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Contribution of the gut microbiota to the regulation of host metabolism and energy balance: a focus on the gut–liver axis

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This review presents mechanistic studies performed *in vitro* and in animal models, as well as data obtained in patients that contribute to a better understanding of the impact of nutrients interacting with the gut microbiota on metabolic and behavioural alterations linked to obesity. The gut microbiota composition and function are altered in several pathological conditions including obesity and related diseases i.e. non-alcoholic fatty liver diseases (NAFLD). The gut–liver axis is clearly influenced by alterations of the gut barrier that drives inflammation. In addition, recent papers propose that specific metabolites issued from the metabolic cooperation between the gut microbes and host enzymes, modulate inflammation and gene expression in the liver. This review illustrates how dietary intervention with prebiotics or probiotics influences host energy metabolism and inflammation. Indeed, intervention studies are currently underway in obese and NAFLD patients to unravel the relevance of the changes in gut microbiota composition in the management of metabolic and behavioural disorders by nutrients interacting with the gut microbiota. In conclusion, diet is among the main triggers of NAFLD and the gut microbiota is modified accordingly, underlining the importance of the concomitant study of the nutrients and microbial impact on liver health and metabolism, in order to propose innovative, clinically relevant, therapeutic approaches.

Gut microbiota: Inflammation: Non-alcoholic fatty liver disease: Prebiotics

Obesity is a complex and multifactorial disorder, involving the balance between energy intake and expenditure⁽¹⁾. Long-term persistence of obesity will increase the prevalence of diabetes but also of non-alcoholic fatty liver disease (NAFLD) that is becoming the most important cause of chronic liver disease in the western countries⁽²⁾. The biological events driving the progression of NAFLD are not clearly elucidated. The hundreds of billions of micro-organisms present in our gut, constituting what is called microbiota, are now considered as potential new therapeutic targets^(1,3). An interesting study reported that the gut microbiota composition (including bacterial

diversity) explains the differential response to dieting in terms of improvement of metabolic disorders and inflammation in obese individuals⁽⁴⁾.

The dysfunction of the microbial ecosystem in obesity contributes to alterations of gut barrier, thereby promoting systemic inflammation, namely through the translocation of lipopolysaccharides (LPS)⁽⁵⁾. This process is called metabolic endotoxaemia. The portal vein directly carries gut-derived products to the liver. This organ functions as a secondary ‘firewall’ and protects the body from intestinal pathogens and other microbial products that have crossed the primary barrier of the intestinal

Abbreviations: AhR, aryl hydrocarbon receptor; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BA, bile acid; FXR, farnesoid X receptor; GLP, glucagon-like peptide; GPR, G protein-coupled receptors; ITF, inulin-type fructans; LPS, lipopolysaccharides; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis.

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tract⁽⁶⁾. The liver is also exposed to bacterial metabolites that may have beneficial effects on metabolic and inflammatory processes, as explained later. Indeed, the intestinal bacteria are able to metabolise nutrients into a wide range of co-metabolites (so-called since they can be further metabolised by host tissues) with metabolic, immune and/or neuroactive properties^(7–9). Although efforts have recently been dedicated to the identification of bacterial by-products, the mechanism behind the beneficial or detrimental effects of key microbial metabolites on host health in the context of NAFLD remains to be studied. In this review, we describe how bacterial metabolites may impact on both the metabolic and inflammatory status of the liver. We discuss novel approaches based on microbiome–nutrient–host interactions (e.g. probiotics, prebiotics) and the need for research and adequate intervention studies to evaluate the feasibility and relevance of these new therapies for NAFLD.

Gut microbial dysbiosis in obesity and non-alcoholic fatty liver disease

Obesity is associated with metabolic alterations related to glucose and lipid homeostasis (e.g. glucose intolerance, type-2 diabetes, insulin resistance, dyslipidaemia) (WHO, 2016, Obesity and overweight; <http://www.who.int/mediacentre/factsheets/fs311/en/>). During the past few decades, the implication of the gut microbiota in the progression of metabolic alterations has been mostly described in rodent models of obesity⁽¹⁰⁾. Indeed, it has been well established that gut microbiota controls the gut barrier function and, therefore, the progression of metabolic endotoxaemia characterised by the translocation of specific microbe-associated molecular patterns such as LPS into the systemic circulation⁽¹¹⁾. In mouse models of obesity (high-fat diet, *ob/ob* or *db/db* mice), the changes in gut microbiota composition are often associated with an increased gut permeability marked by an alteration of the tight junction proteins, occludin and zonula occludens-1^(12,13). The disrupted gut barrier function observed during obesity induces metabolic endotoxaemia leading to a low-grade inflammation^(5,12). This mechanism has been confirmed in human subjects, and more specifically in overweight patients with type-2 diabetes⁽¹⁴⁾. Interestingly, a chronic low grade inflammatory state has been linked to the development of insulin resistance in peripheral tissues during obesity and diabetes⁽¹⁵⁾. This suggests a major role for the gut microbiota in the control of insulin sensitivity in the whole organism.

Data are accumulating in animal models and human subjects suggesting that obesity and type-2 diabetes are associated with bacterial dysbiosis⁽¹⁶⁾. Subjects with a low bacterial richness are characterised by a higher adiposity, insulin resistance, dyslipidaemia and inflammatory phenotype compared with high bacterial richness individuals⁽¹⁷⁾. *Faecalibacterium prausnitzii* was less abundant in obese subjects with diabetes and associated negatively with inflammatory markers. Interestingly, the relative abundance of *F. prausnitzii* significantly

increased in these diabetic patients after by-pass surgery, suggesting that this bacterium could be associated with the reduction in low-grade inflammation⁽¹⁸⁾.

In addition, butyrate-producing bacteria such as *Roseburia intestinalis* were lower in overweight subjects with type-2 diabetes⁽¹⁶⁾. In human subjects, a study demonstrated that the ratio *Bacteroides:Prevotella* was lower in obese patients compared with control subjects and that this ratio was negatively correlated with corpulence⁽¹⁸⁾.

Some studies suggest that gut microbiota composition during childhood could influence the development of obesity. Indeed, early differences in faecal microbiota composition in children, such as the level of bifidobacteria, may predict the appearance of overweight in adolescence. Consistent with this result, a negative correlation between BMI and *Bifidobacterium* spp. was reported in several studies⁽¹⁹⁾. It has been reported that the relative abundance of *Akkermansia muciniphila* that contributes to the homeostasis of the protective mucus layer, is lower in the gut microbiota from obese and diabetic patients⁽¹⁷⁾, together with rodent models of obesity⁽²⁰⁾. It could also be of therapeutic interest as *A. muciniphila* administration prevents the fat mass accumulation and metabolic endotoxaemia in high-fat diet fed mice^(20,21).

NAFLD is a clinical syndrome almost systematically associated with obesity⁽²²⁾, and gut microbial dysbiosis is in a similar manner associated with this pathology. The syndrome is caused by an abnormal accumulation of TAG in hepatocytes (liver parenchymal cells) and can eventually evolve in some patients to non-alcoholic steatohepatitis (NASH), characterised by hepatic inflammation, and in worst cases, to cirrhosis and hepatocellular carcinoma. The implications of the microbiota in this pathology have long been hypothesised⁽²³⁾ and are gradually being explored thanks to next-generation sequencing techniques. In the past few years, it has been demonstrated that the digestive microbial composition of NAFLD and NASH patients differs from healthy individuals to variable degrees⁽²⁴⁾, regardless of the energy intake and the BMI⁽²⁵⁾. In a pioneer study, Le Roy *et al.*⁽²⁶⁾ demonstrated that mice fed a similar high fat diet with similar genetic backgrounds develop varying levels of steatosis that can be correlated with differences in the intestinal microbial composition. The susceptibility to the development of NAFLD is transmissible via gut microbiota transplants in mice, highlighting the direct metabolic impact of the microbiota upon hepatic metabolism and health.

The mechanisms by which the microbiota impacts on hepatic metabolism and health are however poorly understood. The liver is directly connected to the gastrointestinal tract through the gut–liver axis. The portal vein enables the transport of nutrients and bacterial compounds and metabolites from the intestinal lumen through the gut barrier to the liver, contributing to homeostasis under healthy physiological conditions. Under dysbiosis, several mechanisms contribute to the development of hepatic steatosis. As observed during obesity, intestinal barrier integrity can be compromised with an

increased gut permeability associated with increased bacterial translocation⁽²⁷⁾ and endotoxaemia that directly contributes to hepatic lipid metabolism disruption⁽¹¹⁾. Even if endotoxaemia is not characterising all NAFLD patients, it has been observed that endotoxin levels are higher in NASH patients compared with NAFLD patients⁽²⁴⁾ causing an increase in the release of pro-inflammatory cytokines. Intestinal inflammation caused by dysbiosis also favours NAFLD progression as demonstrated by Henao-Mejia *et al.*⁽²⁸⁾. Finally, changes in gut microbial composition alter its function, inducing changes in microbial metabolite production such as SCFA, trimethylamine, ethanol and secondary bile acids (BA) that can modify lipid metabolism and inflammatory processes in the liver⁽²⁹⁾.

Despite the pathophysiological association between obesity and steatosis, the microbial shift in NAFLD distinguishes itself from the one occurring with obesity. NAFLD is not systematically associated with a reduction in bacterial diversity compared with control^(30–33). Boursier *et al.*⁽³⁴⁾ did not evidence any association between microbial diversity and NAFLD severity whereas Hoyle *et al.*⁽³⁵⁾ observed a decrease in diversity with the increase in NAFLD severity. The β diversity represents the species diversity among habitats or sites. Various studies have used β -diversity metrics as tools to assess whether the gut microbiota composition is different between patients with steatosis and healthy controls and contradictory results have been obtained^(30–33,36). An increase in the abundance of the *Prevotella* genus has been observed in NASH and NAFLD children^(33,37) while a decrease in the abundance of this genus is reported in adults^(31,34,36). The relevance of *Prevotella* genus as a risk factor or a marker of steatosis would depend on the age of the patient. Data concerning the abundance of *Roseburia* are also inconsistent: some authors reporting decreases in adult⁽²⁵⁾ and child⁽³³⁾ NASH patients, while others have observed an increase in NAFLD children⁽³⁸⁾, indicating a possible variation in the abundance of this taxon with the severity of the steatosis. Decreases in the *Coprococcus*^(30,33) and *Faecalibacterium*^(25,30,32,36) genera, in particular the *F. prausnitzii*, an anti-inflammatory species⁽³⁰⁾, have been observed in NAFLD and NASH patients. Such modifications are also observed in obese patients, independent of the presence of steatosis⁽¹⁸⁾. Decreases in the abundance of the *Ruminococcaceae* family^(30,31,33,36,38) in particular the *Ruminococcus* genus^(25,30) have also been reported in several studies while increases in the *Lactobacillus* genus have been observed in adult NASH and NAFLD patients^(25,31,38). These two taxa comprise a large variety of species with very different functional implications. Many bacteria from the *Ruminococcaceae* family are butyrate producers; the modulation of their abundance could thus impact on SCFA production and subsequently on liver metabolism. Likewise, the *Lactobacillus* genus comprises different species with important immunological implications that influence liver health, but conclusions on the functional implications of this genus are impossible to make at that taxonomic rank. The lack of consistency in the microbial changes between the data is possibly due to the high

variability in the populations studied (differing in diet, medical treatments, age, environment, sex, severity of the steatosis) and the variability in analytical methods (sequencing, bioinformatics treatment, databases used, statistics). Studies on larger well-characterised cohorts are needed in order to have robust associations between specific bacterial taxa and liver steatosis. The diet is among the main triggers of NAFLD and the gut microbiota is modified accordingly, underlining the importance of the concomitant study of the nutrients and microbial impact on liver health and metabolism.

How do nutrients influence the gut–liver axis?

The gut microbiota has the capacity to produce a diverse range of compounds that play a major role in regulating the activity of distal organs such as the liver through portal circulation and thus modulate hepatic metabolism and health in a broader manner, which will be discussed in detail (Fig. 1). Metabolites are produced through the metabolism of food components by the gut microbiota, such as SCFA, indole- or phenol- derivatives⁽³⁹⁾. Secondary BA are also key metabolites produced from the gut microbiota from primary BA synthesised from cholesterol by host liver⁽⁴⁰⁾.

SCFA

Among SCFA, acetic, propionic and butyric acids are the major products of carbohydrate fermentation by the gut micro-organisms, the gut microbiome producing 50–100 mm daily of these compounds⁽⁴¹⁾. Branched SCFA can be produced from amino acid fermentation by the gut microbes⁽⁴²⁾. The types and amounts of SCFA synthesised in the gut are influenced by the amount of dietary non-digestible carbohydrates, and by the saccharolytic and metabolic characteristics of the gut microbiota. SCFA derived from the gut provide an energy source for different cell types. Butyrate is mostly used as an energy substrate in the colonocyte, whereas acetate and propionate may be used as substrates for glucose and fatty acid synthesis, respectively. Data obtained with labelled SCFA introduced in the colon of healthy subjects using colon delivery capsules and followed by the measurement of plasma levels of ¹³C-SCFA, ¹³C-glucose, ¹³C-cholesterol and ¹³C-fatty acids allowed the quantification of the respective contributions of SCFA as metabolic substrates in human subjects⁽⁴³⁾. Systemic availability of colonic-administered acetate, propionate and butyrate was 36, 9 and 2 %, respectively. Conversion of acetate into butyrate (24 %) was the most prevalent interconversion by the colonic microbiota and was not related to the butyrate-producing capacity in the faecal samples. Less than 1 % of administered acetate was incorporated into cholesterol and <15 % in fatty acids. On average, 6 % of colonic propionate was incorporated into glucose. In rodents, it has been shown that SCFA reduced hepatic cholesterol synthesis and lowered hepatic fatty acid synthase activity and hepatic lipid synthesis whereas there was an increase in hepatic lipid

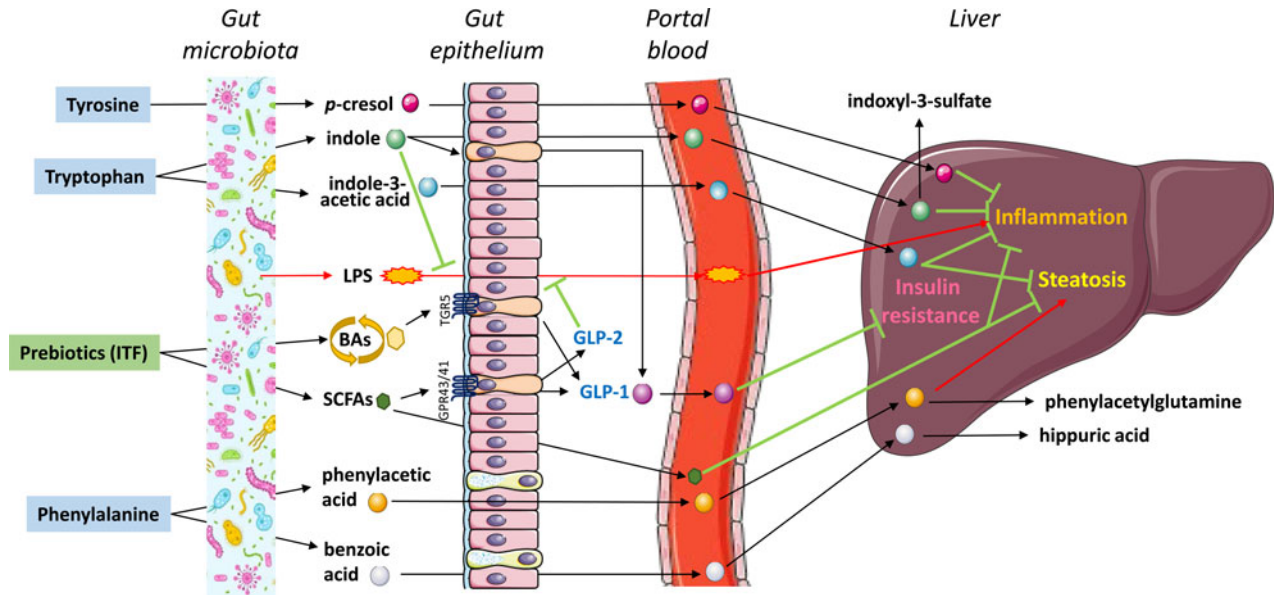


Fig. 1. Contributions of bacterial metabolites derived from amino acids or prebiotics to the gut–liver axis. Gut bacteria catabolise tryptophan into indole and indole-3-acetic acid, phenylalanine into phenylacetic acid and tyrosine into *p*-cresol (among others). SCFA such as acetic, propionic and butyric acids are produced by inulin-type fructans (ITF) fermentation. ITF treatment may affect bile acid (BA) profiles. Some of these metabolites cross the intestinal epithelium and are transported to the liver through the portal circulation whereas others act on intestinal permeability and/or intestinal L cells to produce glucagon-like peptides (GLP)-1 and -2, that improve liver metabolism (insulin sensitivity) and gut barrier (translocation of lipopolysaccharides (LPS)), respectively. Hepatic enzymes produce host–microbiota co-metabolites such as indoxyl-3-sulphate, hippuric acid and phenylacetylglutamine. (Host-) microbiota (co-)metabolites influence various physio(patho)logical processes, namely inflammation and/or lipid accumulation (steatosis) in the liver. For more details, please refer to the main text. Most of the findings illustrated in the figure have been obtained using mouse models of obesity or non-alcoholic fatty liver disease. The figure was produced using Servier MedicalArt (<http://www.servier.com>). GPR, G protein-coupled receptor; TGR5, Takeda G protein-coupled receptor 5.

oxidation, shifting hepatic lipid metabolism towards a more oxidative state^(44–47). SCFA act on the G protein-coupled receptors (GPR) GPR41 and GPR43 of gut enteroendocrine L cells to produce several effects that might impact liver metabolism. These L cells release glucagon-like peptide (GLP)-1, which can act directly on hepatocytes, by activating genes involved in fatty acid β -oxidation and insulin sensitivity⁽⁴⁸⁾. SCFA have also been known to modulate the production of pro- and anti-inflammatory mediators by immune cells, namely through the binding to GPR43 receptors⁽⁴⁹⁾. GPR43 has been implicated in mice in the control of glucose homeostasis⁽⁵⁰⁾ and lipid metabolism⁽⁵¹⁾. Altogether, these data reinforced the hypothesis that GPR43 is involved in metabolic control and could therefore participate in some metabolic disturbances in the liver tissue⁽⁴⁹⁾. Interestingly, a recent study provided the novel insight that butyrate regulated PPAR α to stimulate hepatic fatty acid β -oxidation and inhibited inflammation in high-fat-diet-induced NAFLD⁽⁵²⁾.

Bile acids

BA are important signalling molecules and metabolic regulators that control glucose and lipid homeostasis (for review, see^(41,53)). The majority of BA that are secreted into the intestinal lumen are reabsorbed from the terminal end of the small intestine and return to the liver through the portal blood. The BA that are

synthesised in the liver from cholesterol and conjugated with taurine or glycine on the side chain are called primary BA, which further undergo deconjugation, dehydroxylation, epimerisation and oxidation into secondary BA by intestinal bacteria, in the large intestine. BA are signalling molecules that coordinately regulate metabolism and inflammation via the nuclear farnesoid X receptor (FXR) and the Takeda G protein-coupled receptor 5⁽⁵⁴⁾. These receptors control the expression of genes involved in BA, lipid and carbohydrate metabolism, energy expenditure and inflammation. In rodents, FXR activation may reduce NAFLD, as it lessens steatosis by inhibiting lipogenesis, decreases chemically induced hepatic inflammation and fibrosis; it may help maintaining intestinal barrier integrity, thus protecting the liver from bacteria-derived inflammatory signals⁽⁵⁴⁾. It has been recently shown that in obese patients, the hepatic necro-inflammatory lesions observed in NASH are not associated with alterations in BA metabolism and signalling. BA alterations rather reflect the metabolic phenotype associated with NASH⁽⁵⁵⁾.

Aromatic amino acid derivatives

Recently, bacterial metabolites derived from aromatic amino acids (tryptophan, phenylalanine and tyrosine) emerged as a new class of microbial molecules influencing liver functions. Interestingly, these bacterial metabolites are further metabolised by host intestinal and hepatic

enzymes into host-microbiota co-metabolites that can be detected in the plasma or urine of conventional mice but not of germ-free or antibiotic-treated mice^(56,57). Gut bacteria produce numerous tryptophan catabolites, including indole, indole-3-propionic acid, indole-3-acetic acid, indole-3-aldehyde, tryptamine and 3-methylindole (skatole)^(58,59). Among them, indole is the most abundant (low millimolar concentration in the gut) while the concentrations of the other metabolites are much lower (<10 μM)⁽⁶⁰⁾. Since many tryptophan-derived bacterial metabolites beneficially control intestinal permeability and mucosal immunity^(58,59), these compounds could limit the translocation of harmful microbiota-derived components (LPS, peptidoglycan, etc.) from the gut to the liver. Moreover, indole regulates GLP-1 secretion from L-cells *in vitro* and thus could indirectly impact hepatic metabolism⁽⁶¹⁾. Alternatively, tryptophan catabolites could directly regulate hepatic physiology after transport through the portal blood. Unfortunately, for most of these bacterial metabolites, their concentrations in the portal circulation or in the hepatic tissue are not known. Bacterial indole is known to reach the liver since the hepatic enzymes Cyp2e1 and Sult1a1 convert it into indoxyl-3-sulphate, the main tryptophan derived co-metabolite^(62,63). We recently demonstrated that indole (100 μM) alleviates hepatic inflammation in precision-cut liver slices treated *ex vivo* with bacterial endotoxin or prepared from genetically obese mice, through mechanisms partly involving Kupffer cells⁽⁶⁴⁾. Indole oral administration in mice also prevented inflammation in association with a reduction of LPS-induced alterations of cholesterol metabolism and a regulation of liver X receptor target gene expression⁽⁶⁴⁾. *In vitro*, indole-3-acetic acid reduced pro-inflammatory cytokine expression in macrophages, fatty acid accumulation in hepatocytes and mRNA levels of the key lipogenesis proteins fatty acid synthase and sterol regulatory element-binding protein-1c⁽⁹⁾.

Most of the bacterial metabolites derived from tryptophan activate the aryl hydrocarbon receptor (AhR) which could mediate their hepatic effects⁽⁸⁾. For instance, the reduction of lipid accumulation in hepatocytes by indole-3-acetic acid involves the AhR⁽⁹⁾. Intriguingly, both indole and the related co-metabolite indoxyl-3-sulphate induced the expression of AhR target genes but only indole reduced hepatic inflammation, suggesting a dissociation between AhR activation and the anti-inflammatory effects of indole⁽⁶⁴⁾. Obese individuals have low faecal concentrations of tryptophan-derived metabolites (indole, indole-3-acetic acid, 3-methylindole and tryptamine) and accordingly low AhR agonist activity⁽⁶⁵⁾. High-fat feeding in mice also reduced the intestinal concentration of indole, indole-3-acetic acid and tryptamine⁽⁶⁵⁾. Another study similarly reported low caecal and hepatic concentrations of indole-3-acetic acid in mice fed a high-fat diet⁽⁹⁾. Interestingly, supplementation of diet-induced obese mice with a *Lactobacillus reuteri* strain producing tryptophan catabolites reversed hepatic metabolic alterations through improvement of gut barrier function and incretin secretion in an AhR-dependent manner⁽⁶⁵⁾.

The main bacterial catabolites of phenylalanine are phenylacetic acid, phenylpropionic acid and benzoic acids⁽⁶⁶⁾. Phenylacetic acid and benzoic acid are transported to the liver and metabolised into the co-metabolites phenylacetylglutamine (phenylacetylglutamine in mice) and hippuric acid, respectively⁽⁵⁶⁾. In obese women, plasma phenylacetic acid concentration was positively associated with steatosis severity⁽³⁵⁾. In primary human hepatocytes, treatment with a high concentration of this bacterial metabolite (10 mM) induced lipid accumulation and altered the expression of genes involved in lipid and glucose metabolism⁽³⁵⁾. Moreover, phenylacetic acid supplementation (0.8 %) in a standard diet for 2 weeks increased hepatic TAG levels in mice⁽³⁵⁾. Regarding hepatic inflammation, phenylacetic acid (1 mM) and benzoic acid (1 mM) did not reduce LPS-induced pro-inflammatory gene expression in mouse precision-cut liver slices⁽⁶⁴⁾.

Tyrosine is degraded by the gut microbiota into *p*-cresol, phenol, 4-hydroxyphenylacetic acid, 4-hydroxybenzoic acid, 4-hydroxyphenylpropionic acid and 4-hydroxyphenyllactic acid^(66,67). After absorption by the intestinal epithelium, *p*-cresol and phenol are metabolised by host enzymes into the co-metabolites *p*-cresyl sulphate and phenyl sulphate, respectively⁽⁵⁶⁾. *p*-Cresol (1 mM) prevented LPS-induced proinflammatory gene expression *ex vivo* in mouse precision-cut liver slices⁽⁶⁴⁾. However, since this bacterial metabolite has toxic effects on the intestinal epithelium⁽⁶⁸⁾, more studies are needed to decipher its impact on hepatic tissue.

Altogether, recent studies show that bacterial metabolites derived from aromatic amino acids play a pivotal role in the microbiota-host communication through the gut–liver axis, some of them being protective for hepatic metabolism and inflammation (indole, indole-3-acetic acid) while others (phenylacetic acid) potentially contribute to steatosis progression (Fig. 1). Importantly, the hepatic effects of most aromatic amino acid bacterial catabolites have not been characterised yet. Moreover, *in vivo*, these metabolites reach the liver in combination, which could induce antagonist or synergistic effects between them but this hypothesis remains to be tested. In overweight or obese individuals, high-protein diets increased the faecal concentration of phenylacetic acid and urinary excretion of the co-metabolites phenylacetylglutamine, *p*-cresyl sulphate and indoxyl sulphate, these effects being modulated by the protein source^(69,70). However, the hepatic consequences of this upregulation of aromatic amino acid catabolism by the gut microbiota during high-protein diets were not investigated. Since all bacterial metabolites increased by high-protein diets are not protective for the liver, more specific nutritional strategies (e.g. targeting only indole) would be highly desirable. Increasing the substrate availability for the gut microbiota is the most straightforward approach but remains challenging since free amino acids are efficiently absorbed in the upper part of the gut. A promising approach would be to encapsulate aromatic amino acids to ensure their targeted release in the distal intestine where the gut microbiota could degrade them into bioactive metabolites.

Intervention studies with probiotics and prebiotics in non-alcoholic fatty liver disease

Probiotics are selected micro-organisms which, when given in adequate amount, have a beneficial effect on host health⁽⁷¹⁾. Prebiotics are nutrients selectively utilised by host micro-organisms that confer health benefit to the host^(72,73). A recent systematic review and meta-analysis has been published on prebiotic, probiotic and synbiotic (combination of probiotics and prebiotics) therapies for patients with NAFLD in randomised controlled trials supporting the potential use of microbial therapies in the treatment of NAFLD⁽⁷⁴⁾. Meta-analysis indicated that microbial therapies significantly reduced BMI, hepatic enzymes (aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ -glutamyl transferase), serum cholesterol, LDL-cholesterol and TAG, but not inflammation (based on TNF- α and C-reactive protein determination). Subgroup analysis by treatment category indicated similar effects of prebiotics and probiotics on BMI and liver enzymes but not total cholesterol, HDL-c and LDL-c⁽⁷⁴⁾. One meta-analysis study highlighted that the use of probiotics significantly reduced AST, ALT and ultrasonographic grade of fatty liver⁽⁷⁵⁾. Another study showed that administration during 3 months of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* decreased ALT, AST and γ -glutamyl transferase in patients with NAFLD⁽⁷⁶⁾. Moreover, 6 months of treatment with a probiotic mixture containing *Lactobacillus* and *Bifidobacterium* strains reduced liver fat accumulation and AST in patients with NASH⁽⁷⁷⁾. A 4-month supplementation with VSL#3 probiotics also significantly improves NAFLD in children⁽⁷⁸⁾.

Prebiotic definition now includes a large panel of non-digestible nutrients⁽⁷²⁾. Inulin-type fructans (ITF) were the first to be recognised as prebiotics, together with (galacto-)oligosaccharides. Dietary ITF, which are present in various fruit and vegetables, are fermentable carbohydrates that display prebiotic properties, as their metabolism by gut micro-organisms modulates the composition and/or activity of the gut microbiota, thus conferring a beneficial physiological effect on the host⁽⁷⁹⁾. The beneficial effect of ITF prebiotics on cardiometabolic risk have been demonstrated in many mouse models of obesity, an effect namely linked to gut peptides (GLP-1)^(80,81). Our recent study suggested that changing the microbial composition using ITF impacted largely on the production of secondary BA and could contribute to the improvement of the host's health⁽⁸²⁾. Changes in BA profiles by ITF treatment may also result from the modulation of BA metabolism, including hepatic synthesis (in favour of Cyp7a1) and intestinal reuptake. FXR stimulation seems to suppress NF- κ B and in doing so decreases hepatic inflammation. Of particular interest, muricholic acids, primary BA only present in rodents, with FXR antagonistic properties can be induced by ITF in a dietary model of *n-3* PUFA depletion inducing hepatic steatosis and endothelial dysfunction in mice⁽⁸²⁾.

Animal and human research has demonstrated that ITF improve several NAFLD-associated metabolic risk factors including gut microbiota dysbiosis, intestinal

permeability, endotoxaemia, inflammation, glycaemia and hepatic lipogenesis^(83–87). ITF induce secretion of GLP-2 (co-secreted with GLP-1 by L cells) that is implicated in the lower systemic inflammation occurring in obese mice. The decrease in LPS absorption through an improvement of the expression and activity of proteins involved in gut barrier function (zonula occludens-1 and occludin), occurs in prebiotic-treated animals. In an exploratory, double blind intervention study with ITF in obese women, we demonstrated that the changes in gut microbiota induced by ITF prebiotics are correlated with serum LPS levels, despite a lack of significant effect on body weight⁽⁸⁵⁾. A more recent study demonstrated that ITF decreases serum LPS in adults with overweight/obesity⁽⁸⁸⁾.

Inulin-type prebiotics could have a beneficial impact on hepatic lipogenesis in healthy human subjects⁽⁸⁹⁾. Following this observation, some research studies have focused on the potential interest of prebiotics to regulate hepatic metabolic functions in the context of obesity or associated metabolic disorders such as NAFLD in patients. Our team has previously shown that 8 weeks ITF supplementation significantly decreased the serum AST in patients with NASH⁽⁸⁶⁾. In this context, important human intervention studies are currently ongoing in obese and NAFLD patients. The design of a first clinical trial performed in overweight patients with confirmed NAFLD has been published recently⁽⁹⁰⁾. In this study, patients were asked to consume prebiotics (16 g/d) during 24 weeks. Recently, a multicentre intervention trial in obese patients presenting co-morbidities has been conducted during the FOOD4GUT project devoted to better understand how inulin-type prebiotics present in food play a role on gut microbiota homeostasis and health (<https://sites.uclouvain.be/FOOD4GUT/>). It consists in a single blind randomised control trial in which patients were asked to consume 16 g native inulin daily combined with recipes based on vegetables naturally rich in ITF for 10–16 weeks. The placebo consists in eating 16 g maltodextrin daily, together with recipes favouring vegetables that are not rich in ITF. The primary outcome is to relate the changes in gut microbiome with the metabolic alterations. Such a protocol will allow evaluation of the potential effect on liver disorders, since liver fat accumulation and fibroses will be assessed by ultrasonography (Fibroscan) as well as through the measurement of biomarkers.

Synbiotic approaches, combining prebiotics and probiotics, have also been tested in the NAFLD context. First, in a randomised controlled clinical trial, patients with NAFLD were asked to consume daily 300 g synbiotic yoghurt containing *Bifidobacterium animalis* strain combined with 1.5 g inulin⁽⁹¹⁾. At the end of the protocol, compared with the placebo group, synbiotic formula significantly decreased the grade of NAFLD determined with ultrasonography and improved the serum concentrations of hepatic enzymes in NAFLD patients⁽⁹¹⁾. Likewise, in NASH patients, *Bifidobacterium longum* strain combined with ITF also reduced hepatic steatosis and serum concentrations of hepatic enzymes⁽⁹²⁾. In addition, in patients with NAFLD, synbiotic supplementation

based on several strains of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* combined with ITF ameliorated hepatic fibrosis, improved liver enzymes and attenuated inflammation in the systemic circulation, compared with the placebo group. Finally, another intervention based on the consumption of *L. reuteri* with guar gum and inulin, during 3 months in patients with NASH, significantly reduced hepatic steatosis in the treated group but not in the control⁽⁹³⁾. However, this amelioration of hepatic steatosis was not associated with a regulation of hepatic enzymes (AST, ALT, alkaline phosphatase and γ -glutamyl transferase). Another important clinical trial is currently in progress in NAFLD patients consuming 4 g ITF twice daily combined with *B. animalis* subsp. *lactis* BB-12 (INSYTE study)⁽⁹⁴⁾. The primary outcomes of this study will be to observe the liver fat accumulation by magnetic resonance spectroscopy, to measure a composite liver fibrosis score generated from blood concentrations of three analytes (serum hyaluronic acid, serum amino-terminal pro-peptide of type-III collagen and tissue inhibitor matrix metalloproteinase 1). The gut microbiota composition will also be determined by 16S rRNA sequencing.

In addition to the pre-, pro- and synbiotic approaches, it has been proposed that the replacement of the gut microbiota of ill patients by the microbiome of ‘healthy’ volunteers, could be helpful if the gut microbiota is involved in the evolution of the disease. Faecal transplantation from lean donors into obese/diabetic patients was linked to a marked increase in the proportion of the butyrate producer *R. intestinalis*, and improved insulin sensitivity⁽⁹⁵⁾. The improvement of insulin sensitivity upon faecal microbiota transplantation from lean donor in metabolic syndrome is driven by baseline intestinal microbiota composition since it is more effective in patients with bacterial low diversity⁽⁹⁶⁾. To date, no data are available regarding this kind of approach in NAFLD patients, with a primary outcome being the effect on hepatic disease.

Conclusions

In conclusion, few studies are available concerning the beneficial impact of dietary approaches modulating the gut microbiota composition for the treatment of NAFLD. The available data suggest that modulation of gut microbiota by pre-, pro- and synbiotics could reduce the liver fat accumulation and decrease the serum concentrations of hepatic enzymes. Several clinical trials are ongoing in obese and NAFLD patients (FOOD4GUT, INSYTE studies) in larger cohorts. They could bring mechanistic insight into the role of the gut microbiota in the management of liver disorders by prebiotics and/or probiotics. Further research should consider the limitations of biomarkers currently used for the diagnosis and progression of NAFLD, in addition to the inherent challenges of personalised microbial-based therapies. The identification of new classes of bioactive bacterial metabolites paves the way to development of nutritional strategies aiming to control their

production by the microbiota and ultimately tackle hepatic liver diseases and associated metabolic dysfunctions. Among these metabolites, phenylacetic acid already appears as a strong candidate. Such a metabolite was identified using metabolomics, reinforcing the interest and the relevance of non-targeted metabolomic approaches to think ‘outside the box’ the relationship between the gut microbiota and host health.

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Conflict of Interest

None.

Authorship

N. M. D. conceived, drafted and supervised the manuscript. C. K., M. B., J. R., A. N. and L. B. B. drafted sections of the manuscript.

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