Short chain fatty acids regulate hemodynamics in cirrhosis

by

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Abstract

Advanced cirrhosis is characterized by hemodynamic abnormalities, including increased portal pressure, and decreased arterial pressure, which contribute to the complications of the disease[4]. Short chain fatty acids (SCFAs), produced by the gut microbiota through carbohydrate fermentation, have been shown to regulate blood pressure by interacting with the G-protein coupled receptor GPR41[31]. Impaired liver metabolism, along with dysbiosis in chronic liver disease, can result in elevated circulating levels of SCFAs[35-36]. However, the precise role of SCFAs in cirrhosis-related hemodynamic changes remains unclear. This study aimed to investigate the impact of SCFAs, such as propionate and acetate, on splanchnic vasodilation and hemodynamics in advanced cirrhosis, providing further insights into their potential role in the pathophysiology of the disease.

Methods: A rat model of cirrhosis was established using common bile duct ligation (CBDL), mimicking decompensated cirrhosis. Hemodynamic studies were performed four weeks post-surgery to evaluate mean arterial pressure (MAP), portal pressure (PP), and superior mesenteric artery blood flow (SMABF). The degree of portal systemic shunting (PSS) was determined using colored microspheres, as described by Abraldes et al.[12]. To assess the impact of increased SCFAs, propionate infusion was administered, while metronidazole was used to decrease SCFAs levels.

The results showed that increasing plasma SCFAs through propionate infusion in control rats induced hypotension and splanchnic vasodilation, replicating some hemodynamic features of advanced cirrhosis. In CBDL rats, elevated plasma acetate and propionate levels were observed,

along with hemodynamic abnormalities. Furthermore, propionate infusion in CBDL rats exacerbated splanchnic vasodilation, arterial hypotension, and portal hypertension.

To reduce plasma SCFAs, a subgroup of CBDL rats was treated with metronidazole, leading to improved hemodynamics. Specifically, the metronidazole-treated rats showed a significant 11% increase in mean arterial pressure (MAP), a 12% decrease in portal pressure, and a 36% decrease in superior mesenteric artery blood flow (SMABF). Measurements of SCFA concentration in cecal content revealed no significant differences between CBDL and sham rats, suggesting that elevated plasma levels were not solely attributed to increased colonic production.

In conclusion, this study highlights the potential role of SCFAs in regulating splanchnic vasodilation and its impact on cirrhosis hemodynamics. Increasing circulating SCFAs replicated hemodynamic abnormalities in control rats, while CBDL rats exhibited elevated plasma SCFAs levels and worsened hemodynamics. Decreasing plasma SCFAs through antibiotic treatment improved cirrhosis hemodynamics. These findings contribute to understanding the complex interactions between gut microbiota-derived SCFAs and hemodynamic changes in cirrhosis.

keywords: Short chain fatty acids, cirrhosis, hemodynamics, splanchnic vasodilation, portal hypertension, propionate, metronidazole, gut microbiota

Preface

The study, Role of Propionate as a Vasodilator in Cirrhosis, of which this thesis is a part, received research ethics approval from the University of Alberta Research Ethics Board, ID number AUP00001539.

This work is dedicated to my beloved wife, parents and

Child, for their patience and support.

Thank you.

Acknowledgments

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Without the collective efforts and support from all these individuals, including my supervisor, lab team, supervisory committee, the Department of Medicine, and my loved ones, this study would not have been possible. Thank you once again for your invaluable contributions and for being an integral part of this research endeavour.

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List of Abbreviations and Symbols

SCFAs	Short chain fatty acids
GPR41	G-protein coupled receptor 41
CBDL	Common bile duct ligation
MAP	Mean arterial pressure
PP	Portal pressure
SMABF	Superior mesenteric artery blood flow
PSS	Portal systemic shunting
HRS	Hepato-renal syndrome
LSECs	Liver sinusoidal endothelial cells
NO	Nitric oxide
HRT	Heart rate
CO	Cardiac output
GPR41-/-	GPR41 knockout
sq	Subcutaneous
Kg	Kilogram
I.v.	Intravenous
ml/min/100g	Milliliters per minute per 100 grams
P50	Polyethylene tubing with an internal diameter of 0.5 mm

min	Minutes
mmHg	Millimeters of mercury
µm	Micrometers
g	Grams
µL	Microliters
N	Normal
µg	Micrograms
mL	Milliliters
nmol	Nanomoles
Kg	Kilogram
HCl	Hydrochloric acid
PA	Phosphoric acid g - Grams
°C	Degrees Celsius
SE	Standard error
IQR	Interquartile range
MET	Metronidazole

Introduction

Advanced cirrhosis is associated with profound hemodynamic abnormalities, characterized by a decrease in systemic vascular resistance and an increase in heart rate and cardiac output leading to a progressive *increase in portal pressure* and a progressive *decrease in arterial pressure*[4,5]. These abnormalities account for most complications of cirrhosis. Indeed, portal hypertension and arterial hypotension are strong prognostic factors in these patients[6-8].

The primary and necessary factor leading to increased portal pressure is an increase in hepatic resistance[10]. Endothelial dysfunction in liver sinusoidal endothelial cells (LSECs) reduces the production of vasodilators such as nitric oxide (NO) and favours vasoconstriction, contributing to increased intrahepatic vascular resistance and leading to portal hypertension[90]. But as the disease progresses, endothelial dysfunction develops in the extrahepatic (splanchnic and systemic) circulation, which unlike in LSECs, causes overproduction of vasodilator molecules, leading to *splanchnic arterial vasodilation*. Vasodilation which occurs in the systemic circulation causes arterial hypotension, leading to fluid retention (ascites and edema) and renal hypoperfusion, the cause of hepato-renal syndrome (HRS)[10-11, 84]. This in turn, leads to the hyperdynamic circulation state and contributes to the aggravation of many of the complications of cirrhosis and portal hypertension[81-83] by increasing splanchnic blood inflow. Thus, the development of splanchnic vasodilation plays a central role in hemodynamic abnormalities in cirrhosis[11,12].

A pharmacological reduction in portal pressure and/or an increase in arterial pressure significantly improve the prognosis for cirrhosis patients and are necessary for HRS reversal [6,13-15]. An important concept is that even a moderate reduction in portal pressure (below 12 mmHg or by 10-20% from baseline)[6,16] or a moderate and sustained increase in arterial pressure)[13, 85] has a major impact on the clinical outcomes of these patients.

In the last 25 years, several mediators have been proposed to contribute to splanchnic arterial vasodilation and portal hypertension, including nitric oxide (NO)[4,12], endocannabinoids [86], calcitonin-gene related peptide, adrenomedullin, prostacyclin, carbon monoxide[87], Ang-(1-7)

and urotensin[88-90]. However, unfortunately, this knowledge has not translated into new pharmacologic approaches[9,17]. Thus, the availability of new treatments for patients with cirrhosis and portal hypertension is an unmet medical need.

Short-chain fatty acids (SCFAs; propionate, acetate and butyrate) are produced in the colon through the anaerobic fermentation of carbohydrates by the gut microbiota[22-24]. Acetate, propionate, and butyrate have varying physiologic effects in different body tissues with acetate being the most abundant in the colonic lumen, followed by propionate and butyrate[25,26]. Most butyrate is locally consumed by colonocytes, while the liver metabolizes any remaining gut-derived butyrate, so portal and peripheral concentrations are negligible. Propionate is mainly metabolized by the liver, and peripheral levels are also low in normal subjects[27]. Acetate is the most abundant SCFA in peripheral blood, its levels resulting from the mix of colonic acetate and endogenously produced acetate[27-29]. In chronic liver disease, it has been shown that there are significant differences in the abundance and composition of the microbial taxonomic units between diseased and normal individuals[91]. Therefore, increased circulating SCFA levels is expected in advanced cirrhosis due to dysbiosis in addition to impaired liver metabolism.

SCFAs account for many complex physiological interactions between the gut microbiota and the host, which codevelop from birth and are dependent on many factors, including the host genome, nutrition, and lifestyle[30]. Among these interactions, it was shown that gut-derived SCFA (with the major role for acetate and propionate[31,32]) exerts a chronic effect on the complex array of pathways that regulate blood pressure[33]. Indeed, propionate infusion induced a rapid and dose-dependent hypotensive response in control mice[31]. This effect was likely mediated by activation of the G-protein coupled receptor GPR41 and was completely abrogated in GPR41$^{-/-}$ mice[31]. GPR41$^{-/-}$ mice showed arterial hypertension at baseline[34] suggesting that SCFAs regulate blood pressure at physiological concentrations. More recent studies showed that oral administration of acetate normalized arterial pressure in hypertensive mice [32, 92].

The aim of this study was to assess if circulating short-chain fatty acids (SCFAs) contribute to splanchnic vasodilation (and, consequently, to arterial hypotension and portal hypertension) in advanced cirrhosis.

Methods

Rat model of cirrhosis

Secondary biliary cirrhosis was induced by common bile duct ligation (CBDL)(18). Briefly, under general anesthesia with isoflurane, the common bile duct was isolated, cannulated with a P10 catheter and subsequently injected with 0.1 ml of formalin. Before removing the catheter, two ligatures (5-0 silk thread) were placed, one below the junction of the hepatic ducts and one above the pancreatic ducts. The catheter was removed and the portion between the two ligatures was resected. In sham-operated rats, the common bile duct was dissected but left intact. Following our local protocols for humane animal care, all rats received preoperatively 0.02 mg/Kg of buprenorphine (sq) and 20 mg/kg ampicillin (sq). In the postoperative period, the rats received buprenorphine 0.02 mg/Kg for the first 24 hours, ibuprofen 10 mg/Kg q8 hours orally for 48 hours, and a second dose of ampicillin 20 mg/Kg sq 24 hours after the surgery. On the third day, rats received 40 mg of ampicillin dissolved in drinking water over 24 hours. 14 days after surgery the rats were anesthetized with isoflurane for staples removal and were given a second dose of vitamin K.

Hemodynamic studies

These were performed in all cases 4 weeks after sham or CBDL surgery. Rats were anesthetized with isoflurane. P50 catheters were placed in the femoral artery (for continuous monitoring of mean arterial pressure (MAP)) and the femoral vein (for i.v. infusions and blood sampling). Superior mesenteric artery blood flow (SMABF, ml/min/100g of body weight) was measured with a non-constrictive peri-vascular ultrasonic transit-time flow probe (1RB, 1 mm diameter. Transonic Systems Inc. Ithaca. NY. USA) placed around the superior mesenteric artery close to its aortic origin. The flow probe was connected to a small animal flowmeter (Transonic Systems Inc.,Ithaca, NY. USA). Finally, the ileocolic vein was cannulated with a P50

catheter for portal pressure (PP) measurements. The flow probe and the two pressure transducers were connected to aPower Lab (4SP) linked to a computer using the Chart version 7.2.5 (ADInstruments, Mountain View, LA). The degree of portal-systemic shunting (PSS) was determined as described in Abraldes et al [12]. In brief, 150,000 of 15 µm yellow microspheres (Dye Track, Triton Technology, San Diego, CA) were slowly injected into the spleen. Once the experiment was finished the rat was euthanized and the liver and lungs were dissected and placed into new polypropylene centrifuge tubes. The number of microspheres in each tissue was determined as described previously [12], following the protocol provided by the company. PSS was calculated as total lung microspheres/(total liver microspheres + total lung microspheres).

Interventions to increase and decrease plasma levels of SCFAs

To increase plasma levels of SCFAs, sodium propionate (or saline) was administered as a continuous infusion, 0.2 ml/min, at a dose of 11.2 mg/kg/min (based on Pluznic et al[31]. Propionate was chosen since it is the most potent agonist of the GPR41 receptor[93]. This infusion induces hemodynamic changes at 2 minutes after the onset of the infusion. In 4 rats, samples for SCFA levels were taken at baseline and 2.5 min after the infusion. Propionate infusion induced a 3.92±0.4-fold increase in the plasma levels of total SCFAs. To decrease plasma SCFAs, in a subgroup of rats, 21 days after surgery rats were given 30 mg/Kg of metronidazole in the drinking water. Water consumption was confirmed daily to ensure every rat had a dose of metronidazole within 10% of the target dose.

SCFA measurements

Measurements of SCFA concentration in cecal content were obtained using gas chromatography following the technique described by Laffin et al. [96]. 800 µL of 0.1 N hydrochloric acid and 200 µL of 25% phosphoric acid were used to homogenize 0.2g of stool. 200 µl of 25% phosphoric acid was added and the sample was then centrifuged at 3000g for 10 minutes until obtaining a clear supernatant. An internal standard solution (150 mg of 4-

methyl-valeric acid, S381819, Aigma-Aldrich), 5% phosphoric acid, and the supernatant were added to glass chromatography tubes, mixed well, and stored at room temperature for 30 minutes. The samples were analyzed for SCFA with Varian model 3400 gas chromatograph (Varian, Walnut Creek, CA) using a 30-m x 0.25-mm inner diameter x 0.5 µm film thickness capillary column ((Stabilwax-DA, Restek Corporation, Belfonte, PA). A flame-ionization detector was used with an injector temperature of 170 °C and a detector temperature of 190 °C.

Statistics

Results are expressed as mean ± SE or median (IQR) as appropriate. Since most continuous variables were non-normally distributed, pairwise comparisons between CBDL and sham groups, and between CBDL+MET and CBLD groups were conducted with semi-parametric ordinal regression as described in (Liu Q et al Stat Med. 2017 Nov 30;36(27):4316-4335). This uses the proportional odds model, with no binning of the response variable. The experiments assessing hemodynamics before and after propionate infusion were tested with paired t-test or Wilcoxon signed rank test. A 2-tailed *P* value of less than 0.05 was considered statistically significant. Analysis was conducted in R, with the *rms* package and plots were constructed with the *ggplot* package.

Results

An increase in plasma SCFAs induces hypotension and splanchnic vasodilation in control rats

To explore the potential effects of SCFAs on hemodynamics we first assessed the effects of increasing circulating SCFAs in control (sham-operated) rats. Since among the three main SCFAs, propionate has been shown to be the most potent agonist for GPR41[39, 93], to assess the effect of increasing SCFA concentration on systemic and splanchnic hemodynamics, we challenged sham-operated rats with an infusion of propionate (11.2 mg/Kg/min) and measured mean arterial pressure (MAP), portal pressure (PP) and superior mesenteric artery

blood flow (SMABF, as a surrogate for splanchnic blood flow). We used a saline injection of the same volume to control the effects of volume expansion. Saline infusion did not significantly change hemodynamics, whereas propionate infusion markedly increased SMABF and caused arterial hypotension (Fig. 1A). The lack of significant effects on PP in rats with normal livers, despite the marked increase in portal blood inflow (as assessed by SMABF), was expected, since it is well established that the normal liver (as opposed to the cirrhotic liver[40]) accommodates increases in blood flow without increasing PP[41]. Therefore, these data show that an increase in the concentration of SCFAs in control rats recapitulates some of the splanchnic hemodynamic features of advanced cirrhosis (hypotension and splanchnic vasodilation).

Rats with experimentally induced cirrhosis show increased plasma acetate and propionate levels, and an infusion of propionate aggravates cirrhosis hemodynamic abnormalities.

We then tested whether in the common bile duct ligation (CBDL) rat model of cirrhosis circulating SCFAs have a role in regulating hemodynamics. Four weeks after CBDL, this rat model reproduces many of the features of decompensated cirrhosis,[42,43, 94] including severe portal hypertension, arterial hypotension, splanchnic vasodilation, portal-systemic shunting, and ascites[43-46]. Indeed, these hemodynamic changes were also observed in our CBLD rats, as compared with sham rats (Fig. 1B). In addition, CBLD rats showed increased plasma propionate and acetate as compared to sham rats (Fig. 2B). Butyrate plasma levels were undetectable in most rats (both sham and CBDL rats). To further support the established concept that the microbiome of cirrhotic patients differs from normal controls[91], we tested whether there are differences in cecal SCFA levels between CBLD and sham rats. The results showed no significant differences in the cecal content of SCFAs between sham and CBDL rats (Table 1), though levels of propionate and especially butyrate were numerically lower in CBDL rats. This suggests that increased plasma levels were not due to increased colonic production of SCFAs.

To test if a further increase in SCFA levels would aggravate the already abnormal cirrhosis hemodynamics, after baseline hemodynamics CBLD rats were infused with propionate (11.2 mg/Kg/min.) or saline. Compared with saline infusion, propionate infusion significantly aggravated splanchnic vasodilation, arterial hypotension, and portal hypertension (Fig. 1B).

A decrease in plasma SCFAs induced by antibiotics is associated with an improvement in cirrhosis hemodynamics.

Since gut production accounts for most circulating SCFAs [24,47], the most effective strategy for decreasing circulating SCFAs is to suppress gut production by eliminating producing bacteria with antibiotics[31,48]. Metronidazole has been shown to be very effective in decreasing gut SCFA production[48]. To prove the concept that decreasing circulating SCFAs can improve hemodynamics, CBDL rats (n=20) were given metronidazole or a placebo from day 21 to day 28 (after CBDL) (Fig. 2A). As expected, metronidazole treatment significantly decreased cecum propionate, acetate, and butyrate (Table 1), as well as plasma propionate and acetate (Fig. 2B). In addition, metronidazole treatment significantly increased MAP (by 11%), decreased portal pressure (by 12%) and decreased SMABF (by 36%), whereas there were no changes in portal systemic shunting (Fig. 3).

Since the effects of metronidazole on hemodynamics are likely multifactorial, we further assessed if these effects could be explained, at least in part, by decreased SCFA signalling. After baseline measurements, six metronidazole-treated rats were infused with propionate (11.2 mg/kg/min) to restore high SCFA levels. Propionate infusion reversed the beneficial hemodynamic effects of metronidazole by decreasing arterial pressure ($p=0.005$), increasing portal pressure ($p=0.028$) and increasing SMABF ($p=0.032$) (Fig. 4). Altogether, these results suggest that SCFAs participate in the hemodynamic derangement of CBDL-induced cirrhosis and that improvement in cirrhosis hemodynamics might be achieved by decreasing circulating SCFAs.

Discussion

In this study, we demonstrated that circulating SCFAs contribute to the splanchnic hemodynamic disorder in advanced cirrhosis including splanchnic vasodilation, arterial hypotension, and portal hypertension. Because these are the main features that account for most complications of cirrhosis, the findings of this study are of importance not only to understand the pathophysiology of splanchnic vasodilation and portal hypertension but also to address new pharmacologic approaches for cirrhosis. It has been established that there are significant differences in the microbial taxonomic composition and abundance between normal and cirrhotic patients, where the microbiome in cirrhosis is affected by multiple processes occurring both at the level of the gut and systematically[92,95]. Based on this information, we expected differences in cecal SCFAs levels of CBDL rats and control rats; however, our results showed no significant differences in cecal SCFAs levels, suggesting that increased plasma levels were not due to increased colonic production of SCFAs but likely due to impaired liver metabolism. Additionally, we have shown that improvement in cirrhosis hemodynamics can be achieved by decreasing circulating SCFA, further supporting our hypothesis and providing a meaningful target in the treatment of patients with chronic liver disease.

There is little doubt that the pathogenesis of vasodilation in cirrhosis is multifactorial. Indeed, although Fig. 3 shows that there is a significant association between SCFAs and portal and arterial pressure, there is substantial dispersion, and several other pathogenic factors are likely involved. However, the magnitude of an increase in arterial blood pressure to achieve a therapeutic benefit might be as low as 5 mm Hg[13], and a pharmacological decrease in portal pressure as small as 10% can decrease cirrhosis complications[16]. Major clinical benefits, therefore, can be obtained with partial improvements in hemodynamics, and targeting all involved mediators might not be necessary to achieve a clinical benefit.

The most straightforward approach to targeting SCFA-induced vasodilation would be to block the GPR41 receptor or its downstream signaling. This could have the additional advantage of selectively targeting the vasodilating effects of SCFAs, releasing the compensatory vasoconstrictive effects mediated by Olfr78[31]. At this point, however, there are no selective

pharmacological inhibitors for the GPR41 receptor. Nonetheless, several patents are pending and they will predictably become available in the near future[49].

Moreover, since most SCFAs come from the metabolism of carbohydrates by the gut microbiota[22,24], another strategy would be to target the gut production of SCFAs. Indeed, germ-free animals show negligible plasma levels of SCFAs[47], and our own preliminary data shows the potential of antibiotic treatment to decrease plasma levels of acetate and propionate. This would be a highly feasible treatment, immediately available, for short periods of time (to avoid potential adverse effects of prolonged antibiotic use) in patients with cirrhosis. Long-term treatments, however, would be likely associated with the off-target potential complications of antibiotic therapy, and therefore interventions targeted to the GPR41 receptor would be of high therapeutic interest. Another potential therapeutic option would be the non-antibiotic modulation of SCFA production (with different dietary substrates).

As discussed in the introduction, previous data from non-cirrhotic mice showed that the GPR41 receptor mediates SCFAs-induced vasodilation[31,34]. However, information regarding the pathways underlying SCFA-induced vasodilation is very limited. Acetate was first shown to cause vasodilation in vivo almost 90 years ago, an effect which was proposed to be due to the dilation of small arteries[59]. Subsequent studies confirmed a vasodilator action both in vivo[60-62] and in vitro[63-66]. Seminal studies in the 1990s showed that in rat tail artery (a conduit vessel) [63], human colonic resistance arteries[66], and rat mesenteric resistance arteries, relaxation to acetate occurred independently of the endothelium, possibly through a reduction in the sensitivity of the smooth muscle contractile filaments to Ca^{2+} [67]. On the other hand, propionate has been suggested to induce vasodilation through both direct effects on smooth muscle cells[66] and activation of endothelial K^+ channels[68], although, in the latter study, interpretation of the data is complicated by the use of only one concentration of propionate (10 mM) and the absence of representative traces. More recent studies in mouse tail arteries showed that vasodilation to acetate and propionate is partially endothelium-dependent but NO-independent[34]. Furthermore, *in-vivo* studies showed that GPR41-/- mice showed resistance to propionate-induced hypotension[31]. Finally, vascular expression of

GPR41, at least in the tail artery of mice, is restricted to the endothelium[34]. However, studies assessing these responses in vessels from GPR41$^{-/-}$ have not been conducted and, therefore, it is still unknown if GPR41 has an obligatory role in vasodilation to SCFAs, which would be essential to define GPR41 as a therapeutic target. Additionally, further research is needed to determine the downstream mediators underlying the vascular effects of SCFAs. Future studies should clarify these issues, and narrow down whether GPR41, rather than SCFAs in themselves, are essential for modulating MAP and PP, which can have several beneficial effects for the gut and the rest of the body, should be pursued as a target.

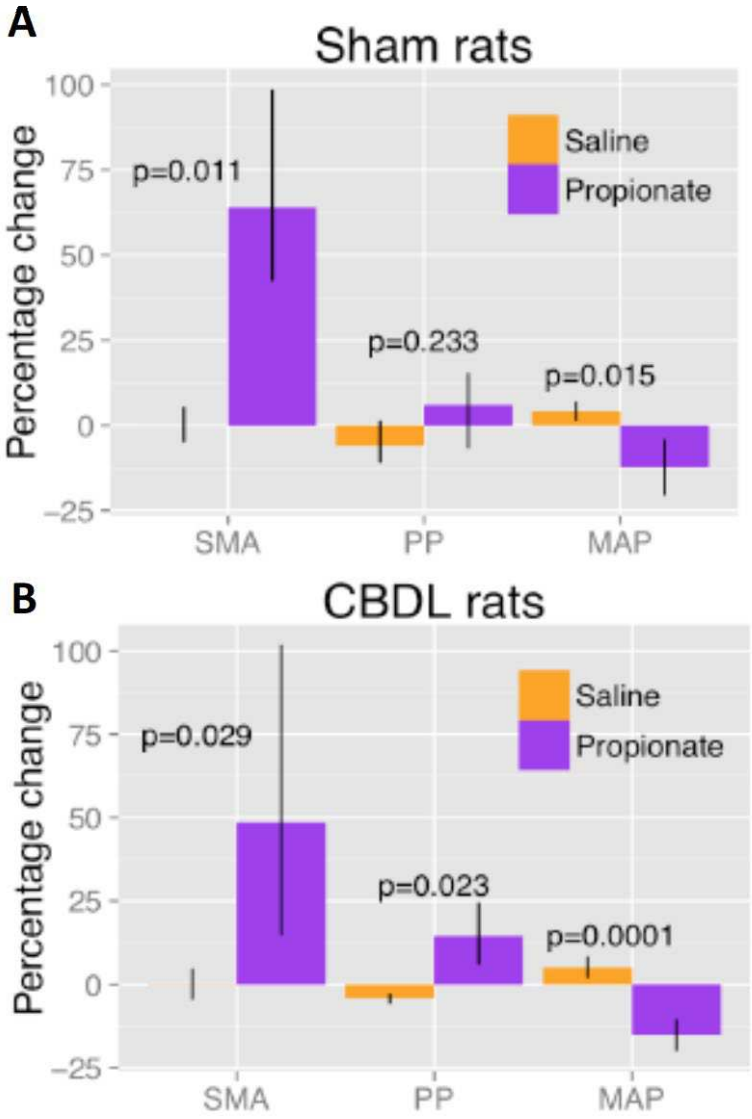

Figure 1. Hemodynamic changes in response to a propionate infusion in sham and CBDL rats. A. In Sham rats, an infusion of propionate (11.2 mg/Kg/min), as compared with an isovolumetric saline infusion, induced splanchnic vasodilation (as shown by an increase in superior mesenteric artery blood flow (SMA), did not significantly change portal pressure (PP) and induced arterial hypotension (as shown by a decrease in mean arterial pressure (MAP)). B. In CBDL rats, propionate infusion aggravated splanchnic vasodilation, portal hypertension and arterial hypotension, as compared with saline infusion.

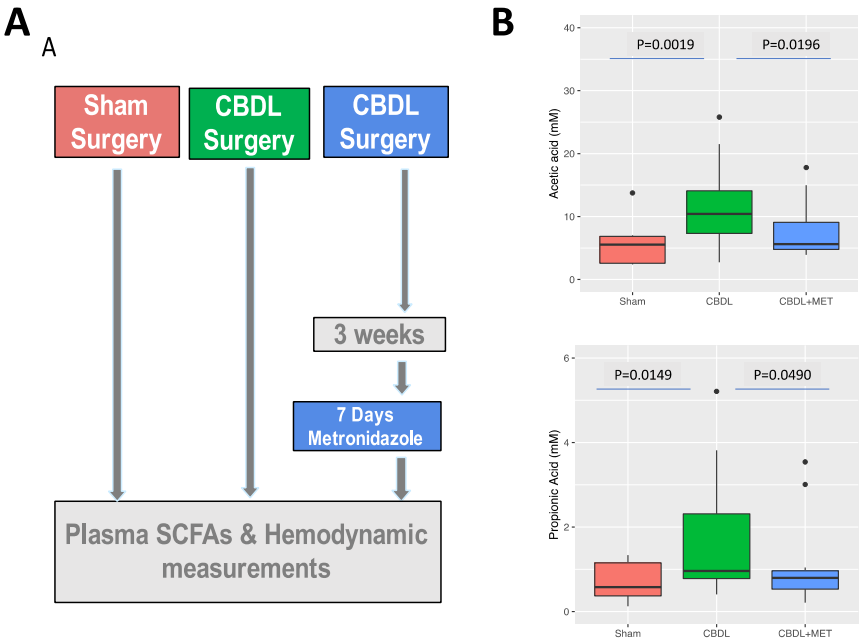

Figure 2A. Schematic representation of the study protocol. Rats underwent common bile duct ligation surgery (CBLD) or sham surgery. Hemodynamic studies were conducted on day 30. In a subgroup of rats, metronidazole (MET) was administered in drinking water from day 21 to day 28. **B. Plasma levels of acetate and propionate in rats from the three groups.** CBDL rats had higher levels of acetate and propionate than sham rats. Metronidazole treatment decreased plasma acetate and propionate (butyrate levels were undetectable in most rats and are not shown).

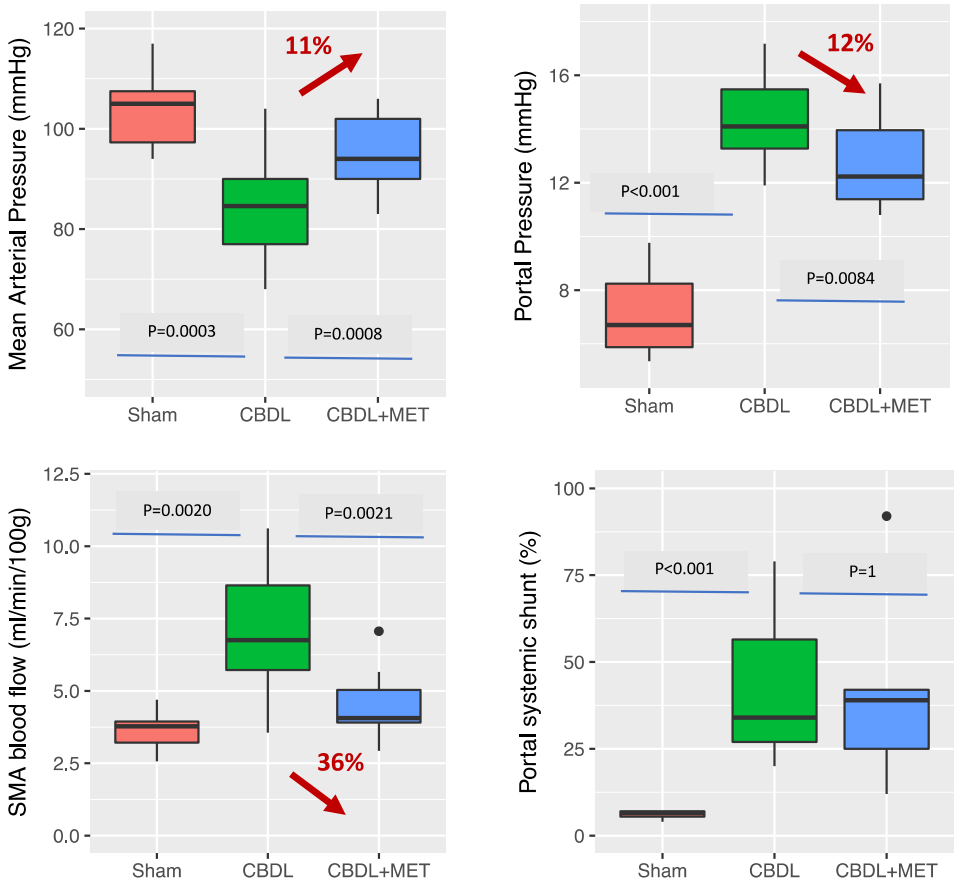

Figure 3. Comparison of the hemodynamics of the three study groups: Sham-operated rats, CBDL rats and CBDL rats treated with metronidazole (MET). CBDL rats had lower mean arterial pressure (MAP), higher portal pressure (PP) and higher superior mesenteric artery (SMA) blood flow than sham rats. Metronidazole treatment partially reverted these hemodynamic abnormalities, increasing MAP and decreasing PP and SMA blood flow. Portal systemic shunt (PSS) was less than 5% in all sham rats, as compared with a median of 35% in CBDL rats. This was not significantly modified by treatment with metronidazole.

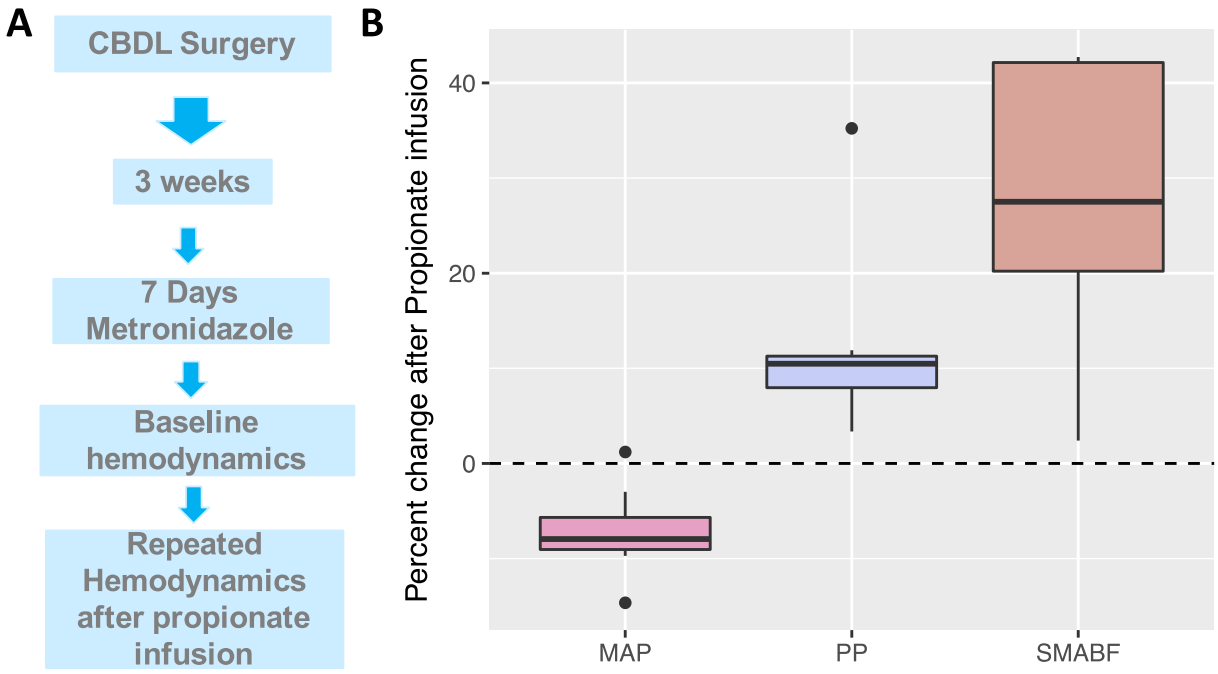

Figure 4. A: Schematic representation of the study protocol aiming at restoring high SCFAs in rats treated with metronidazole. After the baseline measurements, propionate was infused at 11.2 mg/Kg/min. **B. Hemodynamic effects of restoring high SCFAs**. Propionate infusion decreased mean arterial pressure (p=0.005), increased portal pressure (p=0.028) and superior mesenteric artery blood flow (SMABF) (p=0.032).

Table 1. Short chain fatty acid levels in cecal samples from Sham-operated, CBLD and CBLD rats treated with metronidazole (median, IQR)

	Sham (n=8)	CBDL (n=22)	CBDL+MET (n=13)	p-value (CBLD vs Sham)	p-value (CBDL+MET vs CBLD)
Cecal Acetate (median, IQR)	157 (88, 197)	174 (77,240)	47 (27,221)	0.6522	0.0247
Cecal Propionate (median, IQR)	56 (29,72)	33 (18,50)	12 (8,44)	0.3118	0.0241
Cecal Butyrate (median, IQR)	113 (48,153)	68 (32,104)	11 (26,65)	0.1307	0.0064
Cecal total SCFAs (median, IQR)	353 (163,396)	296 (134,396)	79 (48,330)	0.5738	0.0323

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