



## Review Article

# Current insights into the interplay between gut microbiota-derived metabolites and metabolic-associated fatty liver disease

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### ABSTRACT

Metabolic dysfunction-associated fatty liver disease (MAFLD) is a prevalent and challenging disease associated with a significant health and economic burden. MAFLD has been subjected to and widely investigated in many studies; however, the underlying pathogenesis and its progression have yet to understand fully. Furthermore, precise biomarkers for diagnosing and specific drugs for treatment are yet to be discovered. Increasing evidence has proven gut microbiota as the neglected endocrine organ that regulates homeostasis and immune response. Targeting gut microbiota is an essential strategy for metabolic diseases, including MAFLD. Gut microbiota in the gut-liver axis is connected through tight bidirectional links through the biliary tract, portal vein, and systemic circulation, producing gut microbiota metabolites. This review focuses on the specific correlation between gut microbiota metabolites and MAFLD. Gut microbiota metabolites are biologically active in the host and, through subsequent changes and biological activities, provide implications for MAFLD. Based on the review studies, gut-liver axis related-metabolites including short-chain fatty acids, bile acids (BAs), lipopolysaccharide, choline and its metabolites, indole and its derivatives, branched-chain amino acids, and methionine cycle derivatives was associated with MAFLD and could be promising MAFLD diagnosis biomarkers, as well as the targets for MAFLD new drug discovery.

**KEYWORDS:** Gut microbiota-metabolites, Gut-liver axis, Metabolic dysfunction-associated fatty liver disease

### INTRODUCTION

Metabolic dysfunction-associated fatty liver disease (MAFLD), an updated nomenclature of the previous term, nonalcoholic fatty liver disease (NAFLD), is currently the most prevalent chronic liver disease affecting a quarter of the adult population worldwide [1,2]. Recently, MAFLD also becomes prominent as the cause of liver cirrhosis and liver cancer [3,4]. The rising prevalence of MAFLD has been observed globally, with a higher prevalence in the Middle East, South America, and Asia than in Africa. Early large-scale detection of MAFLD and prevention of its related complications have become concerns in many countries [5]. MAFLD was also the most rapidly increasing indication for liver transplantation in the United States [6]. In addition, end-stage liver disease and liver-related mortality have been associated with MAFLD. The modeling system has shown that the global burden of MAFLD will continue to increase, with the most significant increase in prevalence expected in urban populations such as China [7].


MAFLD has been subjected to and widely investigated in many studies. However, the underlying pathogenesis and its progression have yet to be fully understood. MAFLD involves free fatty acids elevation, lipopolysaccharide (LPS) translocation, *de novo* lipogenesis, insulin resistance, oxidative stress, endoplasmic reticulum stress, mitochondria dysfunction, NLRP3 inflammasome activation, and inflammatory cytokines production such as interleukin-1 $\beta$  (IL-1 $\beta$ ) and IL-6 [8,9]. Due to its complexity and lack of comprehensive knowledge, there are currently no FDA-approved drugs to treat MAFLD. According to practice guidance issued by the American Association for the Study of Liver Diseases, only limited pharmacological treatments could be given to selected patients of MAFLD, such as pioglitazone for patients with and without T2DM with biopsy-proven MAFLD and Vitamin E for nondiabetic patients with biopsy-proven MAFLD [10].

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Increasing evidence has proven gut microbiota as the neglected endocrine organ that regulates homeostasis and immune response. Targeting gut microbiota could be an important strategy for metabolic disease and stress-related disorders and opportunities in new drug discovery [11]. The human gut microbiota stands at the intersection between dietary components and health or disease in which microbiota and microbiota-derived molecules interact with the host on the intestinal surface and becomes a signal for biological communication to distant organs in the human body to build the gut-systemic axis [12]. In particular, the gut-liver axis, as part of the gut-systemic axis, plays a vital role in the metabolic system. This gut-liver axis is connected via tight bidirectional links through the biliary tract, portal vein, and systemic circulation. The liver delivers signals to the gut by releasing bile acids (BAs) and bioactive mediators into the biliary tract and systemic circulation. In the gut, microbiota hosts metabolize BAs, amino acids, and other exogenous substrates. These gut microbiota metabolites were translocated to the liver through the portal vein and affected liver functions. This gut-liver axis mediated by gut microbiota could allow us to fully understand the link between gut microbiota and MAFLD pathogenesis [13,14]. Investigating metabolites such as BAs, short-chain fatty acids (SCFAs), LPS, choline and its metabolites, indole derivatives, branched-chain amino acids (BCAAs), methionine cycle derivatives, and other metabolites from gut-liver axis could be a promising approach to discovering the underlying mechanisms of MAFLD and its therapeutic management. This review focuses on the correlation between gut microbiota metabolites and MAFLD through the gut-liver axis. These metabolites could be promising MAFLD diagnosis biomarkers and targets for MAFLD new drug discovery.

## METABOLIC-ASSOCIATED FATTY LIVER DISEASE

MAFLD, previously known as NAFLD, is a new definition for liver disease associated with known metabolic dysfunction. In 2020, a group of international experts and scholars proposed that NAFLD should be renamed MAFLD to emphasize the critical relationship between metabolic dysfunction and this disease and hope to integrate the current concept of MAFLD pathogenesis and seek a better treatment strategy [2]. The diagnosis of NAFLD requires hepatic steatosis of  $\geq 5\%$  without concurrent liver disease, including significant alcohol usage and other known causes of liver diseases. According to the latter definition of MAFLD, type 2 diabetes mellitus and overweight/obesity by ethnic-specific body mass index (BMI) classification are MAFLD metabolic risk drivers. Overweight or obese is defined as BMI  $\geq 25$  kg/m<sup>2</sup> in Caucasians or BMI  $\geq 23$  kg/m<sup>2</sup> in Asians, while type 2 diabetes mellitus is defined according to widely accepted international criteria. The individual who has hepatic steatosis of  $\geq 5\%$  with type 2 diabetes mellitus risk driver or overweight/obesity risk driver diagnosed with MAFLD. In addition, healthy weight individual who has steatosis and meets two of the seven risk factors have abnormalities including increased waist circumference, high blood pressure, increased plasma triglycerides (TG), low

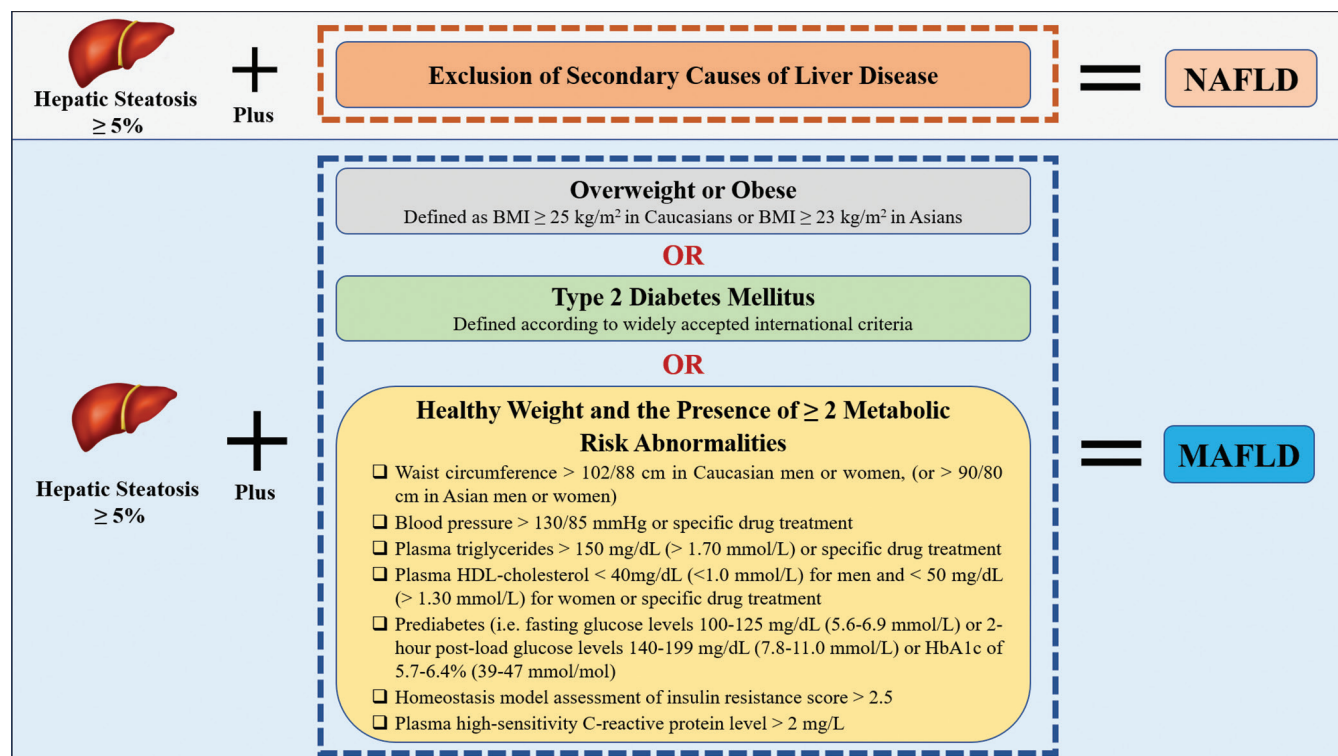
plasma high-density lipoprotein-cholesterol, prediabetes, homeostasis model assessment of insulin resistance score, and high plasma sensitivity C-reactive protein will be categorized as MAFLD. Hepatic steatosis should be detected by imaging techniques, blood biomarkers/scores, or liver histology. Combining hepatic steatosis with one of these three metabolic risk stratifications results in the diagnosis of MAFLD [1,15]. The difference in diagnostic criteria between MAFLD and NAFLD is shown in Figure 1.

MAFLD, as a prevalent and challenging disease, can and often does coexist with other conditions. In addition to the single etiology of MAFLD, international experts and scholars also proposed a dual etiology of concomitant MAFLD with other liver diseases. Patients who meet the criteria to diagnose MAFLD as described above and who also have one of the concurrent conditions, including alcohol-use disorder, viral infection (HIV, HBV, and HCV), autoimmune hepatitis, inherited liver disorders, drug-induced liver injury or other known liver disease should be defined as having dual (or more) etiology fatty liver disease. These individuals with the concomitant condition likely have a different natural history and response to therapy than those with liver disease of a single etiology of MAFLD [1]. In this review, we focus on the single etiology of MAFLD.

Gradually, increasing prevalence of MAFLD has been observed in recent years. It continues to increase, which has brought a significant burden on the health-care system of countries worldwide [16]. MAFLD is a chronic metabolic disease, and its exact pathogenesis is still unclear. At present, precise biomarkers and specific drugs for diagnosing and treating MAFLD still need to be discovered [17]. In addition, currently, invasive biopsy is the most common method in detecting nonalcoholic steatohepatitis (NAS), so there are still many limitations. Suppose the biological biomarkers related to MAFLD can be found to reveal the relationship between them and MAFLD; it may be possible to understand the pathogenesis and prognosis of MAFLD from a new perspective, simultaneously explore its clinical features and provide therapeutic target strategies. The most well-known explanation theory for MAFLD pathogenesis is still the "multiple hit model", which suggests that MAFLD may result from multiple hits on liver cells, including genetic susceptibility, epigenetic alterations, dysregulation of metabolic pathways, cell-cell interactions, hepatic, paracrine, and inter-organ interactions [18,19].

## GUT MICROBIOTA AND METABOLITES

The gastrointestinal tract of adults is estimated to be inhabited by more than 1000 types of microbial flora. Far exceeding the sum of human cells and genes, this microbial flora contains approximately 100 trillion microbial cells and 22 million unique microbial genes [20]; among them, *Firmicutes* and *Bacteroides* accounted for more than 90% of the overall flora [21]. The human gut microbiota can express a variety of metabolic enzymes through these unique genes to produce various metabolites. The gut microbiota metabolites, including SCFAs, BAs, LPS, choline and its metabolites, indole and its derivatives,



**Figure 1:** Diagnostic criteria for metabolic dysfunction-associated fatty liver disease and nonalcoholic fatty liver disease. MAFLD: Metabolic dysfunction-associated fatty liver disease, NAFLD: Nonalcoholic fatty liver disease, The NAFLD requires hepatic steatosis of  $\geq 5\%$  without concurrent liver disease. The MAFLD requires steatosis of  $\geq 5\%$  with type 2 diabetes mellitus and overweight/obesity as metabolic risk drivers. A healthy-weight individual with steatosis who meets two of the seven metabolic abnormalities risk factors is classified as MAFLD.

BCAAs, and methionine cycle derivatives. These metabolites are also biologically active in the host [22] and, through their biological activities, provides implication for cardiovascular and metabolic diseases [23]. Therefore, gut microbiota-derived metabolites can trigger various pathophysiological changes and responses in the host. It is currently known that the human gut microbiota can ferment dietary fibers to produce SCFAs, and SCFAs can be used as the primary energy source of human colonocytes [24]. In addition, other endogenous substances, such as BAs and vitamins, are metabolized by gut bacteria.

On the other hand, gut microbiota also acts as immune defenders. Host bacteria can attach to the surface of intestinal epithelial cells to form a “biological wall” to protect the host from foreign viruses, toxins, or LPS invasion from the intestinal tract. They can also produce antibacterial substances such as microcins or release specific metabolites (such as SCFAs) to decrease pH locally and prevent the proliferation of pathogenic bacteria [25]. All the above examples show that when the human body has good gut microbiota, it is helpful to the health of the human body; otherwise, it is closely related to the emergence of various diseases.

### GUT-LIVER AXIS AND METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE

The liver is the most critical metabolism and detoxification organ in the human body. The portal vein and hepatic artery provide blood supply into the liver. The hepatic portal veins

deliver three-quarters of the liver blood supply, and the hepatic arteries provide the remaining quarters. The gut and liver are closely connected through the portal vein and the biliary system. Gut-derived substances such as digested food fragments (e.g. amino acids, lipid fragments, and simple sugars), gut microbiota products, and exogenous toxins can enter the liver through the portal vein. The liver responds to these gut messages by excreting BAs, cytokines, and other bioactive metabolites into the gut. Therefore, the two-way communication between the gut and the liver is called the “gut-liver axis” [26]. The gut-liver axis is vital to maintaining normal physiological balance in the body. When the gut-liver axis is disturbed, it is associated with the pathogenic mechanism of chronic liver diseases. Rapidly proliferating gut microbiota and their metabolites may penetrate the damaged intestinal wall into the portal vein, activate the immune storm response, cause various liver inflammations, and ultimately promote the occurrence and progression of MAFLD [25]. Recently, many studies have demonstrated that the gut microbiota plays a vital role in the pathogenic progression of MAFLD. Several animal studies have also shown that MAFLD can develop through the gut microbiota of heterogeneous MAFLD mice in the experiment of germ-free mice. These results indicated that the gut microbiota could directly participate in the occurrence and progression of MAFLD [27,28]. In addition, gut microbiota components and their metabolites, such as LPS and BAs, may serve as a bridge for the interaction between gut bacteria and the liver.

## GUT MICROBIOTA-DERIVED METABOLITES AND METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE

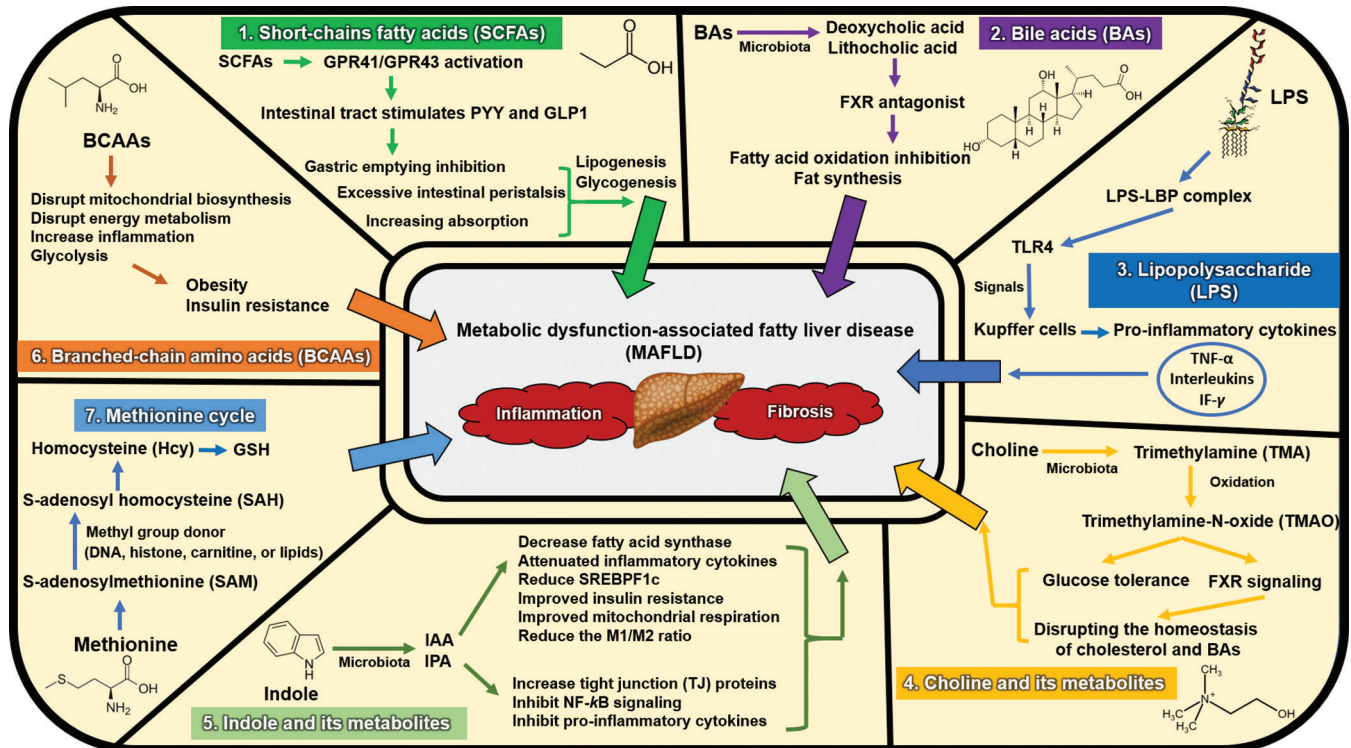
Based on the previous studies to date, there are several groups of gut microbiota-derived metabolites: (1) metabolites synthesized by host cells and then biochemically modified by gut microbiota, such as secondary BAs; (2) Metabolites synthesized directly from dietary fibers, such as SCFAs and indole derivatives by gut microbiota; (3) Metabolites synthesized de novo by gut microbiota, such as polysaccharide A [22,29]. In addition, several other metabolites, such as choline and its metabolites, BCAAs, and the methionine cycle will be discussed in this review. The interplay between gut microbiota-derived metabolites, their subsequent changes, and their implication for MAFLD can be seen in Figure 2.

### SHORT-CHAIN FATTY ACIDS

SCFAs are saturated aliphatic organic acids comprising less than six carbons produced by dietary fiber in gut microbiota's anaerobic fermentation process. The main components include acetate (C2) and propionate (C3), and butyrate (C4) [30]. SCFAs serve as a source of energy for enterocytes and are precursors to lipogenesis and gluconeogenesis. Colonocytes in the proximal colon mainly absorb butyrate as an essential energy source. It is mainly metabolized in the epithelial mucosa to preserve the colon's health by protecting it from some colonic pathologies [24]. GPR41/GPR43 expressed in

adipocytes, immune cells, and enteroendocrine L cells can be activated by SCFA through the host G protein-coupled receptor pathway. The activated GPR41/GPR43 can stimulate the intestinal tract to secrete PYY and GLP-1, thereby inhibiting gastric emptying and excessive intestinal peristalsis, increasing the absorption of nutrients in the intestinal tract, and promoting glycogenesis and lipogenesis in the liver [31]. In addition, when SCFAs bind to GPCRs, this process can induce GPR43 activation on adipocytes, inhibit lipolysis, and reduce the level of free fatty acids in circulation [32].

Moreover, TG synthesis (from acetic acid) and gluconeogenesis (from propionic acid) will be promoted when these SCFAs enter the liver via the portal vein, which may be related to the development of MAFLD [33]. However, the existing results were mixed and were obtained individually due to differences in patient age, dietary influences, environmental factors, sample sources (blood or feces), detection techniques, and different animal models. So, SCFAs levels associated with the development of MAFLD remain controversial. In most animal studies, SCFAs have been considered to be beneficial to host health protection, including supplementing SCFAs *in vitro* or increasing dietary fiber (can be fermented into SCFAs) in the diet or the host can direct use pro-drug of SCFAs, which can improve insulin resistance and alleviate the fatty liver disease or non-alcoholic steatohepatitis (NASH; a more severe form of MAFLD) induced HFD (high-fat diet) mice [27,34-38]. In addition, SCFAs have been shown to possess anti-inflammatory effects by activating GPR41/43



**Figure 2:** The interplay between gut microbiota-derived metabolites and metabolic-associated fatty liver disease. MAFLD: Metabolic-associated fatty liver disease. The human gut microbiota can express a variety of metabolic enzymes to produce various metabolites such as SCFAs, BAs, LPS, choline and its metabolites, indole and its derivatives, BCAAs, and methionine cycle derivatives. These metabolites are biologically active in the host and through their biological activities, provides implication for MAFLD. Abbreviations: G protein-coupled receptor, GPR; glucagon-like peptide, GLP-1; peptide YY, PYY; farnesoid X receptor, FXR; lipopolysaccharide-binding protein (LBP); tumor necrosis factor- $\alpha$ , (TNF- $\alpha$ ); interferon gamma, indole-3-acetic acid (IAA); indole-3-propionic acid (IPA); nuclear factor kappa-light-chain-enhancer of activated B cells, NF- $\kappa$ B

and AMP-activated protein kinase and inhibiting histone deacetylases (HDACs), which will be necessary for the treatment of MAFLD [35,39,40]. The serum levels of acetate and propionate, which are the most abundant SCFAs were also significantly lower in patients with MAFLD, but not butyrate [41]. It has been found that the number of bacteria producing SCFAs in the feces of MAFLD patients was significantly increased compared with normal controls. Higher fecal SCFAs such as acetate and propionate were associated with a higher ratio of Th17/Treg cells in peripheral blood, indicating a higher immune response; this also shows that gut microbiota is indeed related to immune regulation [42]. In addition to that, de la Cuesta-Zuluaga *et al.* have demonstrated that fecal SCFAs levels positively correlate with obesity and metabolic disorders [43]. However, some other scholars pointed out that it should be SCFAs in human circulation rather than SCFAs in feces associated with MAFLD [44].

## BILE ACIDS

BAs are steroid acids mainly synthesized by the liver (primary) and modified by gut microbiota (secondary). Cholic acid and chenodeoxycholic acid are the primary BAs synthesized in the liver cells via CYP7A1 and transported to the intestinal tract. The action of microbiota in the intestine on primary BAs resulted in the formation of secondary BAs, deoxycholic and lithocholic acids [45]. The role and effect of BAs in MAFLD have been widely investigated. BAs control the expression of lipogenesis-related genes in the liver and the metabolism of TG in the liver through the SREBP-1c signaling pathway, thereby reducing the accumulation of TG in the liver and reducing the occurrence of MAFLD [46]. Therefore, fatty liver and steatohepatitis may be induced when the host causes metabolic defects related to carbohydrates and lipids due to BA dysfunction. In previous studies, changes in circulating BAs levels were found in MAFLD patients, as an increase of total BAs in serum and the increase of gut microbiota involved in secondary BAs modification. The serum deoxycholic acid, secondary BA and FXR antagonist increased significantly among them. The level of chenodeoxycholic acid, primary BA and FXR activator was decreased significantly [47,48]. Previous studies also indicated that the FXR signaling pathway in NASH patients might be damaged, inhibiting fatty acid oxidation, thus promoting the increase of fat synthesis and accelerating the progression of MAFLD [48]. Therefore, the levels of both primary and secondary BAs in host blood and the function of their receptors could be potential therapeutic targets for MAFLD treatment management.

## LIPOLYSACCHARIDE

LPS are bacterial toxins consisting of the O polysaccharide, core oligosaccharide, and Lipid A. Typically, the term endotoxin is used synonymously with LPS, however a few endotoxins are not LPS. LPS, produced by most Gram-negative bacteria, provides structural integrity and a permeability barrier to protect the bacterial cell from the entry of harmful molecules such as toxins and bile salts during its inhabitation in the gastrointestinal tract. The role of LPS associated with MAFLD has been widely investigated. In a previous study,

MAFLD patients had higher serum LPS, which increased liver damage by inducing macrophage and platelet activation through the Toll-like receptor 4 (TLR4) pathway [49]. Bacterial strains of endotoxin-producing pathogenic species were also overgrowing in the obese human gut and play as causative agents for MAFLD [50]. Compared to the healthy subjects, the mean LPS was also reported to be significantly higher in MAFLD patients [51,52]. These higher-level clinical specimens indicate the functional role of LPS in MAFLD pathogenesis.

The possible mechanism of LPS in the progression of MAFLD has been reported through TLR4 [49,53]. When LPS enters the liver through the portal vein, it immediately binds to the serum LBP. Then, the LPS/LBP complex activates TLR4. Activated TLR4 delivers the LPS signal to its downstream signal molecules, promoting the production of pro-inflammatory cytokines by Kupffer cells (TNF- $\alpha$ , ILs, and IF- $\gamma$ ) and further hepatic inflammation [54]. More importantly, TLR4 expression is overexpressed in MAFLD patients [55]. Furthermore, the administration of TLR4 inhibitors, such as aspirin in mice with MAFLD and high levels of LPS, resulted in lower levels of inflammation. Since Kupfer cells are responsible for clearing LPS and are activated by TLR4, depletion of Kupfer cells by clodronate liposomes administration prevented increases in TLR-4 and steatohepatitis [56]. Inhibition of TLR4 in macrophages by administration of a cell-permeable molecule TAK-242 could impair endotoxin-mediated IL-17 upregulation, which plays an important role in MAFLD pathogenesis [57]. Given that the LPS-TLR4 pathway plays an important role in MAFLD, this pathway, and its specific inhibitors could be target in MAFLD treatment. However, further studies are needed to assess their pharmacological mechanisms.

## CHOLINE AND ITS METABOLITES

Choline is an essential nutrient that plays an important role in metabolism. Choline is released from lecithin and dietary food intake though humans can *de novo* synthesize. Choline also acts as a source of methyl group needed for many steps in producing acetylcholine, S-adenosylmethionine (SAM), and S-adenosyl homocysteine (SAH). Choline has been studied with MAFLD. A choline-deficient diet often induces experimental MAFLD because choline inhibits excess lipid accumulation in the liver by promoting very low-density lipoprotein production [58]. Once in the digestive tract, gut microbiota covert free choline into trimethylamine (TMA) and further undergo oxidation to form trimethylamine-N-oxide (TMAO) by flavin-containing monooxygenases enzymes in the liver [59]. In addition, choline can also be metabolized into betaine, which both play a role in DNA methylation and histone modification [60]. Several studies have shown that choline and betaine supplementation can attenuate MAFLD induced by high-fat or high-fructose diets, which can be caused by insufficient choline [61-65]. Although betaine can prevent diet-induced MAFLD, it has been reported that the levels of betaine in serum were negatively correlated with MAFLD severity [66]; however, in some studies, high circulating choline levels

were positively associated with metabolic dysfunction and steatosis [67,68]. The positive association between MAFLD and serum choline levels may be attributed to the high level of TMAO in serum [69]. TMAO has been recognized as a risk factor for cardiovascular disease [70]; numerous studies have also pointed out that TMAO is correlated with the effects of metabolic syndrome and MAFLD [67,71-74]. Glucose tolerance was increased in the presence of TMAO by reducing glucose metabolism and insulin signaling [75] and disrupting the homeostasis of cholesterol and BAs by regulating FXR signaling [72]. However, a previous study revealed that oral administration of 120 mg/kg per day of TMAO attenuated steatohepatitis in rats, characterized by altered gut microbiota and decreased serum cholesterol and transaminase levels [76]. Overall, choline appears beneficial in MAFLD, while the precise role of TMAO in MAFLD is yet to be fully elucidated.

## INDOLE AND ITS DERIVATIVES

Indole is an aromatic, heterocyclic, organic compound found in the human body produced by gut microbiota from tryptophan. Indole derivatives mainly include indole-3-acetic acid (IAA), indole-3-propionic acid (IPA), indole-3-lactic acid, indole-3-carboxylic acid, tryptamine, and indoxyl sulfate [77]. Accumulating evidence proves that indole and its derivatives have a protective effect on MAFLD. Oral administration of indole after LPS in mice reduced the levels of pro-inflammatory cytokines IL-1 $\beta$ , IL-6, and IL-15 and the expression of NF $\kappa$ B, thereby attenuating liver inflammation [78]. Other studies have shown that indole supplementation inhibits macrophage activation and proliferation through PFKFB3 and CD68 [79,80]. IAA and tryptamine decreased fatty acid and LPS-stimulated production of pro-inflammatory cytokines in macrophages and inhibited the migration of cells toward a chemokine in mice models. IAA also attenuated inflammatory response in hepatocytes and reduced the expression of fatty acid synthase and sterol regulatory element-binding protein-1c [81]. IAA also attenuated HFD-induced hepatotoxicity in mice with improved insulin resistance, lipid metabolism, oxidative stress, and inflammation, thus alleviating MAFLD [82]. Another previous study showed that IAA improved mitochondrial respiration defects by upregulating peroxisome proliferator-activated receptor gamma coactivator 1-alpha in MAFLD [83]. In addition, a study in patients who had undergone sleeve gastrectomy showed that increased IAA from the intestine was positively correlated with hepatic imaging examinations (liver computed tomography (CT) values or Hounsfield values to measure NAFLD degree) and negatively correlated with liver fat attenuation. An increase in IAA in the intestine could reduce the M1/M2 ratio in the liver, thus promoting the amelioration of MAFLD in obese individuals [84]. IPA has been shown to possess therapeutic potential by reducing cell adhesion, inhibiting migration, and alleviating fibrogenesis in LX-2 cells.

Moreover, circulating IPA levels were lower in patients with liver fibrosis [85]. Furthermore, IPA inhibited gut dysbiosis and endotoxin leakage from attenuating steatohepatitis by increasing tight junction (TJ) proteins such as occluding and

ZO-1 and inhibiting NF- $\kappa$ B signaling and pro-inflammatory cytokines such as TNF $\alpha$ , IL-1 $\beta$ , and IL-6 [86]. IPA also improved the intestinal barrier by enhancing the epithelial barrier and mucus barrier through TJ (claudin-1, occluding, and ZO-1) and mucins (such as MUC2 and MUC4) [87]. On the contrary, another study found that IPA-aggravated CCl<sub>4</sub>-induced liver damage and fibrosis by activating TGF- $\beta$ 1/Smads signaling pathway. However, IPA alone does not produce hepatotoxicity, and oral administration of IPA can restore the gut microbiota structure after liver fibrosis induced by CCl<sub>4</sub> [88]. However, how IPA increases the toxicity of CCl<sub>4</sub> remains unclear.

In addition to IAA and IPA, indoxyl sulfate (InS), another indole derivative, is a low-molecular-weight protein-bound uremic toxin. Indole and indoxyl are also converted into InS by gut microbiota, mainly by *Escherichia coli* from tryptophan in dietary food intake, which is then absorbed by the intestine and redistributed in the blood circulation [79]. Previous studies have indicated that when chronic inflammation develops into chronic kidney disease (CKD), the level of InS in the serum will gradually increase [89], and about 90% of the InS in the serum will be combined with serum proteins, further increasing the difficulty removing uremic toxins by hemodialysis [90]. Overall, InS is involved in CKD progression and activation of inflammation-related biomarkers [91]. Although InS is a well-known predictor of CKD, the association between MAFLD patients and their circulating InS levels remains unclear. Only a few previous reports have indicated that curcumin (curcumin) treatment in MAFLD patients significantly reduces circulating InS levels compared with placebo [92]. Extensive research is needed to elucidate the role and the potency of InS in MAFLD disease.

## BRANCHED-CHAIN AMINO ACIDS

BCAAs are essential nutrients, including leucine (Leu), isoleucine (Ile), and valine (Val). They are amino acids having an aliphatic side chain with a branch. Mammals cannot *de novo* synthesize BCAAs, so they must be obtained through a diet such as meat, dairy, and legumes. However, there is evidence that human gut microbiota can synthesize BCAAs [93]. BCAAs have been used as biomarkers for metabolic diseases, cardiovascular diseases, and cancers, and their metabolites can also regulate gene expression and epigenome [94]. These BCAAs play an important role in the human body. Leu is the main component of protein, and Ile and Val provide carbon for the synthesis process of glucose; the amino acids produced by BCAAs catabolism provide energy for the tricarboxylic acid cycle and provide the cells with the energy they need. Intracellular Leu activates and regulates the mTORC1 pathway related to cell growth and metabolism; integrates signals of nutrients and energy sources in the body; stimulates cell growth when sources are plentiful; and promotes catabolism when the body is starved [95]. The degradation of amino acids in the body mainly occurs in the liver. Since branched-chain aminotransferase, an enzyme that catalyzes the transamination of BCAAs, is not expressed in the liver; therefore, BCAAs enter the systemic circulation directly from the intestinal tract. Therefore, the level of

BCAAs in serum can be altered according to diet. This change level affects several physiological processes, such as mitochondrial biosynthesis, energy metabolism, inflammation, and glycolysis [96]. A previous study has shown that long-term high concentrations of BCAAs in the blood are associated with metabolic disorders such as obesity [97], type 2 diabetes [98], and cardiovascular disease [99]. It has also been observed that there is a strong association between obesity and insulin resistance with increased circulating BCAAs, and these lipid metabolism disorders could be alleviated with low BCAAs diet [100]. Given that there is a strong association of BCAAs with metabolic disorders such as obesity and type 2 diabetes which are also associated with the formation of MAFLD. Another previous study has shown that emerging data indicate the undeniable correlation between elevated circulating BCAA levels and chronic liver diseases, including NAFLD, cirrhosis, and hepatocellular carcinoma [101]. Therefore, further studies related to BCAAs and MAFLD are worth exploring.

## METHIONINE CYCLE AND METABOLIC DYSFUNCTION-ASSOCIATED FATTY LIVER DISEASE

The methionine cycle is an essential metabolic pathway in mammals that regulates methylation and redox balance in cells [102]. It has also been implicated in the pathophysiology of MAFLD [3]. The liver can be regarded as the most active part of the methionine cycle in the body, in which methionine is adenosylated and is then converted to SAM through methionine adenosyltransferases. After that, with the help of various methyltransferases, SAM can provide its methyl group to DNA, histone, carnitine, or lipids, and SAM will then form SAH. SAH is hydrolyzed by SAH hydrolase into homocysteine (Hcy) and adenosine (this step is usually reversible). Then Hcy can participate in the methionine cycle through two biological pathways. Hcy can be catabolized into cystathionine, split into cysteine, and then cysteine forms glutathione, an essential antioxidant in the body. On the other hand, Hcy can be remethylated to form methionine in the presence of Vitamin B<sub>12</sub>.

A previous study has shown that gut microbiota contributes to methionine metabolism in animal model hosts. Methionine levels were elevated in the animal-free germ model; however, when the microbiota cleared with antibiotics resulted in a decrease in intestinal methionine [103]. Another study found that methionine restricted diet can improve gut microbiota by regulating the intestinal microbiota and its metabolite profiles in high-fat-fed mice [104]. Not only in the metabolic pathway of methionine, but the gene families derived from gut microbiota have also been involved in the metabolic pathway of BCAAs [105]. The molecular basis of gut microbiota and methionine metabolism in humans and their relationship with MAFLD still need to be fully understood. On the other hand, since methionine is closely related to DNA and histone methylation, and MAFLD has been subjected to epigenetics, exploring their relationship will also be necessary.

## CONCLUSION AND FUTURE PROSPECTS

The human gut microbiota can express a variety of metabolic enzymes to produce various substances called gut-microbiota-derived metabolites. Increasing evidence supports that microbiota-derived metabolites act as critical mediators of microbiota and the host in maintaining homeostasis and immune response. Gut-liver axis, the two-way communication between the gut and the liver in MAFLD, is becoming increasingly important. Gut-microbiota-derived metabolites, including SCFAs, BAs, LPS, choline and its metabolites, indole and its derivatives, BCAAs, methionine cycle derivatives are associated with MAFLD. However, their function as good and bad key players in MAFLD still vary, as summarized in Table 1. Gut microbiota is directly or indirectly involved in the development and progression of MAFLD through its core components or metabolites. It requires a comprehensive study to understand their roles in MAFLD fully. Due to the sizeable metabolic system of the gut microbiota and the complex regulatory networks, there are many gaps in understanding the mechanism of gut microbiota-derived metabolites in MAFLD. Even though

**Table 1: Circulating levels of gut microbiota-derived metabolites in metabolic dysfunction-associated fatty liver disease patients**

Metabolites	Circulating levels	Effect	Reference(s)
SCFAs	Acetate ↓	Protection	[41]
	Propionate ↓		
	Butyrate ↔		
BAs	Total BAs ↑	Risk or protection	[47,48]
	Deoxycholic acid ↑		
LPS	Chenodeoxycholic acid ↓	Risk	[51,52]
	LPS ↑		
Choline and its metabolites	Choline ↑	Risk or protection	[66-69]
	Betaine ↓		
	TMAO ↑		
Indole and its derivatives	IAA ↓	Protection	[78]
	IPA ↓		
BCAAs	BCAAs ↑	Risk	[84,85] [101]
Methionine cycle membranes	It still needs to be fully understood	Risk or protection?	[103,104]

↑ means increased, ↓ means decreased, and ↔ means no changes, SCFAs: Short-chain fatty acids, BAs: Bile acids, LPS: Lipopolysaccharide, BCAAs: Branched-chain amino acids, IAA: Indole-3-acetic acid, IPA: Indole-3-propionic acid, TMAO: Trimethylamine-N-oxide

there are some contradictory results, given that they have a strong association with MAFLD, these metabolites are promising biomarkers for the early diagnosis of MAFLD and target for MAFLD new drug discovery. However, several issues need to be addressed. Experimental animal study models are useful but may not be transferable to human gut microbiota. More human clinical studies are also necessary with a large sample size. Advanced experimental techniques such as mass spectrometry in identifying and quantifying gut microbial-derived metabolites and other undiscovered markers from human specimens are also important. Finally, a well-design future study is needed to explore the interplay between gut microbiota metabolites and MAFLD.

#### Data availability statement

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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#### Conflicts of interest

There are no conflicts of interest.

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