

2022-05-31

The effect of increasing intestinal shortchain fatty acid concentration on gut permeability and liver injury in the context of liver disease: A systematic review

Pohl, K

<http://hdl.handle.net/10026.1/19451>

10.1111/jgh.15899


Journal of Gastroenterology and Hepatology

Wiley

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

SYSTEMATIC REVIEW

The effect of increasing intestinal short-chain fatty acid concentration on gut permeability and liver injury in the context of liver disease: A systematic review

Keith Pohl,^{*,†}  Prebhashan Moodley,^{*,†}  and Ashwin Dhanda^{*,†} 

^{*}Hepatology Research Group, Faculty of Health, University of Plymouth and [†]South West Liver Unit, University Hospitals Plymouth NHS Trust, Plymouth, UK

Key words

cirrhosis, liver injury, permeability, SCFA.

Accepted for publication 19 May 2022.

Correspondence

Dr Keith Pohl, South West Liver Unit, University Hospitals Plymouth NHS Trust, Plymouth, UK.
Email: keith.pohl@plymouth.ac.uk

Declaration of conflict of interest: The authors declare no conflicts of interest.

Author contribution: Conceptualization: Keith Pohl and Ashwin Dhanda. Data curation: Keith Pohl and Prebhashan Moodley. Formal analysis: Keith Pohl and Prebhashan Moodley. Investigation: Keith Pohl and Prebhashan Moodley. Methodology: Keith Pohl and Ashwin Dhanda. Project administration: Keith Pohl. Supervision: Ashwin Dhanda. Writing - original draft: Keith Pohl and Prebhashan Moodley. Writing - review & editing: Ashwin Dhanda. All authors approve the final manuscript and authorship list. No professional medical writers were involved. All authors confirm that they had full access to the all study data and accept responsibility to submit for publication.

Abstract

Background and Aim: The gut barrier protects the liver through tight junctions, which are disrupted in liver disease either from dysbiosis, inflammation, or the effects of ingested compounds such as alcohol. Strengthening of the gut barrier may ameliorate liver injury of varying etiologies. Short chain fatty acids (SCFAs) have been shown to improve gut barrier function. This systematic review aims to synthesize all studies that have trialed SCFA supplementation as a therapy for liver disease.

Methods: A systematic review assessing the impact of SCFA supplementation on liver injury and intestinal permeability was conducted. All forms of intervention that specifically increased intestinal SCFA concentration and measured both liver injury and permeability were eligible. Two independent reviewers assessed each study for outcomes, risk of bias, and quality using checklists relevant to the study's methodology.

Results: Seventeen studies were identified; two utilized a human model (15 murine). Fifty-eight markers of liver injury were identified, with 26 different measures of permeability. Given the numerous designs, no meta-analysis was possible. SCFA supplements included oral and enteral butyrate, probiotics, and prebiotics. Fourteen studies demonstrated improved permeability. All studies showed a significant amelioration of liver injury.

Conclusions: Short chain fatty acid supplementation to reduce intestinal permeability represents a potential therapy in a variety of liver disease models. A large number of outcome measures were reported however not all are practical in human studies. Future work should evaluate methods to increase luminal SCFA concentrations and the effect of this on gut permeability and liver inflammation in people with liver disease.

Financial support: No specific financial or non-financial support was received.

Guarantor of the article: Keith Pohl.

Registration and protocol: Registered on the PROSPERO database (ID: CRD42021248521). No amendments were made after registration. Available from: https://www.crd.york.ac.uk/prospero/display_record.php?RecordID=248521.

Introduction

Despite the breadth in both acuity and etiology of liver disease, the gut-liver axis is implicated to play a central role in the pathogenesis of all presentations. Defined as the bidirectional relationship between the gut, its microbiome, and the liver,¹ this is enabled by the portal vein, mediated by the gut mucosal barrier and affected by genetic, dietary and environmental factors.²

Cirrhosis (of any etiology) is associated with profound gut dysbiosis.³ Dysbiosis contributes to reduced gut epithelial integrity through the alteration of tight junctions and disruption of the mucous layer and is associated with disease severity.^{3–5} Studies have documented increased gut permeability in patients with cirrhosis, particularly those with advanced cirrhosis or ascites.⁶ Disruption of the gut barrier exposes the liver to inflammatory

signals and pathogen-associated molecular patterns (PAMPs), driving liver damage.¹ Understanding the role of the gut barrier and microbiome in the development of liver disease has identified a potential for therapeutics that target increased intestinal permeability.

Short chain fatty acids (SCFAs) derived from the bacterial fermentation of indigestible fibers act as the main source of energy for colonocytes and have been reliably shown to strengthen gut barrier function.⁷ Supplementation with oral SCFAs as well as transplantation of SCFA producing probiotic bacteria strengthens the integrity of the paracellular junctions between colonocytes.⁸

Interventions that increase intestinal SCFAs may slow or halt the progression of liver disease by reducing the immune-related damage caused by portal transmission of PAMPs and bacterial endotoxins. This systematic review aims to identify, evaluate, and synthesize all literature investigating the effect of treatments designed to increase intestinal SCFA concentration on liver outcomes and gut barrier integrity in the context of liver disease.

Methods

A systematic review was performed.

Eligibility criteria. In order to be eligible for inclusion studies must have met the following criteria:

- **Population** – Human studies or murine models of acute or chronic liver disease of any etiology.
- **Intervention** – Any of administration of SCFAs enterically; high fiber diet with the explicit intention of increasing SCFA concentrations; and microbial interventions (either fecal microbiota transplant, prebiotic, or probiotic therapy) with the explicit intention of increasing SCFA concentrations.
- **Comparisons** – Non-intervention.
- **Outcomes** – Studies must have assessed both changes to intestinal permeability and markers of liver disease.

All study designs were accepted, and given the paucity of research in this area, no minimum or maximum durations were required. Conference abstracts were excluded as were unpublished manuscripts and papers that did not measure both outcomes. Cell culture models are not able to assess both intestinal permeability and liver injury in the context of active liver disease. These were acknowledged if performed alongside a human or animal model but excluded if the sole methodology.

Search strategy. A three-stage search strategy was employed. A basic ‘fact-finding’ search of the PubMed database was first employed to identify key words. The PubMed/MEDLINE and CENTRAL/Cochrane databases were then searched using the PICO search strategy in the supporting information, between January and July 2021 with no constraint on dates of articles. A combination of cirrhosis, short chain fatty acid, permeability, and their synonyms was used.

The reference lists of identified reports and any relevant reviews were examined. The abstracts of potentially eligible titles were screened. Two reviewers independently collected data from each report, with any disagreements resolved by a third reviewer. No specialist software was utilized.

Both direct and surrogate markers of liver injury and intestinal permeability were accepted. A meta-analysis was planned but not possible as the review found that numerous study designs and measures of both permeability and liver injury were used. Patient-level data were not sought.

Risk of bias, quality, and certainty assessment.

Two reviewers independently assessed for bias, with and disagreements settled by a third reviewer. Animal studies, observational studies, and randomized trials were assessed using the ARRIVE 2.0,⁹ STROBE,¹⁰ and CONSORT¹¹ checklists, respectively. Marks were awarded for each checklist item, with two marks awarded if the whole item was clearly reported, one mark if partially reported, and zero mark if missing. Scores were totaled, and a mark of good (> 80%), average (50–80%), or poor (< 50%) awarded.¹²

Results

There were no foreign language reports; 741 records were identified of which 17 met all the selection criteria (Fig. 1). Of the 94 articles assessed for inclusion, 61 were duplicates, 9 utilized an intervention other than SCFAs, 6 used cell culture models only without concurrent liver assessment, and 1 did not measure liver injury. Permeability was significantly reduced by the SCFA intervention in all measures in 13 studies, in some measures in 1 study, and unaffected in 3 studies. Liver injury was significantly ameliorated by the SCFA intervention in all studies, with 12 reporting universal improvements in liver-related outcomes and 5 showing an improvement in some measures only. Table 1 provides a summary of the effects of increasing SCFA concentrations on permeability and liver injury measured in the identified studies. Table 2 summarizes the pathologies and interventions studied along with individual quality assessments.

Table S1 details the specific models and findings of the identified studies. Studies excluded at the full-text screening stage are listed in the supporting information. All of the identified studies measured permeability using outcomes specific to the paracellular pathway. It is this pathway that is governed by tight junctions and felt to be responsible for the “leaking” of PAMPs and inflammatory signals that drive liver injury.

Quality and risk of bias assessment. Fourteen studies scored “average” and three “poor.” In the murine studies, there was a universally poor reporting of blinding and animal care/monitoring leading to lower scores. Full assessments are presented in Tables S2–S4.

As all of the eligible papers utilized varying models and laboratory methods, and a large number of outcome measures were identified (Tables S5 and S6), no meta-analysis was possible. All studies used control groups which did not receive the SCFA intervention.

Effect of short chain fatty acids on permeability and liver injury in murine alcohol related liver disease models. Three studies assessed the impact of SCFA supplementation in alcohol-related liver disease using chronic, short term, and binge alcohol ingestion murine models.^{14,19,28} The

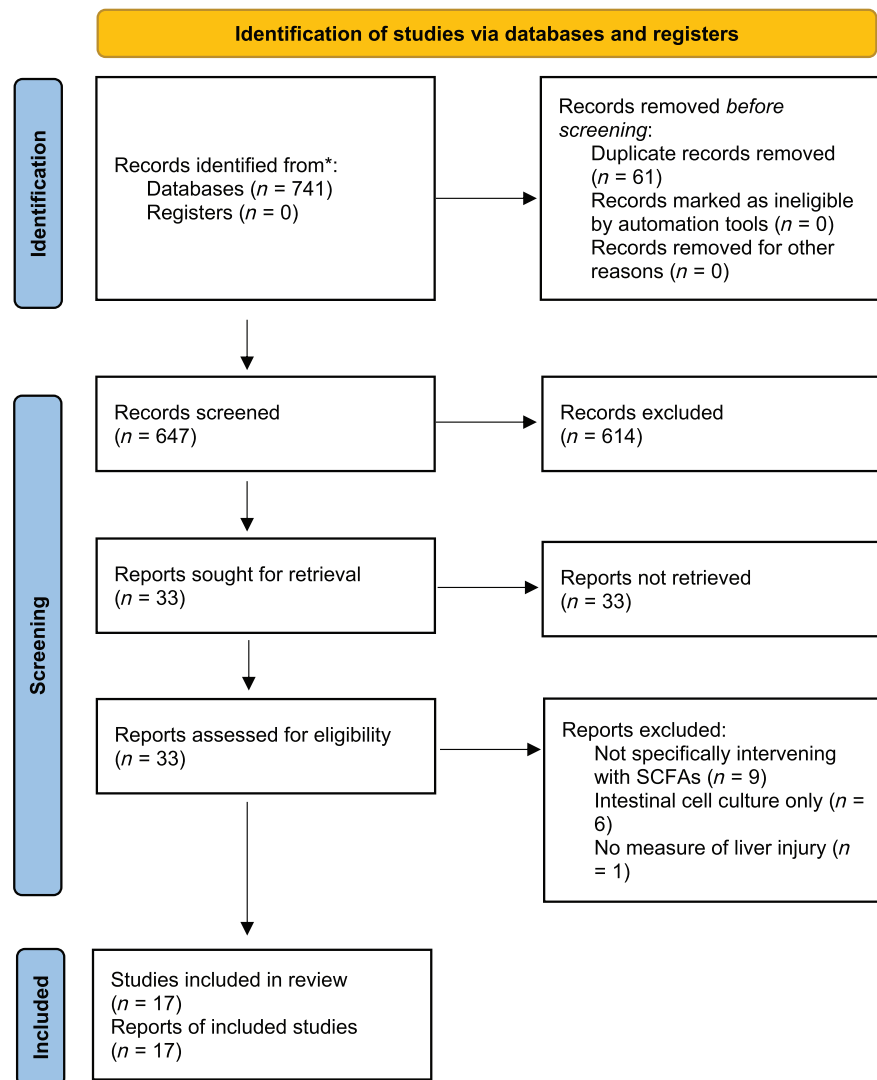


Figure 1 Study selection flow diagram.

SCFA used in two of the studies was tributyrin, and one study used a probiotic containing the SCFA-producing strain *Pediococcus pentosaceus*.

Cresci *et al.*¹⁴

Model. Hepatic triglycerides, tumor necrosis factor alpha (TNF- α), plasma alanine transaminase (ALT), and histological tight junction (TJ) protein expression were compared in a binge model (2 days ad lib alcohol followed by a single large dose of alcohol after an overnight fast) and a chronic model (increasing alcohol over 25 days). Mice either received tributyrin (a pro-drug of butyrate) supplementation or not. Pair fed mice acted as controls.

Effect on liver. Tributyrin pre-treatment blunted binge induced increases in ALT. Tributyrin also prevented histological (liver) tumor necrosis factor (TNF)- α rises in the binge feeding model.

Hepatic triglyceride rises were not mitigated by tributyrin supplementation.

Effect on permeability. Pre-treatment and dietary supplementation with tributyrin protected tight junction protein expression in both models.

Cresci *et al.*¹⁹

Model. The effect of tributyrin was evaluated in a chronic alcohol model (sustained alcohol feeding for 10 days, followed by a single gavage of alcohol).

Effect on liver. Tributyrin protected against caval plasma ALT rises. Histological (liver) TLR4 and TNF- α levels in the tributyrin treated group remained the same as controls. Histologically, tributyrin supplementation did not prevent steatosis or neutrophil infiltration.

Table 1 Summary of key findings on gut permeability and liver injury of eligible studies

Study	Effect on permeability	Effect on liver injury
Endo <i>et al.</i> ¹³	Green	Green
Cresci <i>et al.</i> ¹⁴	Green	Amber
Liu <i>et al.</i> ¹⁵	Green	Green
Yang <i>et al.</i> ¹⁶	Green	Green
Jin <i>et al.</i> ¹⁷	Red	Amber
Ferolla <i>et al.</i> ¹⁸	Red	Amber
Cresci <i>et al.</i> ¹⁹	Green	Amber
Matheus <i>et al.</i> ²⁰	Green	Green
Wu <i>et al.</i> ²¹	Amber	Green
Zhou <i>et al.</i> ²²	Green	Green
Hu <i>et al.</i> ²³	Green	Green
Krawczyk <i>et al.</i> ²⁴	Green	Green
Peng <i>et al.</i> ²⁵	Green	Green
Ye <i>et al.</i> ²⁶	Green	Green
Baumann <i>et al.</i> ²⁷	Red	Amber
Jiang <i>et al.</i> ²⁸	Green	Green
Yang <i>et al.</i> ²⁹	Green	Green

Specific findings for each study are summarized in Table S1. Green: significantly reduced in all measures. Amber: significantly reduced in some measures. Red: not significantly altered.

Table 2 The liver injuries and interventions studied with quality/bias assessments

Study	Liver injury studied	Intervention	Quality/bias assessment
Endo <i>et al.</i> ¹³	NASH	<i>Clostridium butyricum</i>	Amber
Cresci <i>et al.</i> ¹⁴	Alcohol	Tributylin	Red
Liu <i>et al.</i> ¹⁵	Ischemia	NaB	Amber
Yang <i>et al.</i> ¹⁶	Toxin	NaB	Amber
Jin <i>et al.</i> ¹⁷	NASH	NaB	Amber
Ferolla <i>et al.</i> ¹⁸	NASH	Prebiotic	Amber
Cresci <i>et al.</i> ¹⁹	Alcohol	Tributylin	Amber
Matheus <i>et al.</i> ²⁰	NASH	NaB	Amber
Wu <i>et al.</i> ²¹	AIH	NaB	Amber
Zhou <i>et al.</i> ²²	NASH	NaB	Amber
Hu <i>et al.</i> ²³	AIH	High fiber diet + NaB	Amber
Krawczyk <i>et al.</i> ²⁴	NASH	High fiber diet	Amber
Peng <i>et al.</i> ²⁵	NASH	NaB	Red
Ye <i>et al.</i> ²⁶	NASH	NaB	Amber
Baumann <i>et al.</i> ²⁷	NASH	NaB	Amber
Jiang <i>et al.</i> ²⁸	Alcohol	<i>Pediococcus pentosaceus</i>	Amber
Yang <i>et al.</i> ²⁹	NASH	<i>C. butyricum</i> + NaB	Amber

Amber: average. Red: poor.

AIH, auto-immune hepatitis; NaB, sodium butyrate; NASH, non-alcoholic steatohepatitis.

Effect on permeability. Tributyrin led to increased expression of TJ proteins zonula occludens (ZO-1), occludin and claudin in intestinal sections compared with the group that only received

ethanol. This model inflicted minimal damage to the intestinal mucosa.

Jiang *et al.*²⁸

Model. The SCFA-producing probiotic *P. pentosaceus* was tested in a chronic alcohol model (alcohol containing diet for 10 days).

Effect on liver. *P. pentosaceus* alleviated ethanol induced inflammation, attenuated steatosis, and decreased hepatic triglycerides. It also significantly correlated with reduced levels of plasma ALT, aspartate aminotransferase (AST), and hepatic neutrophil infiltration. Pro-inflammatory cytokines in plasma significantly decreased in the probiotic group (interleukin [IL]-5, TNF α , TLR4, macrophage inflammatory protein [MIP]-1 α , monocyte chemoattractant protein [MCP]-1, Granulocyte-Colony Stimulating Factor [G-CSF]).

Effect on permeability. *P. pentosaceus* treatment increased ZO-1 mRNA and mucin mRNA expression (MUC-1, MUC-2, and MUC-4) compared with both the alcohol only group and control group. The probiotic group also demonstrated decreased plasma lipopolysaccharide binding protein (LBP; a marker of endotoxemia and surrogate for permeability) compared with the alcohol group.

Effect of short chain fatty acids on permeability and liver injury in animal non-alcoholic steatohepatitis models. Eight studies assessed the impact of SCFA supplementation in varying models of non-alcoholic fatty liver disease (NAFLD)/NASH.^{13,17,20,22,25–27,29} Six studies supplemented sodium butyrate (NaB) orally, and another study supplemented MIYAIRI 588, a butyrate-producing probiotic. One study supplemented sodium butyrate in one experimental group and a butyrate-producing probiotic (*Clostridium butyricum*) in another.

Endo *et al.*¹³

Model. Rats were fed a choline deficient amino acid (CDAA) diet for 2 weeks to induce steatosis, and then supplemented with MIYAIRI 588 (a probiotic containing *C. butyricum*) for up to 50 weeks.

Effect on liver. Rats supplemented with probiotics demonstrated improved markers of liver inflammation, oxidative stress and intestinal integrity compared with controls. Probiotic supplemented rats also demonstrated decreased levels of 4-hydroxynonenal (4-HNE) and malondialdehyde (markers of oxidative stress), significantly improved ALT levels, hepatic levels of TNF α , nuclear factor kappa B (NF- κ B) and improved intestinal expression of ZO-1 and occludin levels compared with controls. MIYAIRI 588 treated rats had greater expression of nuclear factor-erythroid factor 2-related factor 2 (Nrf2), a transcriptional activator that induces expression of many cytoprotective genes against oxidative damage.

Effect on permeability. Probiotic supplemented rats demonstrated suppressed portal venous endotoxemia and improved intestinal expression of ZO-1 and occludin levels compared with controls.

Jin *et al.*¹⁷

Model. Mice were fed a control and western standard diet (WSD) with or without NaB supplementation for 6 weeks.

Effect on liver. ALT levels did not differ among the groups. NaB supplemented mice had reduced steatosis, inflammatory foci, neutrophils and infiltrating immune cells in liver specimens compared with mice on the WSD. NaB supplementation in WSD groups led to significantly lower levels of inducible nitric oxide synthase (iNOS) and 4-HNE. NaB-treated mice had lower levels of TLR4 mRNA and MyD88 in liver sections compared with controls.

Effect on permeability. NaB supplementation did not increase expression of ZO-1 and occludin in the duodenum of mice fed the WSD.

Matheus *et al.*²⁰

Model. Mice were fed a high fat diet for 60 days with or without NaB supplementation throughout.

Effect on liver. NaB supplementation led to a significant decrease in hepatic steatosis.

Effect on permeability. Increased expression of tight junction protein claudin-1 and decreased intestinal permeability as measured by FITC dextran test.

Zhou *et al.*²²

Model. Mice were fed a standard diet, a high fat diet or a high fat diet with NaB for 16 weeks.

Effect on liver. NaB supplementation ameliorated rises in ALT and AST. NaB-treated mice had lower levels of pro-inflammatory cytokines (MCP-1, TNF α , IL-1, IL-2, IL-6, interferon [IFN]- γ , TLR4, and MyD88) in liver sections compared with the high fat diet group.

Effect on permeability. NaB supplementation increased expression of small intestinal ZO-1. Reduced intestinal villus height was ameliorated by NaB supplementation. Plasma and liver endotoxin also decreased in the NaB compared with the high fat diet group.

Peng *et al.*²⁵

Model. Mice were fed a high fat diet and supplemented with traditional Chinese medicine geniposide/chlorogenic acid (GC) or NaB for 12 weeks, alongside a control. GC is theorized to modulate the gut microbiota to treat NAFLD.

Effect on liver. NaB supplementation was associated with reductions in plasma ALT and hepatic triglycerides.

Effect on permeability. Liver tissue LBP, plasma lipopolysaccharide (LPS), and plasma D-Lactate (markers of endotoxemia and increased permeability) were lower in the NaB group. NaB restored expression of ZO-1 and occludin in the intestine indicating improved intestinal integrity.

Ye *et al.*²⁶

Model. A methionine choline deficient (MCD) diet was used to induce NASH in mice with or without NaB supplementation for 6 weeks.

Effect on liver. NaB supplementation was associated with reduced liver fibrosis and steatosis and decreased levels of plasma AST and ALT compared with controls. IL-4 and IL-10 were up-regulated in the NaB group.

Effect on permeability. NaB supplementation increased Claudin-1 and ZO-1 mRNA expression and reduced plasma LPS compared with mice fed the MCD diet alone.

Baumann *et al.*²⁷

Model. Mice were fed a fat, fructose and cholesterol (FFC) diet to induce steatosis (or control diet). After 8 weeks, the animals were either sustained on this or supplemented with NaB for 5 weeks.

Effect on liver. NaB supplementation had no significant effect on ALT and AST. NaB treatment in the FFC fed group decreased liver inflammation on histology evidenced by lower NAFLD Activity Score and decreased number of infiltrating immune cells. Liver TNF α , IL-6, iNOS, and 4-HNE was similar in NaB supplemented mice and controls.

Effect on permeability. Histological ZO-1, occludin, and portal endotoxemia were not significantly different compared with mice fed the FFC diet alone.

Yang *et al.*²⁹

Model. Diabetic mice (C57BLKS/J) were fed a normal diet for 6 weeks, with one group supplemented with NaB, a second with *C. butyricum* and a third control.

Effect on liver. NaB and *C. butyricum* supplementation was associated with decreased AST, ALT, and alkaline phosphatase (ALP), and lower systemic inflammatory markers (IL-1 β , IL-6, and TNF α). TLR4, Myd88, and NF- κ B in liver sections were significantly down-regulated in mice treated with either intervention.

Effect on permeability. Both interventions restored TJ proteins (ZO-1 and occludin), decreased plasma LPS, and improved intestinal histological appearances.

Effect of short chain fatty acids on permeability and liver injury in other murine models. The remaining eligible studies investigated animal models of autoimmune hepatitis (two studies), ischemia reperfusion injury (one study), and toxin induced acute liver failure (one study).^{15,16,21,23} The SCFA supplementation was either orally, intravenously or through a high fiber diet.

Lui *et al.*¹⁵

Model. Ischemia reperfusion injury was induced in rats by 30-min tourniquet of the portal triad. The treatment group was given intravenous NaB pre-operatively.

Effect on liver. NaB pre-treatment decreased plasma AST, ALT, intrahepatic TNF α , IL-6, and TLR4 expression, macrophage activation and neutrophil infiltration.

Effect on permeability. NaB attenuated intestinal injury, prevented ultrastructural alterations of tight junctions, and improved expression of ZO-1.

Yang *et al.*¹⁶

Model. Acute on chronic toxin-induced liver injury was induced with 14 weeks of human plasma albumin injection followed by acute intraperitoneal injection of endotoxins (D-Gal and LPS). Intervention groups received intravenous NaB at either 12- or 24-h post induction of acute liver injury.

Effect on liver. NaB treated groups had less cellular necrosis compared with the control group. Rises in plasma ALT, AST, total bilirubin, TNF α , IFN- γ , and HMGB1 were ameliorated in both intervention groups compared with controls.

Effect on permeability. Intestinal permeability (measured using everted gut sac method) and intestinal damage was reduced with NaB treatment. NaB groups demonstrated significantly lower plasma endotoxin levels than controls.

Wu *et al.*²¹

Model. Autoimmune hepatitis (AIH) was induced through intra-peritoneal injection of Freud's adjuvant on days 1 and 8.

The treatment group was supplemented by daily gavage of NaB for 3 weeks.

Effect on liver. NaB treatment attenuated rises in plasma ALT and AST as well as liver IL-6, TNF α , TLR4, and MyD88 compared with controls. NaB treatment ameliorated lymphocyte infiltration and hepatocyte lesions.

Effect on permeability. NaB increased expression of the ZO-1, occludin, and claudin-1 and reduced intestinal villus disruption and *Escherichia coli* protein in the liver compared with controls.

Hu *et al.*²³

Model. Autoimmune hepatitis was induced with intraperitoneal injections of Freud's adjuvant and S100 antigen in mice fed a standard diet, high fiber diet, or diet supplemented with NaB for 4 weeks.

Effect on liver. The high fiber diet and NaB reduced plasma ALT, AST, IL-17A, and IL-6 and ameliorated the increased ratio of Th17 cells to Treg cells compared with controls.

Effect on permeability. Both interventions decreased expression of occludin, claudin-1, and ZO-1 in intestinal sections, decreased intestinal tract lesions, and restored villus height to crypt depth ratios. NaB and high fiber diet interventions also resulted in decreased *E. coli* protein in the liver compared with controls.

Effect of short chain fatty acids on permeability and liver injury in human models. Two papers using human models were identified.^{18,24}

Krawczyk *et al.*²⁴

Model. A retrospective analysis of a selection of patients from the larger Nutrient-Induced Insulin Output Ratio (NIOR) study.³⁰ The NIOR study randomized patients with NAFLD to one of three dietary strategies with compliance measured through questionnaires and self-reporting at 1, 2, and 6 months. All three strategies mandated a high insoluble fiber intake (29.24 g \pm 10.97 g/day), higher than the average estimated for the population (19 g/day). Fifty-six patients were excluded for non-compliance and from the remaining 110 and 32 were randomly selected for analysis at 6 months.

Plasma ZO-1 was measured as a surrogate marker of intestinal permeability. Liver injury was measured using laboratory liver enzyme measurements (ALT/AST/gamma-glutamyl transferase [GGT]) and ultrasound calculation of the Hamaguchi score for liver steatosis.³¹ Diet was self-reported using short (72 h) food diaries completed prior to follow-up.

Effect on liver. The dietary intervention led to significant amelioration of liver disease as measured by serum AST, ALT, and GGT and by Hamaguchi score.

Effect on permeability. Serum ZO-1 levels were correlated with Hamaguchi score, AST, and ALT. Spearman's rank correlation found that fiber was the only dietary component significantly correlated with ZO-1 levels.

Ferolla *et al* (2016)¹⁸. A randomized unblinded trial of a synbiotic (*Lactobacillus reuteri* with guar gum and inulin) with monthly dietary advice versus dietary advice alone (no placebo). Patients were required to have a diagnosis of NASH on biopsy, and the primary outcome was degree of steatosis on MRI. Secondary measures included serum ALT/AST/ALP/GGT/bilirubin/platelets, and permeability was assessed using serum LPS and urinary lactulose/mannitol excretion ratio.

Effect on liver. After 12 weeks, the intervention was associated with a significant reduction in steatosis and a lower proportion of patients with moderate/severe steatosis. The degree of fibrosis and serum transaminases was unchanged.

Effect on permeability. There was no difference between the groups in the measures of permeability, and LPS levels were found to be raised after 12 weeks regardless of the intervention.

Discussion

This systematic review identified 17 eligible studies evaluating the effect of interventions designed to increase intestinal SCFA concentration in liver disease. No two studies employed the same model, intervention or outcomes making meta-analysis inappropriate. NaB delivered orally was the most commonly used intervention and expression of TJ proteins in intestinal tissue or serum the commonest marker of permeability. Liver injury was mostly determined by histology and liver biochemistry (ALT and AST). Liver injury is hypothesized to be in part a consequence of disrupted gut barrier function, with immune mediated liver damage caused by translocation of PAMPs into the portal circulation. Several studies therefore measured pro-inflammatory cytokines such as TNF- α and IL-6 in liver tissue as a surrogate marker of barrier dysfunction-induced liver damage. A full list of measures is provided in Tables S5 and S6.

The available evidence supports the hypothesis that SCFA supplementation in liver disease ameliorates liver injury through the maintenance of gut epithelial integrity. All of the included studies show significant differences between treatment and control groups. However, five studies demonstrated no benefit of SCFAs in multiple measures.^{14,17–19,27}

Baumann *et al.* found that plasma ALT/AST levels were not affected by NaB supplementation.²⁷ It is worth noting that their other measures of hepatic inflammation were ameliorated. Given that ALT/AST rises are often minimal in NASH this may be a poor measure of liver injury. They also found no improvement in markers of permeability (endotoxemia and TJ protein expression). The lack of TJ protein expression may be explained by the use of whole intestinal sections rather than epithelium, such that TJ proteins are relatively “diluted” in the analysis. The persistent endotoxemia may be explained by relatively low circulating levels of endotoxin in controls (0.4 EU/mL). Jin *et al.*¹⁷ also found that NaB ameliorated liver injury but with no effect on TJ protein

expression, although with some changes in markers of endotoxemia. Both of these studies used the shortest duration of treatment with SCFAs (5 and 6 weeks, respectively), which could also explain the lack of benefit.

In the first of the two studies by Cresci *et al.*,¹⁴ tributyrin supplementation did not reduce liver inflammation or TJ protein expression in the chronic alcohol consumption model. In contrast, their second study is more supportive of the hypothesis. They demonstrated improvement in TJ expression and hepatic inflammation although this was not completely ameliorated.¹⁹ These differences are likely explained by the duration of alcohol consumption in the two models: the former was 25 days and the latter only 10. The shorter duration resulted in reduced liver injury and minimal intestinal mucosal injury.

The study by Ferolla *et al.* showed significant amelioration of liver injury with synbiotic treatment; however, it is not clear if this was due to the probiotic component or the SCFA precursor (inulin). Furthermore, they were not able to show any improvement in permeability. There are several possible explanations for this, including the lack of power, a relatively short intervention, and the lack of placebo. The authors also state doubts around their LPS assay as they are unable to explain the rise in levels among all groups (which equally may have been the result of the dietary advice which all groups received). A similar study by Malaguarnera *et al.*³² used a synbiotic (*Bifidobacterium longum* with fructo-oligosaccharides) versus placebo model for a longer period, and demonstrated both reduction in liver injury (on repeat biopsy and standard liver biochemistry) and in permeability measured by serum endotoxemia. Although not explicitly using SCFAs (and therefore not eligible for inclusion in this review), the fructo-oligosaccharides may have played a similar role to inulin in this study.³³ A longer period of intervention and the use of placebo may have strengthened the findings of Ferolla *et al.*

The gut-liver axis has been identified as an important area for further research and as a target for therapy of liver disease.³⁴ One potential strategy involves modulating the gut microbiome to promote intestinal barrier integrity. This has already been tested in the treatment of hepatic encephalopathy. Two studies have shown promising results with probiotic therapy, significantly reducing venous ammonia levels and improving encephalopathy.^{35,36} Another approach has investigated fecal microbiota transplant, with a phase 2 study demonstrating safety in the settings of decompensated cirrhosis and alcohol misuse.^{37,38} The latter trial also documented improvement in gut microbial dysbiosis with an increase in SCFA-producing bacteria and increased serum SCFA after transplantation.³⁸

This review is limited by the lack of meta-analysis. The 17 eligible studies reported the effects of 7 different SCFA preparations/interventions in 15 different animal models and 1 human disease model (NASH). Furthermore, as the majority used animal models, several of the interventions (e.g. gavage) and measures (e.g. portal blood samples and everted gut sacs) are less applicable to human disease.

The majority of the studies used plasma ALT/AST levels as a marker of liver injury, but these can remain normal in some etiologies. Direct measurement of gut permeability is challenging, and there is no gold standard.³⁹ Intestinal epithelial structure can be assessed by electron microscopy, but this cannot measure functional alterations. Functional assessments such as measurement

of radiolabeled tracers in the urine or by trans-epithelial permeation of FITC-dextran can provide accurate estimates of gut permeability. Indirect markers of permeability including serum levels of TJ proteins, endotoxin, and LBP may be influenced by other factors such as the presence of infection. Additionally, increased serum levels of TJ proteins may represent increased production rather than functional improvement. Krawczyk *et al.* hypothesize that ZO-1 levels may have potential as a non-invasive diagnostic marker for liver inflammation. The findings of Ram *et al.* that ZO-1 was significantly elevated in HCC patients would support this.⁴⁰ Only two studies reported a functional assessment of gut permeability.^{18,20}

All studies were of low or average quality (Table 2). None of the studies were blinded and the majority of animal studies provided insufficient detail on sample size calculations, limitations, and statistical methods. The human studies were a retrospective subgroup analysis of a larger trial (limited by participant self-report of diet), and an unblinded non-placebo-controlled trial lacking significant power.

In summary, this review has identified that there have been few studies investigating the effect of interventions to increase intestinal SCFA concentration in the context of liver disease. Studies were heterogeneous in population, intervention, design and outcome measures. Those that have been performed are of low or average quality and only 2 were in humans with liver disease. Fourteen of the 17 eligible studies demonstrated a positive effect of the intervention on both gut permeability and liver inflammation. This review demonstrates that strategies that increase intestinal SCFA concentration have biologically relevant effects in *in vivo* and *in vitro* models of liver disease. Future studies should examine the most effective methods to increase luminal SCFA concentrations, particularly in adults with liver disease. Furthermore, more work is needed to identify the most appropriate outcome measures in this population.

Data availability statement. Extracted data available with publication upon reasonable request to the corresponding author.

References

- Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. *J. Hepatol.* 2020; **72**: 558–77.
- Pohl K, Moodley P, Dhanda AD. Alcohol's Impact on the Gut and Liver. *Nutrients* 2021; **13**: 3170.
- Bajaj JS, Heuman DM, Hylemon PB *et al.* Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J. Hepatol.* 2014; **60**: 940–7.
- Bajaj JS, Idilman R, Mabudian L *et al.* Diet affects gut microbiota and modulates hospitalization risk differentially in an international cirrhosis cohort. *Hepatology* 2018; **68**: 234–47.
- Schwenger KJ, Clermont-Dejean N, Allard JP. The role of the gut microbiome in chronic liver disease: the clinical evidence revised. *JHEP Rep.* 2019; **1**: 214–26.
- De Munck TJI, Xu P, Verwijs HJA *et al.* Intestinal permeability in human nonalcoholic fatty liver disease: A systematic review and meta-analysis. *Liver Int.* 2020; **40**: 2906–16.
- Parada Venegas D, De la Fuente MK *et al.* Short Chain Fatty Acids (SCFAs)-Mediated Gut Epithelial and Immune Regulation and Its Relevance for Inflammatory Bowel Diseases. *Front. Immunol.* 2019; **10**: 277.
- Liu P, Wang Y, Yang G, Zhang Q *et al.* The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol. Res.* 2021; **165**: 105420.
- Percie du Sert N, Hurst V, Ahluwalia A *et al.* The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. *PLoS Biol.* 2020; **18**: e3000410.
- von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *J. Clin. Epidemiol.* 2008; **61**: 344–9.
- Schulz KF, Altman DG, Moher D, CONSORT Group. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ* 2010; **340**: e332.
- Leung V, Rousseau-Blass F, Beauchamp G, Pang DSJ. ARRIVE has not ARRIVED: Support for the ARRIVE (Animal Research: Reporting of in vivo Experiments) guidelines does not improve the reporting quality of papers in animal welfare, analgesia or anesthesia. Pritchett-Corning KR, editor. *PLOS ONE* 2018; **13**: e0197882.
- Endo H, Niioka M, Kobayashi N, Tanaka M, Watanabe T. Butyrate-Producing Probiotics Reduce Nonalcoholic Fatty Liver Disease Progression in Rats: New Insight into the Probiotics for the Gut-Liver Axis. Zang M, editor. *PLoS ONE* 2013; **8**: e63388.
- Cresci GA, Bush K, Nagy LE. Tributyrin Supplementation Protects Mice from Acute Ethanol-Induced Gut Injury. *Alcohol. Clin. Exp. Res.* 2014; **38**: 1489–501.
- Liu B, Qian J, Wang Q, Wang F, Ma Z, Qiao Y. Butyrate Protects Rat Liver against Total Hepatic Ischemia Reperfusion Injury with Bowel Congestion. Bruns H, editor. *PLoS ONE* 2014; **9**: e106184.
- Yang F, Wang LK, Li X, Wang LW, Han XQ, Gong ZJ. Sodium butyrate protects against toxin-induced acute liver failure in rats. *Hepatobiliary Pancreat. Dis. Int.* 2014; **13**: 309–15.
- Jin CJ, Sellmann C, Engstler AJ, Ziegenhardt D, Bergheim I. Supplementation of sodium butyrate protects mice from the development of non-alcoholic steatohepatitis (NASH). *Br. J. Nutr.* 2015; **114**: 1745–55.
- Ferolla S, Couto C, Costa-Silva L *et al.* Beneficial Effect of Synbiotic Supplementation on Hepatic Steatosis and Anthropometric Parameters, But Not on Gut Permeability in a Population with Nonalcoholic Steatohepatitis. *Nutrients* 2016; **8**: 397.
- Cresci GA, Glueck B, McMullen MR, Xin W, Allende D, Nagy LE. Prophylactic tributyrin treatment mitigates chronic-binge ethanol-induced intestinal barrier and liver injury: Tributyrin and chronic-binge ethanol. *J. Gastroenterol. Hepatol.* 2017; **32**: 1587–97.
- Matheus V, Monteiro L, Oliveira R, Maschio D, Collares-Buzato C. Butyrate reduces high-fat diet-induced metabolic alterations, hepatic steatosis and pancreatic beta cell and intestinal barrier dysfunctions in prediabetic mice. *Exp. Biol. Med.* 2017; **242**: 1214–26.
- Wu JL, Zou JY, Hu ED *et al.* Sodium butyrate ameliorates S100/FCA-induced autoimmune hepatitis through regulation of intestinal tight junction and toll-like receptor 4 signaling pathway. *Immunol. Lett.* 2017; **190**: 169–76.
- Zhou D, Pan Q, Xin FZ *et al.* Sodium butyrate attenuates high-fat diet-induced steatohepatitis in mice by improving gut microbiota and gastrointestinal barrier. *World J. Gastroenterol.* 2017; **23**: 60.
- Hu ED, Chen DZ, Wu JL *et al.* High fiber dietary and sodium butyrate attenuate experimental autoimmune hepatitis through regulation of immune regulatory cells and intestinal barrier. *Cell. Immunol.* 2018; **328**: 24–32.
- Krawczyk M, Maciejewska D, Ryterska K *et al.* Gut Permeability Might be Improved by Dietary Fiber in Individuals with Nonalcoholic

- Fatty Liver Disease (NAFLD) Undergoing Weight Reduction. *Nutrients* 2018; **10**: 1793.
- 25 Peng J, Leng J, Tian H *et al.* Geniposide and Chlorogenic Acid Combination Ameliorates Non-alcoholic Steatohepatitis Involving the Protection on the Gut Barrier Function in Mouse Induced by High-Fat Diet. *Front. Pharmacol.* 2018; **9**: 1399.
 - 26 Ye J, Lv L, Wu W *et al.* Butyrate Protects Mice Against Methionine–Choline-Deficient Diet-Induced Non-alcoholic Steatohepatitis by Improving Gut Barrier Function, Attenuating Inflammation and Reducing Endotoxin Levels. *Front. Microbiol.* 2018; **9**: 1967.
 - 27 Baumann A, Jin CJ, Brandt A *et al.* Oral Supplementation of Sodium Butyrate Attenuates the Progression of Non-Alcoholic Steatohepatitis. *Nutrients* 2020; **12**: 951.
 - 28 Jiang XW, Li YT, Ye JZ *et al.* New strain of *Pediococcus pentosaceus* alleviates ethanol-induced liver injury by modulating the gut microbiota and short-chain fatty acid metabolism. *World J. Gastroenterol.* 2020; **26**: 6224–40.
 - 29 Yang T, Yang H, Heng C *et al.* Amelioration of non-alcoholic fatty liver disease by sodium butyrate is linked to the modulation of intestinal tight junctions in db/db mice. *Food Funct.* 2020; **11**: 10675–89.
 - 30 Stachowska E, Ryterska K, Maciejewska D *et al.* Nutritional Strategies for the Individualized Treatment of Non-Alcoholic Fatty Liver Disease (NAFLD) Based on the Nutrient-Induced Insulin Output Ratio (NIOR). *Int. J. Mol. Sci.* 2016; **17**: 1192.
 - 31 Hamaguchi M, Kojima T, Itoh Y *et al.* The Severity of Ultrasonographic Findings in Nonalcoholic Fatty Liver Disease Reflects the Metabolic Syndrome and Visceral Fat Accumulation. *Am. J. Gastroenterol.* 2007; **102**: 2708–15.
 - 32 Malaguarnera M, Vacante M, Antic T *et al.* Bifidobacterium longum with Fructo-Oligosaccharides in Patients with Non Alcoholic Steatohepatitis. *Dig. Dis. Sci.* 2012; **57**: 545–53.
 - 33 Khoshbin K, Camilleri M. Effects of dietary components on intestinal permeability in health and disease. *Am J Physiol-Gastrointest Liver Physiol.* 2020; **319**: G589–608.
 - 34 Angeli P, Bernardi M, Villanueva C *et al.* EASL Clinical Practice Guidelines for the management of patients with decompensated cirrhosis. *J. Hepatol.* 2018; **69**: 406–60.
 - 35 Liu Q, Duan ZP, Ha DK, Bengmark S, Kurtovic J, Riordan SM. Synbiotic modulation of gut flora: Effect on minimal hepatic encephalopathy in patients with cirrhosis. *Hepatology* 2004; **39**: 1441–9.
 - 36 Xia X, Chen J, Xia J *et al.* Role of probiotics in the treatment of minimal hepatic encephalopathy in patients with HBV-induced liver cirrhosis. *J. Int. Med. Res.* 2018; **46**: 3596–604.
 - 37 Woodhouse CA, Patel VC, Goldenberg S *et al.* PROFIT, a PROspective, randomised placebo controlled feasibility trial of Faecal mIcrobiota Transplantation in cirrhosis: study protocol for a single-blinded trial. *BMJ Open* 2019 Feb; **9**: e023518.
 - 38 Bajaj JS, Gavis EA, Fagan A *et al.* A Randomized Clinical Trial of Fecal Microbiota Transplant for Alcohol Use Disorder. *Hepatology* 2021; **73**: 1688–700.
 - 39 Pijls KE, Jonkers DMAE, Elamin EE, Masclee AAM, Koek GH. Intestinal epithelial barrier function in liver cirrhosis: an extensive review of the literature. *Liver Int.* 2013; **33**: 1457–69.
 - 40 Ram AK, Pottakat B, Vairappan B. Increased systemic zonula occludens 1 associated with inflammation and independent biomarker in patients with hepatocellular carcinoma. *BMC Cancer* 2018; **18**: 572.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1: The main findings of the identified studies.

Table S2: Risk of bias/quality assessment for murine model studies.

Table S3: Risk of bias/quality assessment for observational studies.

Table S4: Risk of bias/quality assessment for randomized trials.

Table S5: Markers of liver injury in the identified studies.

Table S6: Markers of intestinal permeability in the identified studies.