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Review Article

The Multifaceted Roles of Gut Microbiota and Their Metabolites in Metabolic Dysfunction-associated Steatotic Liver Disease: A Literature Review

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Abstract

Metabolic dysfunction-associated steatotic liver disease (MASLD) represents a major global health concern and encompasses a spectrum ranging from hepatic steatosis and metabolic dysfunction-associated steatohepatitis to liver fibrosis, cirrhosis, and ultimately hepatocellular carcinoma. Insulin resistance, the pathogenic cornerstone of MASLD, drives enhanced peripheral lipolysis and increased hepatic de novo lipogenesis, thereby overloading the liver with lipids and inducing steatosis. Subsequent lipotoxicity, inflammation, and gut microbiota dysbiosis further exacerbate disease progression. The gut microbiota and their metabolites communicate with the liver via the gut-liver axis, forming a complex signaling network that directly or indirectly modulates hepatic metabolism, systemic immune responses, oxidative stress, and intestinal barrier integrity. In this review, we synthesize evidence for the beneficial and detrimental effects of the major human gut microbial communities and their metabolites during the course of MASLD. We delineate how these gut-derived factors regulate hepatic function through an integrated tripartite "gut-liver axis–oxidative stress–metabolic reprogramming" mechanism. These insights may inform microbiome-based precision interventions and accelerate the development of therapeutic strategies targeting MASLD.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), a chronic liver disease, had a global prevalence rate exceeding 30% in 2019, and its prevalence has continued to show a year-on-year upward trajectory.[1] MASLD can be delineated into four stages with distinct histological features. It initially manifests as hepatic steatosis, which is characterized by relatively mild hepatic inflammation. As inflammatory activity progresses, it is accompanied by ballooning degeneration and potential fibrosis, and the disease transitions to metabolic dysfunction-associated steatohepatitis (MASH). If MASH is not effectively controlled, it ultimately progresses to hepatic fibrosis, at which point the risk of hepatocellular carcinoma increases. In patients with MASH cirrhosis, the estimated annual incidence of hepatocellular carcinoma ranges from 0.5% to 2.6%, whereas in non-cirrhotic MASLD it is much lower, at approximately 0.1–1.3 cases per 1,000 patient-years.[2]

MASLD was previously termed nonalcoholic fatty liver disease. Owing to the former label's lack of pathophysiologic specificity and its stigmatizing connotations, a Delphi consensus recommended adopting "MASLD" as the updated nomenclature, thereby underscoring its identity as a metabolic disease.[3] MASLD is widely understood within a multiple-hit framework. An unhealthy lifestyle, a high-fat diet (HFD), and insulin resistance trigger hepatic lipid accumulation. Lipotoxicity, inflammation, cellular injury, and gut microbiota dysbiosis then drive progression to MASH and fibrosis.[4,5] Under the updated nomenclature, MASLD is now positioned within a broader framework of steatotic liver disease (SLD).[6,7] This framework also includes metabolic dysfunction and alcohol-related/associated liver disease (MetALD) for individuals with MASLD and moderate alcohol consumption, and alcohol-related liver disease.[8] Distinguishing MetALD from cryptogenic SLD is important because differences in alcohol exposure and etiology may affect disease progression, microbial signatures, and treatment response.[9]

The gut microbiota has emerged as an important component of MASLD pathogenesis. A meta-analysis showed that the gut microbial composition is significantly altered in MASLD. The relative abundances of *Escherichia*, *Prevotella*, and *Streptococcus* were increased, with pooled standardized

mean differences of 1.55 (95% CI, 0.57–2.54), 1.89 (95% CI, 0.02–3.76), and 1.33 (95% CI, 0.62–2.05), respectively. By contrast, the relative abundances of *Coprococcus* and *Ruminococcus* were reduced, with standardized mean differences of −1.75 (95% CI, −3.13 to −0.37) and −1.84 (95% CI, −2.41 to −1.27), respectively.[10] These compositional shifts are linked to impaired barrier integrity, inflammation, and hepatic injury along the gut-liver axis. Microbiota-targeted interventions have become a major focus of MASLD research. However, microbiota alterations show marked interindividual and regional heterogeneity, and most available evidence remains largely associative. The precise causal relationships underlying these findings still require further validation through functional approaches, such as germ-free animal models.

This review focuses on gut microbial taxa and metabolites for which mechanistic evidence, quantitative clinical associations, or early interventional data are available. We assess how these microbial factors influence barrier integrity, inflammatory signaling, oxidative stress, and hepatic metabolic reprogramming, with particular attention to their translational relevance, major limitations, and therapeutic potential in MASLD.

Intestinal microbiota dysbiosis in MASLD

Escherichia coli and its subtypes in MASLD pathogenesis

Under the pathological state of "triple damage" to the intestinal defense function via (1) reduced mucus layer thickness, (2) disruption of the gut vascular barrier, and (3) downregulated expression of tight junction proteins, *E. coli* breaches its normal biogeographic boundaries and translocates via the portal venous system, resulting in aberrant trafficking along the gut-liver axis.[11]

Enterotoxigenic *E. coli* can directly impair the intestinal barrier. It disrupts tight junctions and mucosal layer integrity by downregulating claudin-1, occludin, MUC1, and MUC2, thereby increasing intestinal permeability.[12] From a spatial tissue perspective, *E. coli*-associated barrier injury may show regional specificity, as different intestinal regions have distinct local microenvironments and signaling features.[13] Spatial host-microbiome sequencing further showed that the intestine contains regional niches defined by host cellular composition and microbial distribution. A total of 39 genera were detected across 124 tissue sections and 15,321 spatial spots from three mouse conditions, including 22 with relative abundances greater than 1%. The taxonomic profiles obtained were significantly correlated with those generated by conventional 16S rRNA sequencing ($r = 0.69$, $P < 0.0001$).[14] These findings suggest that *E. coli*-associated barrier disruption and inflammatory activation may show spatial heterogeneity.

Notably, the NF73-1 strain isolated from patients with MASH displays a distinct pathogenic mechanism. Rather than signaling through the classical Toll-like receptor (TLR) 4 pathway, this strain signals via the TLR2/TLR6 heterodimer, resulting in a 65% increase in NLR family pyrin domain containing 3 (NLRP3) inflammasome assembly efficiency. This strain-specific activation promotes polarization of hepatic macrophages toward the M1 phenotype. *In vitro*, NF73-1 increased the proportion of $CD11b^+F4/80^+$ macrophages from approximately 15% to 23% ($P < 0.01$) and raised the $CD86^+CD206^-$ M1 subset from about 30% to 35% ($P < 0.05$). NF73-1 was also associated with increased phosphorylation of mTOR at Ser2448 and S6K1 at Thr389, indicating activation of a pathway related to lipid metabolic reprogramming.[15]

In addition to other bacterial structural components, flagellin—the major structural protein of bacterial flagella—induces endothelial-to-mesenchymal transition in liver sinusoidal endothelial cells via the TLR5/myeloid differentiation primary response 88 (MyD88) signaling axis. Mechanistic studies demonstrate that TLR5/MyD88-dependent nuclear factor kappa B (NF-κB) activation (p65 phosphorylation and nuclear translocation) transcriptionally upregulates Twist family bHLH transcription factor 1 (*TWIST1*), thereby driving an endothelial-to-mesenchymal transition program characterized by a cadherin switch and increased mesenchymal features. This phenotypic transition concurrently upregulates α-smooth muscle actin expression, establishing a foundation for extracellular matrix remodeling in liver fibrosis.[16]

The pathogenic processes mediated by *E. coli* and its key structural components in MASLD form a continuous pathological axis linking intestinal barrier disruption, amplified hepatic inflammation, fibrotic tendency, and metabolic imbalance. This pattern reflects their coordinated pathogenic role within the tripartite framework.

Dual role of Prevotella in MASLD

The role of *Prevotella* in MASLD is significantly strain-specific and host microenvironment-dependent, and the coexistence of its pathogenic and protective effects warrants further investigation. A *Prevotella*-dominated enterotype (ET-P) is associated with MASLD progression, and this association appears to be more pronounced in Asian populations. In an enterotype-based analysis, the proportion associated with MASLD was higher in Asian than in Caucasian samples within ET-P (98.6% vs. 65.1%, $P = 0.049$). In the same analysis, enterotype, age, obesity, and ethnicity were all associated with MASLD occurrence (all $P < 0.05$). Functional prediction further showed that *Prevotella* had a markedly higher pathogenicity score than *Akkermansia muciniphila* (0.868 vs. 0.006) and was positively correlated with lipopolysaccharide (LPS) biosynthesis ($P < 0.05$). These findings support the possibility that ET-P promotes liver injury progression by enhancing endotoxin-related inflammatory responses.[17–19] Moreover, *Prevotella* promotes the maturation of dendritic cells and the differentiation of Th17 cells, thereby disrupting intestinal immune homeostasis and contributing to the establishment of a chronic low-grade inflammatory state within the gut-liver axis.[20] Within this tripartite framework, ET-P acts upstream to disrupt barrier integrity and immune homeostasis, promoting hepatic inflammation and fibrosis.

Prevotella exerts strain-specific effects on host metabolism and does not uniformly act as a pathogenic genus. A study isolated 39 *Prevotella* strains from 27 healthy donors and selected three representative strains, HF2123, HF1478, and HF2130, for testing in db/db mice. All three strains increased glucagon-like peptide-1 (GLP-1) secretion ($P < 0.05$). HF2130 produced an approximately twofold greater increase in GLP-1 than the other strains ($P < 0.001$). In addition, HF1478 increased the intestinal abundance of *A. muciniphila* ($P = 0.03$). These findings suggest that selected *Prevotella* strains may improve glucose metabolism by reshaping the gut microbiota and enhancing GLP-1-related signaling.[21] Nevertheless, evidence for these beneficial effects is largely limited to animal studies and shows marked strain specificity, which limits its generalization to the entire *Prevotella* genus.

In contrast, the *Prevotella* DSM 18205 strain used for transplantation was significantly associated with obesity in a pediatric cohort ($P = 0.003$). In HFD-fed mice, it increased fasting blood glucose and fasting insulin levels (both $P < 0.01$), elevated interleukin-1 beta levels ($P < 0.05$), reduced

A. muciniphila abundance (P < 0.01), and disrupted the bile acid profile. These findings suggest that this strain is more likely to aggravate obesity-related insulin resistance and hepatic metabolic dysfunction.[22] Therefore, selected beneficial *Prevotella* strains may be more suitable for glucose metabolic disorders characterized mainly by hyperglycemia. For obesity-related MASLD/MASH, a more feasible strategy may be to suppress the expansion of harmful *Prevotella*-dominant communities and restore *A. muciniphila* abundance and bile acid homeostasis.

In summary, *Prevotella* should not be regarded as a uniformly pathogenic or protective factor in MASLD, as its effects are jointly shaped by strain variation, gut microbial configuration, and host metabolic status. At present, *Prevotella* may be better considered a microbial marker for risk stratification and precision intervention screening rather than a universal therapeutic target.

A. muciniphila effectively ameliorates MASLD through multiple mechanisms

A. muciniphila is a beneficial gut commensal with therapeutic potential in MASLD. Through the gut-liver axis, it supports barrier integrity, suppresses inflammatory and oxidative stress pathways, and improves metabolic homeostasis, thereby helping to alleviate hepatic steatosis, insulin resistance, and systemic inflammation. Its protective effects are mainly reflected in four aspects:

1. Intestinal barrier repair, reconstruction, and microbiota homeostasis: In HFD-induced mouse models, *A. muciniphila* ameliorates intestinal barrier dysfunction by upregulating the gene and protein expression of tight junction proteins, including zonula occludens-1 and occludin (both $P < 0.05$).[23] *A. muciniphila* intervention improved gut microbial diversity in HFD-fed mice, with the Sobs index increasing from approximately 270 to 340, the Shannon index from approximately 2.4 to 3.1, and the Simpson index decreasing from approximately 0.17 to 0.09. In the same study, treatment with the Amuc_1100 outer membrane protein further reshaped the microbiota by reducing *Coriobacteriaceae_UCG-002* and increasing beneficial short-chain fatty acids (SCFAs)-associated taxa, including *Blautia* and *norank_f__Ruminococcaceae.*[24]
2. Suppression of the inflammatory cascade: *A. muciniphila* alleviates hepatic inflammation by improving intestinal barrier function, with this effect being associated with inhibition of the TLR2/γδT17 cell axis. In MASH model mice, compared with those without *A. muciniphila* intervention, treatment with *A. muciniphila* reduced the proportion of hepatic γδT cells from 12.15% to 4.33% and that of interleukin-17-producing^{+}γδT cells from 7.36% to 0.31%. Hepatic *tumor necrosis factor* and *Tlr2* mRNA expression was also reduced (both $P < 0.05$). Stimulation with lipoteichoic acid weakened these protective effects, suggesting that the anti-inflammatory effect of *A. muciniphila* partly depends on attenuation of TLR2-related immune activation secondary to improved intestinal barrier function.[25]
3. Reprogramming of lipid metabolism: *A. muciniphila* inhibited hepatic lipogenesis, as reflected by lower hepatic expression of sterol regulatory element-binding protein (SREBP) than in the untreated HFD group ($P < 0.05$). In HFD-fed mice, *A. muciniphila* treatment reduced hepatic triglyceride and diacylglycerol levels to approximately 40% and 55% of those in the untreated HFD group, respectively. This was accompanied by activation of the liver kinase B1-AMP-activated protein kinase pathway and an approximately 1.5- to 2.0-fold increase in carnitine palmitoyltransferase (CPT)-1β expression.[26,27]
4. Amelioration of insulin resistance: In HFD-fed mice, *A. muciniphila* treatment reduced the area under the curve of the insulin tolerance test from approximately 0.55×10^3 to 0.40×10^3. This protective effect may be related to enhanced secretion of the G protein-coupled receptor (GPR) 43-associated incretins GLP-1 and peptide YY, together with reduced serine phosphorylation of insulin receptor substrate-1 (IRS-1) and restoration of the phosphatidylinositol 3-kinase (PI3K)/phosphatidylinositol (3,4,5)-trisphosphate (PIP3)/protein kinase B (Akt) signaling pathway. Specifically, hepatic PIP3 increased from approximately 1.6 to 2.1 ng/g protein, phosphorylated IRS-1 decreased from approximately 2.0 to 1.3, and phosphorylated Akt S473 and the PI3K p110/β-actin ratio increased from approximately 0.6 to 1.0 and from 0.8 to 1.1.[28,29]

A. muciniphila may have therapeutic relevance by restoring intestinal barrier integrity and modulating immune and metabolic functions. Its potential clinical applications are in patients with MASLD and type 2 diabetes, particularly those whose disease is driven by insulin resistance and increased intestinal permeability. Components like *Amuc_1100* may provide an additional basis for translational and clinical development. Further studies are needed to optimize dosing and delivery strategies in preclinical models, followed by early-phase clinical trials to assess safety and efficacy.

Table 1 summarizes the roles of *E. coli*, *Prevotella*, and *A. muciniphila* in MASLD, highlighting their effects on pro-inflammatory, anti-inflammatory, lipid metabolism, and insulin resistance functions. Table 2 shows the quantitative changes in key gut microbial taxa in MASLD population.

Gut microbiota metabolic products affect MASLD

The multifaceted role of gut microbiota-derived endogenous ethanol in the pathogenesis and progression of MASLD

Endogenous ethanol generated by the gut microbiota via the glycolytic pathway has emerged as an independent risk factor for MASLD. The primary ethanol-producing microbial taxa include opportunistic pathogens such as *Klebsiella pneumoniae* and *E. coli* as well as commensal genera such as *Lactobacillus* and *Bifidobacterium*.[30–32] Colonization of germ-free mice with high-ethanol-producing *K. pneumoniae* induces hepatic steatosis in a dose-dependent manner.[31,33]

Clinical studies further support an association between endogenous ethanol burden and disease progression. Portal vein ethanol concentrations are approximately 2.1 mmol/L in individuals without fatty liver, 8.0 mmol/L in patients with MASLD, and 21.0 mmol/L in patients with MASH. Notably, portal vein ethanol concentrations in patients with MASLD are approximately 187-fold higher than fasting peripheral blood ethanol concentrations.[32] In another study, high-alcohol-producing *K. pneumoniae* was associated with up to 60% of MASLD cases, and transfer of clinical isolates or introduction of microbiota containing high-alcohol-producing strains induced new-onset fatty liver in mice.[31]

Endogenous ethanol promotes MASLD progression mainly through three pathways. First, acetaldehyde, a metabolite of ethanol, disrupts intestinal epithelial tight junctions by activating protein phosphatase 2A, facilitating the hepatic translocation of LPS and other gut-derived harmful substances.[34] Second, ethanol metabolism induces superoxide production through electron transport chain leakage, whereas cytochrome P450 2E1 (CYP2E1) directly generates reac-

Table 1. The role of intestinal microbiota in MASLD

Intestinal microbiota	Pro–inflammatory	Anti–inflammatory	Lipid metabolism	Insulin resistance
Escherichia coli	1. Activating the TLR4/NF–κB pro–inflammatory pathway; 2. Activating the TLR2/NLRP3 pathway promotes the activation of M1 macrophages	–	1. Activating the mTOR/S6K1/SREBP–1/PPAR–α pathway promotes triglyceride synthesis	1. Promoting inflammatory responses indirectly leads to insulin resistance
Prevotella	1. Weakening of the intestinal barrier leads to the entry of bacterial products into the liver via the bloodstream, activating the TLR4/NF–κB pro–inflammatory pathway; 2. Inducing the proliferation of pro–inflammatory T lymphocytes	–	1. Preferring high–carbohydrate substrates lead to increased propionate production, hepatic propionyl–CoA accumulation, inhibition of mitochondrial β–oxidation, and enhanced triglyceride synthesis; 2. Reshaping the gut microbial community and altering the bile acid profile aggravate MASLD–related lipid metabolic disturbances	1. Promoting inflammatory responses indirectly leads to insulin resistance; 2. Representative strains exacerbate obesity–related insulin resistance by altering bile acid metabolism and microbiota homeostasis; 3. Specific subtypes can increase the abundance of beneficial bacteria such as Akkermansia, promote GLP–1 secretion, and improve insulin sensitivity
Akkermansia muciniphila	–	1. Enhancing intestinal barrier function to block the activation of the TLR2/γδT17 axis	1. Inhibiting the expression of sterol regulatory element–binding proteins and reducing lipid synthesis; 2. Activating the LKB1–AMPK axis and upregulating the expression of lipid decomposition and transport proteins	1. Activating GPR43 increases the secretion of GLP–1 and PYY; 2. Inhibiting the phosphorylation of IRS–1; 3. Elevating the level of PIP3 and activating the PI3K/PIP3/Akt signaling pathway

Akt, protein kinase B; AMPK, AMP-activated protein kinase; GLP-1, glucagon-like peptide-1; GPR43, G protein-coupled receptor 43; IRS-1, insulin receptor substrate-1; LKB1, liver kinase B1; M1, M1 macrophage; MASLD, metabolic dysfunction-associated steatotic liver disease; mTOR, mechanistic target of rapamycin; NF-κB, nuclear factor kappa B; NLRP3, NLR family pyrin domain containing 3; PI3K, phosphatidylinositol 3-kinase; PIP3, phosphatidylinositol (3,4,5)-trisphosphate; PPAR-α, peroxisome proliferator-activated receptor-α; PYY, peptide YY; S6K1, ribosomal protein S6 kinase beta-1; SREBP-1, sterol regulatory element-binding protein 1; TLR2, Toll-like receptor 2; TLR4, Toll-like receptor 4; γδT17, interleukin-17-producing γδ T cells.

Table 2. Quantitative evidence for gut microbial taxa changes in MASLD: effect sizes, statistical indicators, and evidence levels

Microbial taxa	Quantitative data (abundance changes in MASLD patients)	Effect size/Statistical indicators	Evidence type	Evidence level
Escherichia coli	Increased in MASLD patients	SMD = 1.30/95% CI = [0.60, 2.00]	Systematic Review and Meta–Analysis	High[79]
Prevotella	Decreased in MASLD patients	SMD = −0.41/95% CI = [−0.74, −0.08]	Systematic Review and Meta–Analysis	High[79]
Akkermansia muciniphila	Decreased in MASLD patients	MASLD/HC ≈ 0.6–fold/$P < 0.05$	Observational case–control / cross–sectional study	Moderate[80]
Lactobacillus	No significant difference	SMD = −0.05/95% CI = [−0.82, 0.72]	Systematic Review and Meta–Analysis	High[79]
Faecalibacterium prausnitzii	Decreased in MASLD patients	SMD = −1.34/95% CI = [−2.17, −0.52]	Systematic Review and Meta–Analysis	High[79]
Ruminococcaceae	Decreased in MASLD patients	SMD = −0.70/95% CI = [−1.36, −0.05]	Systematic Review and Meta–Analysis	High[79]
Coprococcus	Decreased in MASLD patients	SMD = −1.66/95% CI = [−3.04, −0.28]	Systematic Review and Meta–Analysis	High[81]
Bacteroidetes	Decreased in MASLD patients	SMD = −0.67/95% CI = [−1.13, −0.01]	Systematic Review and Meta–Analysis	High[79]
Firmicutes	No significant difference	$P = 0.052$	Systematic Review and Meta–Analysis	High[79]

CI, confidence interval; HC, healthy control; MASLD, metabolic dysfunction-associated steatotic liver disease; SMD, standardized mean difference.

tive oxygen species during ethanol oxidation. Endogenous ethanol promotes mitochondrial fission, further aggravating electron transport chain dysfunction and amplifying oxidative damage.[35–37] Third, the conversion of ethanol to acetate by alcohol dehydrogenase, CYP2E1, and aldehyde dehydrogenase causes an imbalance in NADH/NAD^+, which activates SREBP-1c-driven lipogenesis and suppresses peroxisome proliferator-activated receptor-α (PPAR-α)-mediated fatty acid oxidation, ultimately promoting triglyceride accumulation.[38,39]

Microbiota-derived endogenous ethanol promotes the development and progression of MASLD through the pathogenic cascade of acetaldehyde production, intestinal barrier disruption, hepatic oxidative stress, and metabolic reprogramming. However, the current evidence in this field remains mainly mechanistic and preclinical, and therapeutic evidence is still limited. This mechanism may be of greater clinical relevance in patients with elevated postprandial peripheral ethanol levels and enrichment of high-ethanol-producing strains. Compared with non-specific microbiota modulation, selective removal of high-ethanol-producing strains and inhibition of bacterial ethanol-producing pathways may have greater clinical therapeutic significance in these patients.

Bile acid metabolism disorders: From dysregulation of enterohepatic circulation to dual modulation of farnesoid X receptor (FXR) signaling

Bile acids are synthesized in hepatocytes from cholesterol through the classical and alternative pathways and subsequently secreted into the small intestine via the bile salt export pump. Approximately 95% of intestinal bile acids are reabsorbed in the terminal ileum by the apical sodium-dependent bile acid transporter, thereby completing the enterohepatic circulation.[40] At present, most studies on bile acids are based on plasma, feces, or tissue homogenates. These preparations treat the intestine or liver as a homogeneous reactor. However, bile acid synthesis, transport, and transformation are highly dependent on anatomical compartmentalization and local microenvironments, and reliance on bulk specimens alone can obscure critical regional differences.[41]

In MASLD, dysregulation of the gut-liver axis is closely associated with abnormal gut microbiota-mediated bile acid conversion, which is characterized by remodeling of the bile acid pool and relative enrichment of deoxycholic acid (DCA).[42] Clinical studies further support this association, with significantly higher serum total bile acid levels in patients with MASLD than in controls ($P < 0.01$), together with increased levels of cholic acid ($P < 0.01$), chenodeoxycholic acid ($P < 0.05$), and ursodeoxycholic acid ($P < 0.05$).[43] A study compared 28 individuals with advanced fibrosis and 26 without advanced fibrosis and found that total plasma bile acids were significantly increased in the advanced fibrosis group ($P < 0.01$), whereas total stool bile acids did not differ significantly between the two groups ($P = 0.36$). Chenodeoxycholic acid family bile acids were also higher in individuals with advanced fibrosis ($P < 0.05$), while fibroblast growth factor 19 (FGF19) and GLP-1 showed no significant changes.[44] Spatial omics studies help explain this phenomenon. Spatial metabolomics has shown significant metabolite gradients in the liver and small intestine, indicating that bile acid-related signals are not uniformly distributed but instead exhibit clear spatial organization.[45] Integrated spatial transcriptomic and spatial metabolomic analyses have further shown that MASLD tissues contain region-specific transcriptional programs, metabolite modules, and pathological lesions, with signals related to bile acid dysregulation spatially corresponding to areas of local inflammation and fibrosis.[46]

High-fat and high-cholesterol diets exacerbate MASLD through two distinct pathways. DCA and chenodeoxycholic acid activate the purinergic receptor P2X7 on hepatocyte membranes, leading to the suppression of PTEN-induced kinase 1/Parkin RBR E3 ubiquitin ligase (Parkin)-mediated mitophagy; the subsequent accumulation of damaged mitochondria promotes pyroptosis via the thioredoxin-interacting protein-NLRP3 signaling axis.[47] Serum levels of unconjugated bile acids in mice fed an HFD for 24 weeks were increased by 3.2-fold, and hepatic interleukin-1 beta messenger RNA (mRNA) expression was elevated by 5.7-fold.[48] In contrast, secondary bile acids suppress activation of the NLRP3 inflammasome through the Takeda G protein-coupled receptor 5 (TGR5)/cAMP/protein kinase A signaling cascade, thereby attenuating inflammatory responses.[49] Therefore, alterations in bile acid composition may shift the net biological effect from TGR5-mediated anti-inflammatory signaling toward mitochondrial injury and enhanced activation of the innate immune response.

Primary bile acids (cholic acid, chenodeoxycholic acid) and secondary bile acids (DCA, lithocholic acid) modulate glucose and lipid metabolic homeostasis through the activation of FXR and TGR5.[50] Along the lipid metabolism axis, FXR activates the hepatic FXR/small heterodimer partner pathway, suppresses the expression of SREBP-1c, and reduces triglyceride synthesis.[51] Along the glucose metabolism axis, FXR improves insulin sensitivity. Its agonists promote the conversion of hypertrophic adipocytes into small, differentiated adipocytes by enhancing PPAR-γ activity and suppressing Wnt/β-catenin signaling, thereby restoring adipocyte function and alleviating insulin resistance.[52] FXR also carries a pro-fibrotic risk. Chronic activation of FXR leads to miR-29a-mediated suppression of PPAR-γ, thereby promoting hepatic stellate cell activation.[52] However, paradoxical phenomena have also been observed. Despite elevated total bile acid levels in patients with MASLD, the FXR downstream gene *CYP7A1* is aberrantly upregulated, indicating receptor desensitization; this observation may be attributed to competitive antagonism of FXR by DCA.[42]

The FXR and FGF19 pathway has shown therapeutic potential in clinical studies. Current benefits are mainly reflected in improvements in liver enzymes, liver fat, and surrogate fibrosis endpoints, rather than in consistent histological reversal. Aldafermin showed an antifibrotic signal in compensated MASH cirrhosis, and tropifexor improved alanine aminotransferase and liver fat content. The available studies still rely mainly on noninvasive or surrogate endpoints and report class-related adverse effects such as diarrhea or pruritus.[53,54] Overall, bile acid-related therapies are more likely to benefit patients with active MASH and fibrosis risk, rather than being regarded as a universal strategy for all patients with MASLD.

For bile acid-related therapies to achieve better clinical translation, the key is to move from pathway-based intervention to precise patient stratification. Current evidence suggests that the patients most likely to benefit are those with active MASH and fibrosis risk, especially those with F2–F3 disease and some patients with compensated cirrhosis. Most of these patients have more pronounced bile acid dysregulation, inflammatory activation, and fibrogenic drive. By contrast, the benefit in patients with simple steatosis may be limited. The next step should be to establish a patient selection system based on bile acid profiles, FGF19-related markers, and fibrosis stage, while also improving the management of adverse events such as pruritus. This approach would better support the translation of bile acid signaling into a sustainable therapeutic strategy for fibrotic MASH.

Regulation of SCFA metabolism: From intestinal barrier restoration to maintenance of multi-organ metabolic homeostasis

SCFAs are a class of water-soluble free fatty acids that consist of six or fewer carbon atoms and are generated through the microbial fermentation of dietary fiber in the gut. The primary SCFAs include acetate, propionate, butyrate, and valerate.[55] SCFAs exert protective effects by reinforcing intestinal barrier function, suppressing inflammatory responses, and improving hepatic lipid metabolism.

Among SCFAs, butyrate is the most extensively studied. It enhances intestinal barrier function through three complementary mechanisms. After antibiotic treatment reduced gut microbial abundance, SCFA levels decreased by more than 90%, accompanied by impaired hypoxia-inducible factor signaling in the mouse colon. Supplementation with tributyrin restored hypoxia-inducible factor-1α and its target gene expression to near-normal levels. Mechanistic studies further showed that butyrate directly inhibits prolyl hydroxylase domain protein 2, with a noncompetitive Ki value of 5.3 ± 0.5 mM, and that restoration of hypoxia-inducible factor-related signaling was statistically significant.[56] Second, butyrate contributes to mucus barrier repair. In a dextran sulfate sodium model, oral administration of butyrate at 20 mg/kg increased goblet cell numbers and enhanced the expression of mucin 2 and SAM pointed domain-containing ETS transcription factor. In co-culture experiments, butyrate showed a stronger mucus-repair effect after pretreatment of macrophages with 500 μM butyrate, suggesting that this process is mediated in part through the macrophage/Wnt/extracellular signal-regulated kinase axis.[57] In LPS-stimulated Caco-2BBe cells, butyrate reduced the ratios of phosphorylated NF-κB p65 subunit to total p65 and phosphorylated inhibitor of NF-κB alpha to total inhibitor of NF-κB alpha. It also increased the expression of solute carrier family 26 member 3, zonula occludens-1, occludin, and claudin-1. These changes were statistically significant, and some endpoints reached $P < 0.0001$. These findings suggest that butyrate restores intestinal epithelial tight junction integrity through the histone deacetylase 8/NF-κB pathway.[58] These results indicate that butyrate has an upstream regulatory role in MASLD, which can enhance epithelial hypoxia signals, promote mucosal repair, and maintain the integrity of tight junctions, thereby limiting the translocation of intestinal-derived microbial products.

In an HFD-induced rat model, oral butyrate at 20 mg/kg/day attenuated hepatic inflammatory signaling, significantly reducing nuclear p50 NF-κB levels compared with the HFD group ($P < 0.01$) and restoring cytosolic IκBα expression ($P < 0.05$). Butyrate also downregulated hepatic *Tlr2* mRNA expression ($P < 0.05$), while *Tlr4* and *Tlr9* showed a downward trend.[59] Butyrate also exerts anti-inflammatory effects through the modulation of lymphocyte function. Treatment of rat lymphocytes with 1.5 mM butyrate resulted in an approximately 99% reduction in thymidine uptake, indicating potent suppression of lymphocyte proliferation; furthermore, butyrate significantly inhibited the secretion of interleukin-2 and interferon-γ from Th1 cells.[60] Butyrate promotes an anti-inflammatory immune response by enhancing M2 macrophage polarization without an increase in M1 macrophage-associated markers. In bone marrow-derived macrophages, 50 μg/mL butyrate significantly enhanced interleukin-4-induced Arg1, Fizz1, and Ym1 expression, with all three markers showing significant increases ($P < 0.05$ to $P < 0.001$). Consistently, in HFD-fed mice, sodium butyrate increased the CD206+ M2 macrophage population ($P < 0.05$) and upregulated anti-inflammatory genes, including interleukin-10 ($P < 0.001$) and interleukin-4 ($P < 0.01$), whereas the CD11c+ M1 macrophage population showed no significant change.[60,61]

In a randomized double-blind placebo-controlled trial, a butyrate-based formula given for 12 weeks improved plasma lipid profiles in individuals with liver steatosis and metabolic syndrome. In the active-treatment group, total cholesterol decreased from 228 ± 13 to 221 ± 11 mg/dL, and triglycerides decreased from 221 ± 24 to 207 ± 23 mg/dL.[62] Sodium butyrate at a low concentration (2 mM) suppresses the expression of adipogenic genes while upregulating fatty acid oxidation-related genes via the activation of GPR41/GPR43-calcium/calmodulin-dependent protein kinase II/HDAC1-cAMP response element-binding protein signaling. In addition, sodium butyrate enhances fatty acid oxidation and energy metabolism through upregulation of CPT1 expression and increased cellular maximal oxygen consumption rate, thereby significantly ameliorating hepatic lipid accumulation induced by HFD.[63] These lipid-directed mechanisms provide a metabolic endpoint for the aforementioned gut-liver and inflammatory modulation, shifting hepatic metabolism toward enhanced oxidative capacity and reduced lipid storage.

Although animal studies have demonstrated that supplementation with SCFAs exerts significant therapeutic effects against MASLD, their low molecular weight and rapid systemic clearance pose challenges in maintaining stable and effective drug concentrations, thereby limiting clinical applicability.[64] To address this challenge, Babita Shashni and colleagues developed a prodrug strategy based on amphiphilic block copolymers, in which SCFAs are conjugated via ester linkages to enable sustained *in vivo* release of SCFAs, thereby significantly enhancing their clinical translatability. The clinical value of this prodrug strategy lies mainly in converting SCFAs into an oral therapeutic form that can maintain a longer duration of action in the liver. After oral administration, free butyrate declined to physiological levels in the liver within 24 h. In contrast, butyrate released by this prodrug strategy remained detectable for up to 72 h. This strategy also markedly ameliorated lipogenesis and fibrosis in a choline-deficient, L-amino acid-defined, HFD-induced mouse model of MASH with liver fibrosis. This approach is most likely to be developed as a sustained-release therapeutic strategy for active MASH, especially in patients with metabolic syndrome and a risk of early-to-moderate fibrosis, rather than as a general intervention for simple steatosis or end-stage cirrhosis. This is because the strategy not only improves the pharmacokinetic limitations of SCFAs but also enhances their integrated regulatory effects on barrier homeostasis, metabolic reprogramming, and fibrosis progression (Fig. 1).[65]

The dual nature of choline metabolism: From preservation of membrane homeostasis to pathological activation of the gut microbiota–trimethylamine N-oxide (TMAO) axis

Dietary choline deficiency is an important contributor to MASLD progression by promoting hepatic lipid accumulation, endoplasmic reticulum stress, insulin resistance, and downstream inflammatory injury.[66] In murine models, a methionine- and choline-deficient diet increased hepatic uptake of radiolabeled oleic acid by 3-fold in db/m mice ($P < 0.001$) and by 2-fold in db/db mice ($P < 0.01$), while very-low-density lipoprotein secretion was significantly reduced in db/m mice. These findings indicate that choline deficiency enhances intrahepatic lipid accumulation.[67] Conversely, in *Pemt*$^{-/-}$*; Ldlr*$^{-/-}$ mice fed a HFD, hepatic free cholesterol, cholesteryl esters, and triglycerides increased by 30%, 1.1-fold, and 3.1-fold, respectively. Choline supplementation normalized hepatic cholesterol metabolism and markedly attenuated liver injury, although hepatic triglyceride accumulation was

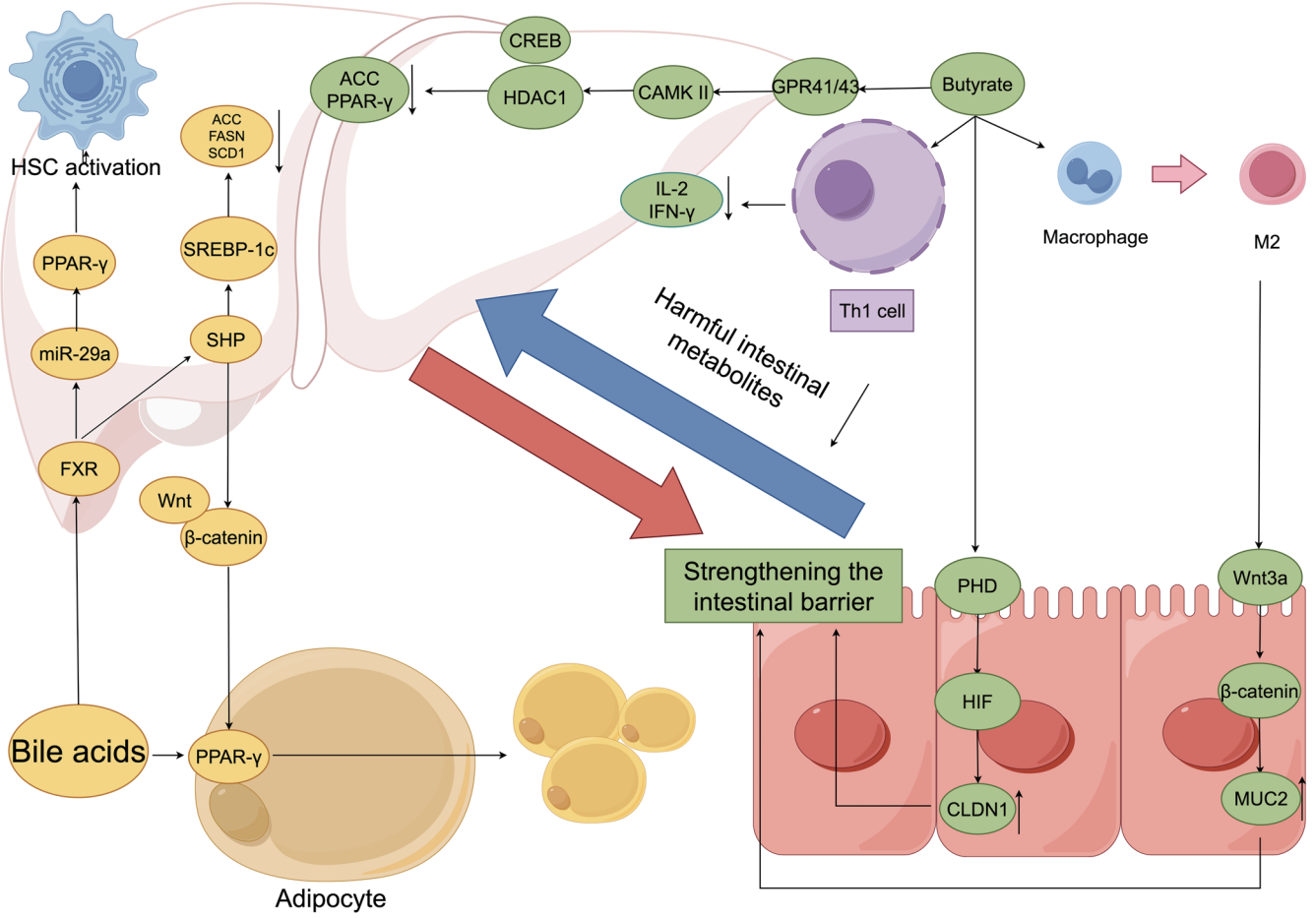

Fig. 1. Butyrate- and bile acid-associated pathways in MASLD. The schematic shows FXR-centered bile acid signaling and butyrate-linked GPR41/43-CaMKII-HDAC1-CREB signaling. It also includes the miR-29a/PPAR-γ and SHP/SREBP-1c/Wnt/β-catenin branches, together with Th1 cell, M2-macrophage, hepatic stellate cell, adipocyte, and intestinal barrier components. ACC, acetyl-CoA carboxylase; CaMKII, calcium/calmodulin-dependent protein kinase II; CLDN1, claudin-1; CREB, cAMP response element-binding protein; FASN, fatty acid synthase; FXR, farnesoid X receptor; GPR41/43, G protein-coupled receptor 41/43; HDAC1, histone deacetylase 1; HIF, hypoxia-inducible factor; HSC, hepatic stellate cell; IFN-γ, interferon-γ; IL-2, interleukin-2; MUC2, mucin 2; miR-29a, microRNA-29a; M2, M2 macrophage; PHD, prolyl hydroxylase domain-containing protein; PPAR-γ, peroxisome proliferator-activated receptor-γ; SCD1, stearoyl-CoA desaturase 1; SHP, small heterodimer partner; SREBP-1c, sterol regulatory element-binding protein 1c; Th1, T helper 1; Wnt, Wingless/Integrated; Wnt3a, Wnt family member 3A; ↓, decrease; ↑, increase. Created with Figdraw.

not fully corrected.[68]

Choline is also a key substrate for the gut microbiota-mediated trimethylamine (TMA) and TMAO pathway.[69] At the hepatic level, spatial transcriptomics can resolve lobular zonation and cell-specific transcriptional programs and localize metabolic signals related to TMA–TMAO conversion. It can also link these signals to local microenvironmental features, such as inflammatory infiltration and fibrosis, thereby revealing spatial coupling between local transcriptional programs and the pathological microenvironment.[70] Notably, because hepatic flavin-containing monooxygenase 3 (*Fmo3*) shows low basal expression in male mice, whole-liver Western blotting may fail to detect its low-abundance signal and may mask regionally enriched changes.[71] In this setting, spatial transcriptomics can first localize *Fmo3* mRNA-enriched lobular regions on tissue sections and then correlate these regions with metabolite spatial maps from the same or adjacent sections, thereby identifying microregions in which TMA–TMAO conversion is more likely to be active.[72]

TMAO contributes to the progression of MASLD through multiple mechanisms. A case-control study showed that plasma TMAO levels were significantly higher in patients with MASLD than in healthy controls. Compared with the lowest tertile, the highest tertile of TMAO was associated with significantly higher odds of MASLD (OR = 2.02, 95% CI 1.04–3.92; *P* for trend = 0.003). Further analysis showed that, after TMAO and related metabolites were added to the conventional risk model, the area under the receiver operating characteristic curve increased from 0.685 to 0.769, suggesting their potential value for risk stratification.[73] TMAO can directly activate the protein kinase R-like endoplasmic reticulum kinase (PERK)-eukaryotic initiation factor 2α (eIF2α) pathway, thereby inducing endoplasmic reticulum stress. This process suppresses global protein synthesis and activates the unfolded protein response, ultimately leading to the accumulation of misfolded proteins and sustained endoplasmic reticulum stress.[74]

TMAO promotes hepatic lipid accumulation through PERK-dependent endoplasmic reticulum stress. In zebrafish liver, TMAO significantly increased the expression of activating transcription factor 4 (ATF4) and C/EBP homologous protein (CHOP). At 20 weeks, *Atf4* mRNA reached approximately 6-fold and 4-fold of control levels in the 1% and 3% TMAO groups, respectively, whereas CHOP increased to about

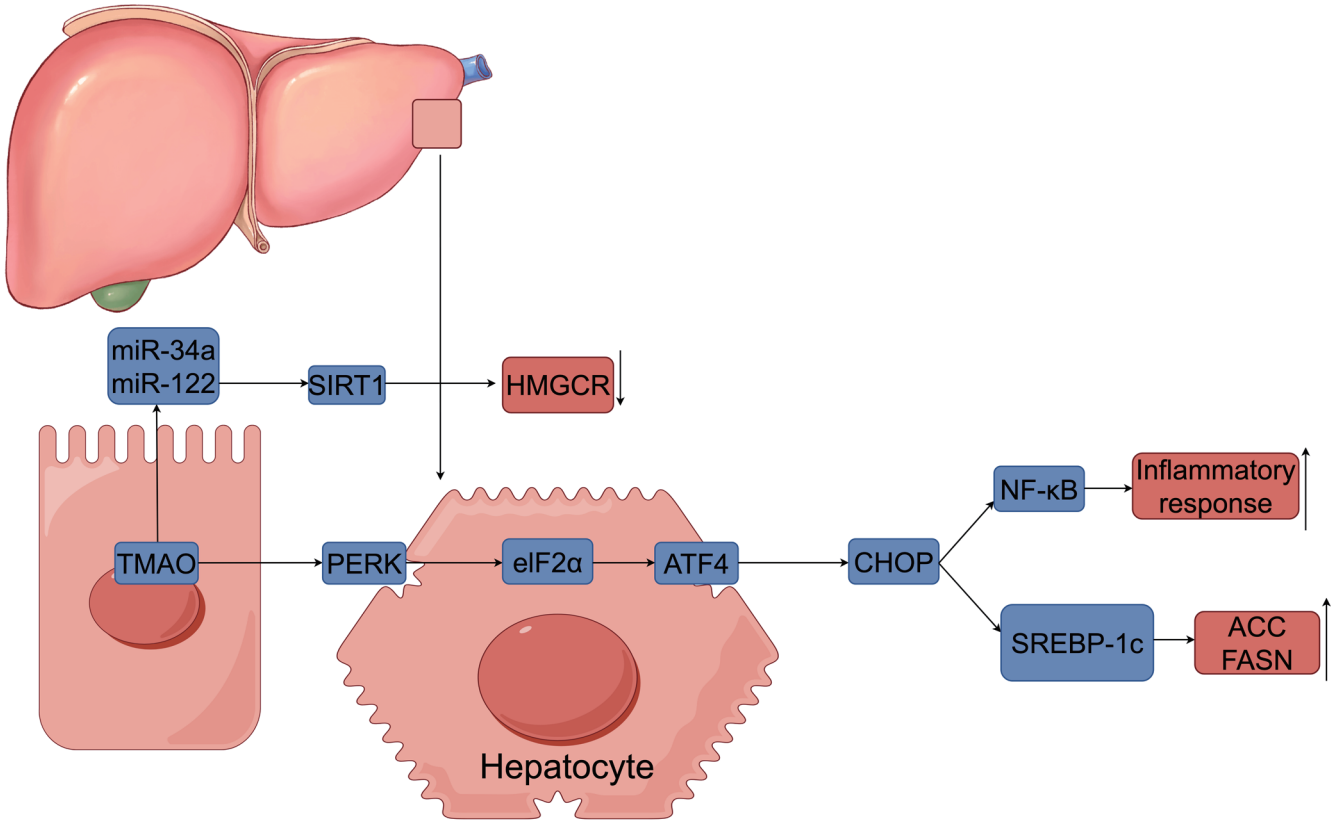

Fig. 2. TMAO-associated pathways in MASLD. This schematic illustrates two TMAO-related pathways in hepatocytes. The miR-34a/miR-122-SIRT1-HMGCR axis is associated with hepatic lipid metabolism, whereas the PERK-eIF2α-ATF4-CHOP pathway is associated with endoplasmic reticulum stress and is connected to the downstream NF-κB/inflammatory response and SREBP-1c/ACC/FASN branches. ACC, acetyl-CoA carboxylase; ATF4, activating transcription factor 4; CHOP, C/EBP homologous protein; eIF2α, eukaryotic initiation factor 2α; FASN, fatty acid synthase; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; miR-34a, microRNA-34a; miR-122, microRNA-122; NF-κB, nuclear factor kappa B; PERK, protein kinase R-like endoplasmic reticulum kinase; SIRT1, sirtuin 1; SREBP-1c, sterol regulatory element-binding protein 1c; TMAO, trimethylamine N-oxide; ↓, decrease; ↑, increase. Created with Figdraw.

1.5-fold and 2-fold. In HepG2 cells, 50 μM TMAO increased triglyceride content at 24 h and transiently upregulated *Srebp1* mRNA during early exposure. The PERK inhibitor GSK2606414 attenuated these changes. Overall, these results support a role for PERK-mediated endoplasmic reticulum stress in TMAO-induced hepatic lipid accumulation.[75]

PERK-driven endoplasmic reticulum stress may amplify inflammatory signaling in MASLD. PERK phosphorylation promotes eIF2α phosphorylation, increases ATF4 and CHOP expression, and facilitates NF-κB activation.[76] TMAO may further aggravate MASLD through selective dysregulation of disease-related microRNAs. In a steatotic HepG2 model, TMAO at 75 and 150 μM significantly increased miR-34a expression ($P = 0.006$ and $P = 0.003$, respectively). TMAO at 75 μM also increased miR-122 ($P < 0.05$), whereas the fibrosis-related miR-192 remained unchanged.[77] These changes are consistent with the reported association of miR-34a and miR-122 with sirtuin 1 suppression and disturbed hepatic lipid metabolism, as illustrated in Figure 2.

TMAO is expected to become a quantifiable biomarker for risk stratification and treatment evaluation in MASLD patients.[78] Machine learning-assisted decision-making provides a new perspective for MASLD treatment. By integrating plasma TMAO levels and other related metabolic indicators, machine learning can dynamically analyze disease progression and assess treatment responses. However, current TMAO detection mainly relies on liquid chromatography-mass spectrometry, a method that requires expensive equipment and complex operation, limiting its widespread use in clinical practice. Developing novel TMAO detection methods, such as enzyme-linked immunosorbent assays or portable sensors, is key to overcoming this technical challenge.

Conclusions

The gut microbiota and its metabolites are integral to MASLD pathogenesis. Within the tripartite framework of the gut-liver axis, oxidative stress, and metabolic reprogramming, detrimental microbial factors promote intestinal barrier disruption, activation of inflammatory and cellular stress pathways, and hepatic lipid accumulation, whereas protective microbial factors help preserve barrier integrity and metabolic homeostasis. Bile acids and choline also exhibit dual roles. Under physiological conditions, they are essential for lipid transport, maintenance of hepatocellular membrane homeostasis, and hepatic metabolic regulation. Under conditions of gut microbial dysbiosis and metabolic imbalance, however, their aberrant transformation and signaling disturbances can drive inflammatory amplification, endoplasmic reticulum stress, lipid reprogramming, and fibrogenic progression. This dual role indicates that microbiome-based interventions in MASLD should be guided by disease stage, metabolic phenotype, and key dysregulated metabolic pathways.

The "multiple hits" hypothesis is widely accepted in MASLD,

suggesting that several primary metabolic abnormalities may jointly drive disease progression. Notably, gut microbial dysbiosis and metabolic disturbances are heterogeneous across patients, and a dominant dysregulated metabolic pathway may underlie disease progression in a given individual. Future studies should therefore move beyond descriptive associations and combine metagenomics, metabolomics, and transcriptomics with functional validation and causal inference to identify the dominant pathways driving disease progression. Such pathway-oriented stratification may provide a basis for more individualized microbiome-based interventions in MASLD.

Although substantial advances have been achieved in current research, numerous unresolved questions persist. In this context, the rapid development of spatially resolved technologies has created new opportunities to address MASLD-related questions. Applying spatial transcriptomics together with imaging mass spectrometry-based spatial metabolomics to MASLD can localize, at the tissue microregional scale, the spatial origins of key pathological processes such as inflammation, fibrosis, and metabolic reprogramming, and delineate their propagation across distinct cellular niches. Building on this, the spatial distribution of metabolites such as bile acids and lipids can be co-registered with receptor signaling, stress responses, and transcriptional programs in the corresponding cells, thereby linking "metabolite exposure–cell–state transition–tissue injury progression" into a continuous spatial causal chain. This approach may further enable the identification of zonated biomarkers with defined tissue origin and microenvironmental specificity, providing more precise subtyping and earlier warning signals for the diagnosis and management of MASLD.

Future gut-liver axis studies should stratify MASLD, MetALD, and cryptogenic SLD, because alcohol exposure and underlying etiology may influence disease trajectory, gut microbiota signatures, and therapeutic response. Once etiology and subtype are clearly defined, microbiome and metabolite abnormalities that are truly related to disease progression may be identified more accurately, thereby reducing signal distortion arising from mixed cohorts with different etiologic backgrounds. This, in turn, may improve the accuracy of patient selection, help identify patients more likely to benefit from microbiome-based interventions, and increase the reliability of treatment-response evaluation.

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Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Study concept and design (JZ, QZ), literature search and acquisition of data (YZ, DW, BZ), analysis and interpretation of data (FZ, CT), drafting of the manuscript (YZ, DW), critical revision of the manuscript for important intellectual content (JZ, QZ, BZ), and administrative and technical support (QZ). All authors have read and approved the final manuscript.

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