healthy controls has been recently described in a study by Jin *et al.* (33). This reduction was, in turn, more evident for butyrate production in patients with more advanced liver disease.

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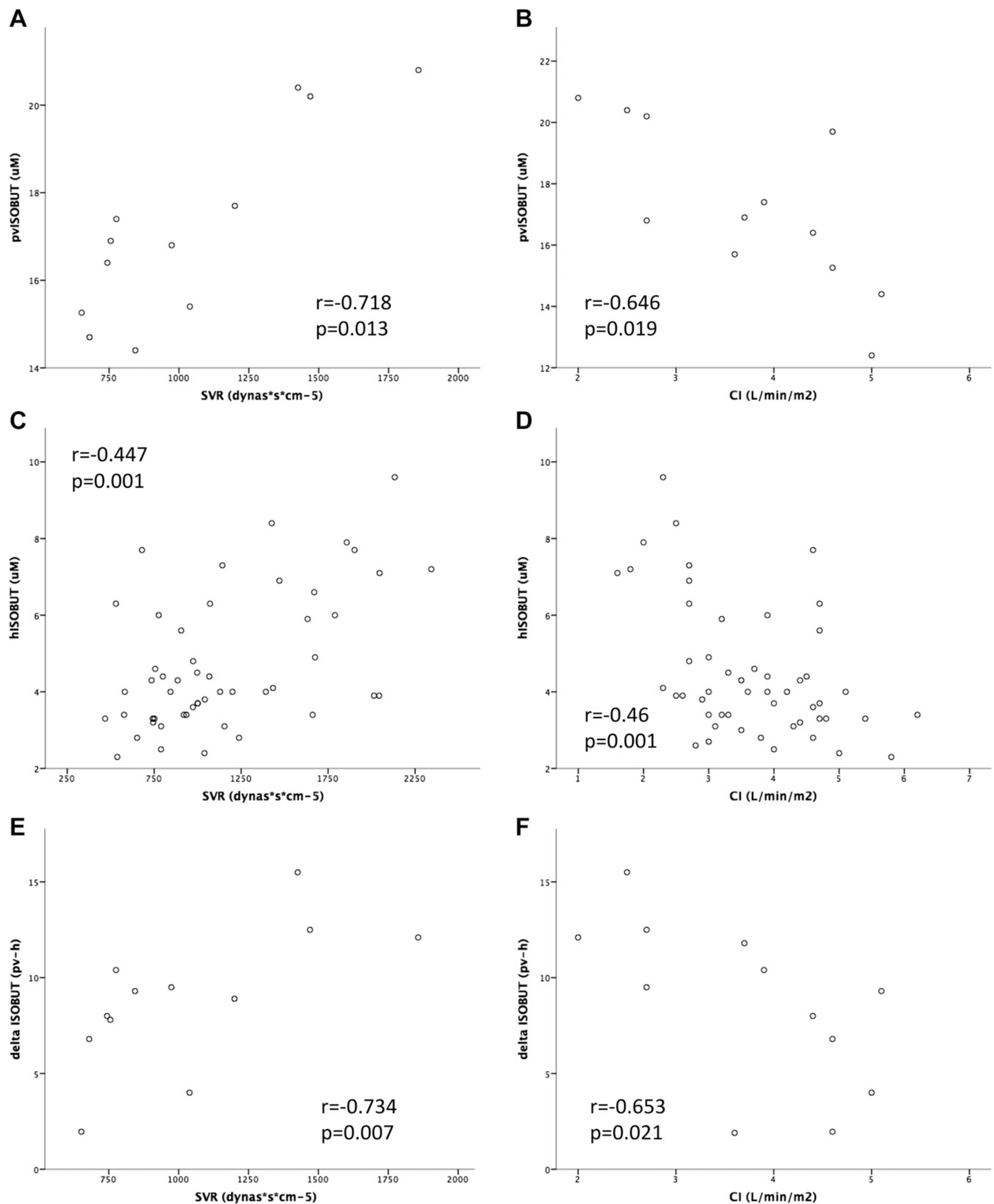

Figure 3. *A*, *B*) Correlation between portal levels of isobutyric acid and SVR (*A*) and cardiac index (*B*). *C*, *D*) Correlation between hepatic levels of isobutyric acid and SVR (*C*) and cardiac index (*D*). *E*, *F*) Correlations between the difference in portal minus hepatic isobutyric acid levels and SVR (*E*) and cardiac index (*F*) in the subgroup of patients with TIPS. BUT, butyric acid level; CI, cardiac index; h, hepatic; ISOBUT, isobutyric acid; pv, portal.

Also, previous episodes of decompensation, such as ascites, SBP, or hepatic encephalopathy (HE), have shown a correlation with low levels of butyric acid. Although inflammation, gut barrier disruption, and dysbiosis toward pathogenic microbiota caused by progression of disease may link these aspects, a common mechanism

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TABLE 3. *Serum endotoxin, TNF-α, IL-6, and NO levels in samples from hepatic and peripheral blood in the overall series of patients and also in portal blood in the subgroup of patients who later underwent a TIPS*

Variable	All patients (n = 62)		Patients later treated with TIPS (n = 12)		
	Hepatic	Peripheral	Portal	Hepatic	Peripheral
Endotoxin (UE/ml)	1.3 ± 0.4	1.4 ± 0.7	1.7 ± 0.4*	1.3 ± 0.4	1.4 ± 0.8
TNF-α (pg/ml)	15.6 ± 7.8	15.5 ± 7.8	19.2 ± 3.8*	16 ± 6.6	15.3 ± 8.9
IL-6 (pg/ml)	17.2 ± 9.2	21.2 ± 10.8	25.9 ± 3.6*	17.2 ± 8.8	21.1 ± 11.3
NO_x (nmol/ml)	17 ± 7.3	16.6 ± 7.7	20.6 ± 2.8*	15.6 ± 6	16.6 ± 8.2

*$P < 0.05$ compared with levels in hepatic and peripheral blood.

based on butyrate concentrations may underlie. For instance, dietary supplementation with butyrate in a methionine-choline–deficient diet–induced nonalcoholic steatohepatitis mice alleviates liver injury, improves fibrosis, and stabilizes the gut barrier (34). Also, glycerol phenylbutyrate has been described to decrease HE events in patients with cirrhosis in a randomized, double-blind controlled trial (35). In our study, the fact that butyric acid levels were significantly lower in patients with previous episodes of HE (Supplemental Table

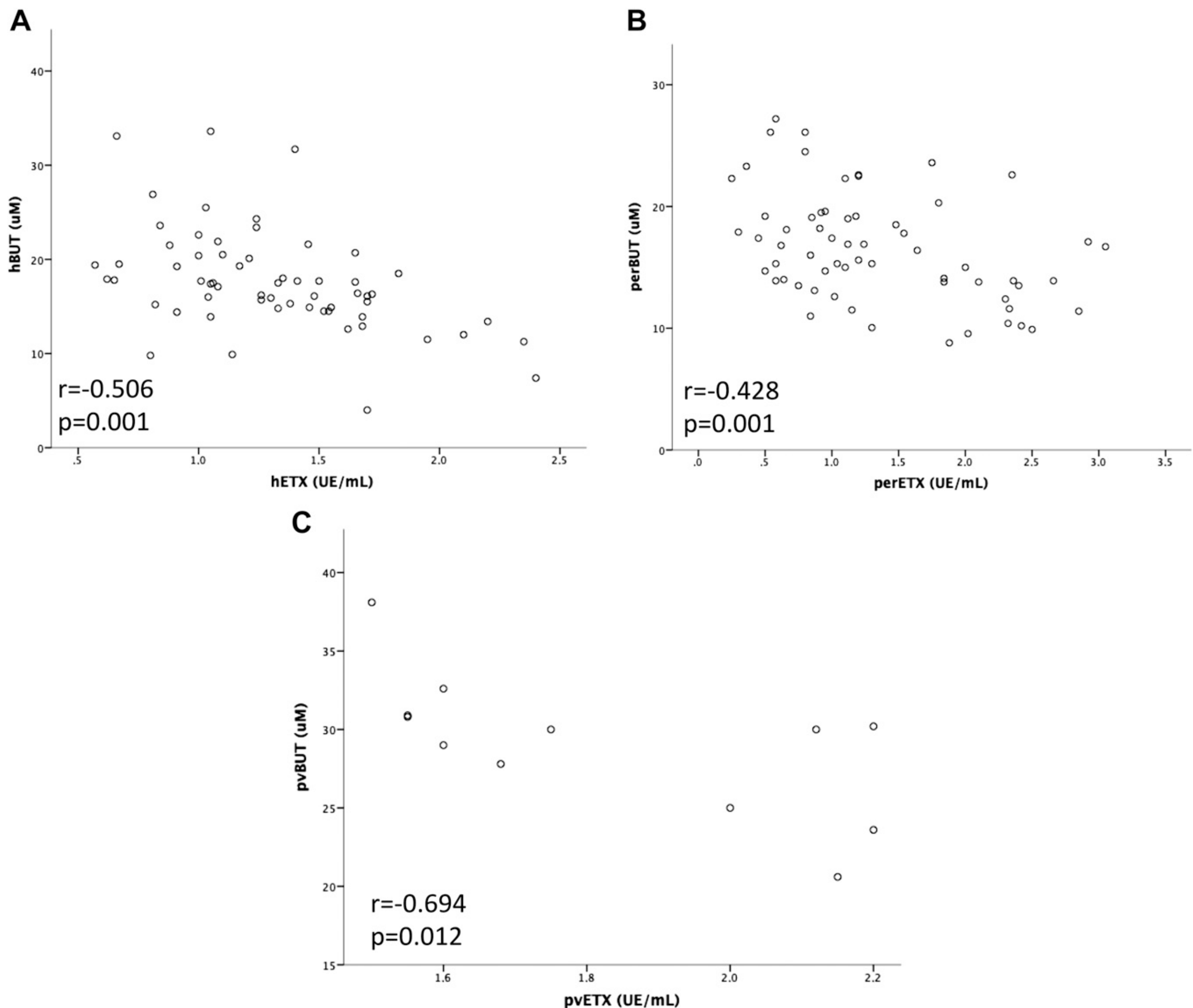

Figure 4. *A*, *B*) Correlation between hepatic (*A*) and peripheral (*B*) levels of butyric acid and serum endotoxin values in these territories in the overall series of patients. *C*) Correlation between portal levels of butyric acid and serum endotoxin in the subgroup of patients with TIPS. BUT, butyric acid; ETX, endotoxin; h, hepatic; per, peripheral; pv, portal.

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S2) might support that butyrate could play a role in the development of these events.

In addition to changes in SCFA-producing gut microbiota, an impaired uptake of SCFAs by rectal mucosa has been claimed to also contribute to the reduced SCFA levels observed in patients with more severe liver disease (36). Despite the lower entrance of SCFAs in the liver, their concentration in hepatic blood decreases to a lesser extent, as shown for butyric acid (Fig. 2*D*), suggesting a deficient SCFA liver metabolization in patients with more advanced liver disease. In this regard, because SCFAs are metabolized to acetyl coenzyme A to generate ATP and NADH and contribute to ketogenesis in the liver (37), reduced SCFA levels may compromise energy expenditure in decompensated cirrhosis (38, 39).

An inverse relationship was also found between the concentration of different SCFAs and inflammatory markers TNF-α, IL-6, and NO. The anti-inflammatory capacities of SCFAs have been extensively documented (4–8) and suggested to be related to activation of GPCR receptors (40). The interaction between GPR43 and SCFAs has been involved in the suppression of inflammatory responses in models of colitis, arthritis, and asthma, as demonstrated by the amelioration of exacerbated inflammatory reactions in germ-free models of these diseases after SCFA restoration (9). Because GPR43 is expressed both in colonic and liver tissue (41), SCFAs may limit hepatic damage by suppressing mucosal inflammation and regulating liver inflammatory progression that will be reduced in states of SCFA deficiency as observed in our patients with cirrhosis. In this line, we observe higher levels of endotoxin levels and proinflamatory cytokines in patients with lower SCFA concentrations, a situation that facilitates the development of complications in cirrhosis. In accordance, levels of different SCFAs were significantly lower in patients with previous episodes of hepatic encephalopathy, ascites, or SBP.

In addition to GPCR activation, SCFAs such as butyrate have been shown to produce immune modulation and oxidative stress reduction through the inhibition of histone deacetylases. This activity helps favor intestinal barrier and motility regulation and therefore the maintenance of gut homeostasis (42). In fact, histone deacetylase inhibitors are being tested in several fibrosis-related diseases (43). Considering also that butyric, propionic, and acetic acid contribute to improve metabolism of glucose and lipids (14, 44, 45) and that butyrate restores high-fat diet–induced metabolic adaptations in obese mice (46), results presented herein provide new rationale for exploring prebiotic supplementation in patients with cirrhosis and chronic liver conditions leading to cirrhosis, such as nonalcoholic fatty liver disease or steatohepatitis.

Despite the promising body of knowledge arising around gut-targeted therapies in cirrhosis, a set of physiologic and experimental limitations remains to be addressed when interpreting the results described in the present study as well as in others. Firstly, diet has been proven to directly affect gut microbiota (47), and therefore, its ability to produce SCFAs may be affected beyond disease. Secondly, the use of rifaximin, lactulose, β-blockers, and proton pump inhibitors may also constitute additional confounders because they have an impact on microbiota composition (48–51). Nevertheless, in the present study, no correlations have been found between any of these treatments and SCFA levels in any vascular territory. Finally, although the unavailability of fecal samples to catalog their microbiota content constitutes a limitation of our study, gut microbiota content may differ from that in stools. Accordingly, neither fecal nor circulating levels of SCFAs may represent actual SCFA gut production (52).

In summary, a global descent in SCFA levels in the blood of patients with cirrhosis is associated with more advanced liver disease. Among all SCFAs, butyric acid has consistently and inversely correlated with markers of an impaired gut-liver axis, suggesting a prime role for this molecule in its relationship with the gut epithelial barrier, the maintenance of the host's immune response, and the containment of BT in cirrhosis. FJ

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AUTHOR CONTRIBUTIONS

O. Juanola, P. Zapater, J. M. González-Navajas, and R. Francés evaluated inflammatory markers and endotoxemia in patients' samples, and performed statistical analysis of results; J. Ferrusquía-Acosta, M. Magaz, P. Olivas, A. Baiges, P. Bellot, F. Turón, V. Hernández-Gea, and J. C. García-Pagán included patients and performed hemodynamic procedures; R. García-Villalba, A. Marín, and F. A. Tomás-Barberán performed short-chain fatty acid measurements in patients' samples; and J. C. García-Pagán and R. Francés designed the study and wrote the manuscript.

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