Proceedings of the Nutrition Society (2019), **78**, 319–328 doi:10.1017/S0029665118002756
 First published online 10 January 2019

The Nutrition Society Summer Meeting was held at the University of Leeds, UK on 10–12 July 2018

Conference on 'Getting energy balance right'
Symposium 2: Molecular mechanisms contributing to energy balance

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 homoeostasis (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 homoeostasis 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 homoeostasis 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

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

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 Hoyles *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 ^{13}C-SCFA, ^{13}C-glucose, ^{13}C-cholesterol and ^{13}C-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

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

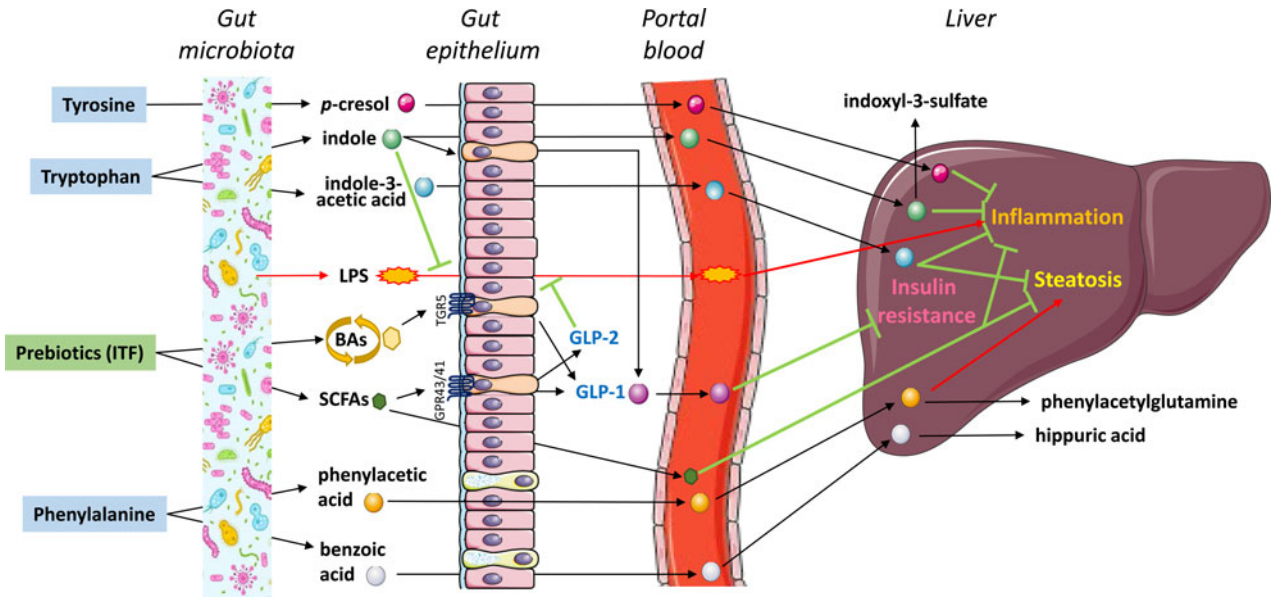

Fig. 1. Contributions of bacterial metabolites derived from amino acids or prebiotics to the gut–liver axis. Gut bacteria catabolise tryptophan into indole and indole-3-acetic acid, phenylalanine into phenylacetic acid and tyrosine into *p*-cresol (among others). SCFA such as acetic, propionic and butyric 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 homoeostasis[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 PPARα 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 homoeostasis (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

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

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 indoxy-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 (phenylacetylglycine 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.

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

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 metabolisation 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-κB 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 homoeostasis 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

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

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.

Acknowledgements

This work is supported by Wallonia (FOOD4GUT project, convention 1318148). N. M. D. is a recipient of an EU grant (613979 MyNewGut, Project) and Belgium National Scientific Research Fund (FRS-FNRS, Belgium) grants. C. K. is a beneficiary of the AgreenSkills+ fellowship program which has received funding from the EU Seventh Framework Programme under grant agreement No. FP7-609398.

Financial support

None.

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.

References

1. Maruvada P, Leone V, Kaplan LM *et al.* (2017) The human microbiome and obesity: moving beyond associations. *Cell Host Microbe* **22**, 589–599.
2. Perumpail BJ, Khan MA, Yoo ER *et al.* (2017) Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World J Gastroenterol* **23**, 8263–8276.
3. Torres-Fuentes C, Schellekens H, Dinan TG *et al.* (2017) The microbiota-gut-brain axis in obesity. *Lancet Gastroenterol Hepatol* **2**, 747–756.
4. Cotillard A, Kennedy SP, Kong LC *et al.* (2013) Dietary intervention impact on gut microbial gene richness. *Nature* **500**, 585–588.
5. Cani PD, Bibiloni R, Knauf C *et al.* (2008) Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* **57**, 1470–1481.
6. Brandl K, Kumar V & Eckmann L (2017) Gut-liver axis at the frontier of host-microbial interactions. *Am J Physiol Gastrointest Liver Physiol* **312**, G413-g419.
7. Husted AS, Trauelsen M, Rudenko O *et al.* (2017) GPCR-mediated signaling of metabolites. *Cell Metab* **25**, 777–796.

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

8. Hubbard TD, Murray IA & Perdew GH (2015) Indole and tryptophan metabolism: endogenous and dietary routes to Ah Receptor Activation. *Drug Metab Dispos* **43**, 1522–1535.
9. Krishnan S, Ding Y, Saedi N *et al.* (2018) Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. *Cell Rep* **23**, 1099–1111.
10. Cani PD, Osto M, Geurts L *et al.* (2012) Involvement of gut microbiota in the development of low-grade inflammation and type 2 diabetes associated with obesity. *Gut Microbes* **3**, 279–288.
11. Cani PD, Amar J, Iglesias MA *et al.* (2007) Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772.
12. Cani PD, Possemiers S, Van de Wiele T *et al.* (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103.
13. Neyrinck AM, Van Hee VF, Piront N *et al.* (2012) Wheat-derived arabinoxylan oligosaccharides with prebiotic effect increase satietogenic gut peptides and reduce metabolic endotoxemia in diet-induced obese mice. *Nutr Diabetes* **2**, e28.
14. Al-Attas OS, Al-Daghri NM, Al-Rubeaan K *et al.* (2009) Changes in endotoxin levels in T2DM subjects on anti-diabetic therapies. *Cardiovasc Diabetol* **8**, 20.
15. Galic S, Oakhill JS & Steinberg GR (2010) Adipose tissue as an endocrine organ. *Mol Cell Endocrinol* **316**, 129–139.
16 . Tilg H & Moschen AR (2014) Microbiota and diabetes: an evolving relationship. *Gut* **63**, 1513–1521.
17. Le Chatelier E, Nielsen T, Qin J *et al.* (2013) Richness of human gut microbiome correlates with metabolic markers. *Nature* **500**, 541.
18. Furet JP, Kong LC, Tap J *et al.* (2010) Differential adaptation of human gut microbiota to bariatric surgery-induced weight loss: links with metabolic and low-grade inflammation markers. *Diabetes* **59**, 3049–3057.
19. Castaner O, Goday A, Park Y-M *et al.* (2018) The gut microbiome profile in obesity: a systematic review. *Int J Endocrinol* **2018**, 9.
20. Everard A, Belzer C, Geurts L *et al.* (2013) Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc Natl Acad Sci U S A* **110**, 9066–9071.
21. Plovier H, Everard A, Druart C *et al.* (2017) A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* **23**, 107–113.
22. Haas JT, Francque S & Staels B (2016) Pathophysiology and mechanisms of nonalcoholic fatty liver disease. In *Annual Review of Physiology*, vol **78**, pp. 181–205. [Julius D, editor]. Palo Alto: Annual Reviews.
23. Tilg H, Cani PD & Mayer EA (2016) Gut microbiome and liver diseases. *Gut* **65**, 2035–2044.
24. Abdou RM, Zhu L, Baker RD *et al.* (2016) Gut microbiota of nonalcoholic fatty liver disease. *Dig Dis Sci* **61**, 1268–1281.
25. Duarte SMB, Stefano JT, Miele L *et al.* (2018) Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: A prospective pilot study. *Nutr Metab Cardiovasc Dis* **28**, 369–384.
26. Le Roy T, Llopis M, Lepage P *et al.* (2013) Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. *Gut* **62**, 1787–1794.
27. Miele L, Valenza V, La Torre G *et al.* (2009) Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* **49**, 1877–1887.
28. Henao-Mejia J, Elinav E, Jin C *et al.* (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. *Nature* **482**, 179.
29. Bashiardes S, Shapiro H, Rozin S *et al.* (2016) Non-alcoholic fatty liver and the gut microbiota. *Mol Metab* **5**, 782–794.
30. Da Silva HE, Teterina A, Comelli EM *et al.* (2018) Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* **8**, 1466.
31. Jiang W, Wu N, Wang X *et al.* (2015) Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. *Sci Rep* **5**, 8096.
32. Wong VW, Tse CH, Lam TT *et al.* (2013) Molecular characterization of the fecal microbiota in patients with non-alcoholic steatohepatitis – a longitudinal study. *PLoS One* **8**, e62885.
33. Zhu L, Baker SS, Gill C *et al.* (2013) Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. *Hepatology* **57**, 601–609.
34. Boursier J, Mueller O, Barret M *et al.* (2016) The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* **63**, 764–775.
35. Hoyles L, Fernandez-Real JM, Federici M *et al.* (2018) Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* **24**, 1070–1080.
36. Shen F, Zheng RD, Sun XQ *et al.* (2017) Gut microbiota dysbiosis in patients with non-alcoholic fatty liver disease. *Hepatobiliary Pancreat Dis Int* **16**, 375–381.
37. Michail S, Lin M, Frey MR *et al.* (2015) Altered gut microbial energy and metabolism in children with non-alcoholic fatty liver disease. *FEMS Microbiol Ecol* **91**, 1–9.
38. Raman M, Ahmed I, Gillevet PM *et al.* (2013) Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* **11**, 868–875. e861–e863.
39. Postler TS & Ghosh S (2017) Understanding the holobiont: how microbial metabolites affect human health and shape the immune system. *Cell Metab* **26**, 110–130.
40. Ridlon JM, Kang DJ & Hylemon PB (2006) Bile salt biotransformations by human intestinal bacteria. *J Lipid Res* **47**, 241–259.
41. Leung C, Rivera L, Furness JB *et al.* (2016) The role of the gut microbiota in NAFLD. *Nat Rev Gastroenterol Hepatol* **13**, 412–425.
42 . Nyangale EP, Mottram DS & Gibson GR (2012) Gut microbial activity, implications for health and disease: the potential role of metabolite analysis. *J Proteome Res* **11**, 5573–5585.
43. Boets E, Deroover L, Houben E *et al.* (2015) Quantification of in vivo colonic short chain fatty acid production from inulin. *Nutrients* **7**, 8916–8929.
44. Wong JM, de Souza R, Kendall CW *et al.* (2006) Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* **40**, 235–243.
45. Nicholson JK, Holmes E, Kinross J *et al.* (2012) Host-gut microbiota metabolic interactions. *Science* **336**, 1262–1267.
46. Wright RS, Anderson JW & Bridges SR (1990) Propionate inhibits hepatocyte lipid synthesis. *Proc Soc Exp Biol Med* **195**, 26–29.
47. Demigne C, Morand C, Levrat MA *et al.* (1995) Effect of propionate on fatty acid and cholesterol synthesis and on acetate metabolism in isolated rat hepatocytes. *Br J Nutr* **74**, 209–219.

https://doi.org/10.1017/S0029665118002756 Published online by Cambridge University Press

48. Svegliati-Baroni G, Saccomanno S, Rychlicki C *et al.* (2011) Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. *Liver Int* **31**, 1285–1297.
49. Bindels LB, Dewulf EM & Delzenne NM (2013) GPR43/FFA2: physiopathological relevance and therapeutic prospects. *Trends Pharmacol Sci* **34**, 226–232.
50. Bjursell M, Admyre T, Goransson M *et al.* (2011) Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* **300**, E211–E220.
51. Kimura I, Ozawa K, Inoue D *et al.* (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* **4**, 1829.
52. Sun B, Jia Y, Hong J *et al.* (2018) Sodium butyrate ameliorates high-fat-diet-induced non-alcoholic fatty liver disease through peroxisome proliferator-activated receptor alpha-mediated activation of beta oxidation and suppression of inflammation. *J Agric Food Chem* **66**, 7633–7642.
53. Fu ZD & Cui JY (2017) Remote sensing between liver and intestine: importance of microbial metabolites. *Curr Pharmacol Rep* **3**, 101–113.
54. Chavez-Talavera O, Tailleux A, Lefebvre P *et al.* (2017) Bile acid control of metabolism and inflammation in obesity, type 2 diabetes, dyslipidemia, and nonalcoholic fatty liver disease. *Gastroenterology* **152**, 1679–1694. e1673.
55. Legry V, Francque S, Haas JT *et al.* (2017) Bile acid alterations are associated with insulin resistance, but not with NASH, in obese subjects. *J Clin Endocrinol Metab* **102**, 3783–3794.
56. Wikoff WR, Anfora AT, Liu J *et al.* (2009) Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc Natl Acad Sci U S A* **106**, 3698–3703.
57. Zheng X, Xie G, Zhao A *et al.* (2011) The footprints of gut microbial-mammalian co-metabolism. *J Proteome Res* **10**, 5512–5522.
58. Agus A, Planchais J & Sokol H (2018) Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* **23**, 716–724.
59. Roager HM & Licht TR (2018) Microbial tryptophan catabolites in health and disease. *Nat Commun* **9**, 3294.
60. Jin UH, Lee SO, Sridharan G *et al.* (2014) Microbiome-derived tryptophan metabolites and their aryl hydrocarbon receptor-dependent agonist and antagonist activities. *Mol Pharmacol* **85**, 777–788.
61. Chimerel C, Emery E, Summers DK *et al.* (2014) Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep* **9**, 1202–1208.
62. Banoglu E, Jha GG & King RS (2001) Hepatic microsomal metabolism of indole to indoxyl, a precursor of indoxyl sulfate. *Eur J Drug Metab Pharmacokinet* **26**, 235–240.
63. Banoglu E & King RS (2002) Sulfation of indoxyl by human and rat aryl (phenol) sulfotransferases to form indoxyl sulfate. *Eur J Drug Metab Pharmacokinet* **27**, 135–140.
64. Beaumont M, Neyrinck AM, Olivares M *et al.* (2018) The gut microbiota metabolite indole alleviates liver inflammation in mice. *FASEB J* **32**, 6681–6693.
65. Natividad JM, Agus A, Planchais J *et al.* (2018) Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. *Cell Metab* **28**, 737–749.
66. Russell WR, Duncan SH, Scobbie L *et al.* (2013) Major phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of protein. *Mol Nutr Food Res* **57**, 523–535.
67. Windey K, De Preter V & Verbeke K (2012) Relevance of protein fermentation to gut health. *Mol Nutr Food Res* **56**, 184–196.
68. Andriamihaja M, Lan A, Beaumont M *et al.* (2015) The deleterious metabolic and genotoxic effects of the bacterial metabolite p-cresol on colonic epithelial cells. *Free Radic Biol Med* **85**, 219–227.
69. Beaumont M, Portune KJ, Steuer N *et al.* (2017) Quantity and source of dietary protein influence metabolite production by gut microbiota and rectal mucosa gene expression: a randomized, parallel, double-blind trial in overweight humans. *Am J Clin Nutr* **106**, 1005–1019.
70. Russell WR, Gratz SW, Duncan SH *et al.* (2011) High-protein, reduced-carbohydrate weight-loss diets promote metabolite profiles likely to be detrimental to colonic health. *Am J Clin Nutr* **93**, 1062–1072.
71. Hill C, Guarner F, Reid G *et al.* (2014) Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat Rev Gastroenterol Hepatol* **11**, 506–514.
72. Gibson GR, Hutkins R, Sanders ME *et al.* (2017) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* **14**, 491–502.
73 . Gibson GR & Roberfroid MB (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* **125**, 1401–1412.
74. Loman BR, Hernandez-Saavedra D, An R *et al.* (2018) Prebiotic and probiotic treatment of nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Nutr Rev* **76**, 822–839.
75. S Lavekar A, V Raje D, Manohar T *et al.* (2017) Role of probiotics in the treatment of nonalcoholic fatty liver disease: a meta-analysis. *Euroasian J Hepatogastroenterol* **7**, 130–137.
76. Aller R, De Luis DA, Izaola O *et al.* (2011) Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. *Eur Rev Med Pharmacol Sci* **15**, 1090–1095.
77. Wong VW, Won GL, Chim AM *et al.* (2013) Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. *Ann Hepatol* **12**, 256–262.
78. Alisi A, Bedogni G, Baviera G *et al.* (2014) Randomised clinical trial: the beneficial effects of VSL#3 in obese children with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* **39**, 1276–1285.
79. Bindels LB, Delzenne NM, Cani PD *et al.* (2015) Towards a more comprehensive concept for prebiotics. *Nat Rev Gastroenterol Hepatol* **12**, 303–310.
80. Roberfroid M, Gibson GR, Hoyles L *et al.* (2010) Prebiotic effects: metabolic and health benefits. *Br J Nutr* **104** Suppl 2, S1–63.
81. Delzenne NM, Cani PD, Everard A *et al.* (2015) Gut microorganisms as promising targets for the management of type 2 diabetes. *Diabetologia* **58**, 2206–2217.
82. Catry E, Bindels LB, Tailleux A *et al.* (2018) Targeting the gut microbiota with inulin-type fructans: preclinical demonstration of a novel approach in the management of endothelial dysfunction. *Gut* **67**, 271–283.
83. Mokhtari Z, Gibson DL & Hekmatdoost A (2017) Nonalcoholic fatty liver disease, the gut microbiome, and diet. *Adv Nutr* **8**, 240–252.
84. Parnell JA, Raman M, Rioux KP *et al.* (2012) The potential role of prebiotic fibre for treatment and management of

non-alcoholic fatty liver disease and associated obesity and insulin resistance. *Liver Int* **32**, 701–711.
85. Dewulf EM, Cani PD, Claus SP *et al.* (2013) Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **62**, 1112–1121.
86. Daubioul CA, Horsmans Y, Lambert P *et al.* (2005) Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. *Eur J Clin Nutr* **59**, 723–726.
87 . Kellow NJ, Coughlan MT & Reid CM (2014) Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr* **111**, 1147–1161.
88. Parnell JA, Klancic T & Reimer RA (2017) Oligofructose decreases serum lipopolysaccharide and plasminogen activator inhibitor-1 in adults with overweight/obesity. *Obesity* (*Silver Spring*) **25**, 510–513.
89. Letexier D, Diraison F & Beylot M (2003) Addition of inulin to a moderately high-carbohydrate diet reduces hepatic lipogenesis and plasma triacylglycerol concentrations in humans. *Am J Clin Nutr* **77**, 559–564.
90. Lambert JE, Parnell JA, Eksteen B *et al.* (2015) Gut microbiota manipulation with prebiotics in patients with non-alcoholic fatty liver disease: a randomized controlled trial protocol. *BMC Gastroenterol* **15**, 169.
91. Bakhshimoghaddam F, Shateri K, Sina M *et al.* (2018) Daily consumption of synbiotic yogurt decreases liver steatosis in patients with nonalcoholic fatty liver disease: a randomized controlled clinical trial. *J Nutr* **148**, 1276–1284.
92. Malaguarnera M, Vacante M, Antic T *et al.* (2012) *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. *Dig Dis Sci* **57**, 545–553.
93. Ferolla SM, Couto CA, Costa-Silva L *et al.* (2016) Beneficial effect of synbiotic supplementation on hepatic steatosis and anthropometric parameters, but not on gut permeability in a population with nonalcoholic steatohepatitis. *Nutrients* **8**, 397.
94. Scorletti E, Afolabi PR, Miles EA *et al.* (2018) Design and rationale of the INSYTE study: a randomised, placebo controlled study to test the efficacy of a synbiotic on liver fat, disease biomarkers and intestinal microbiota in non-alcoholic fatty liver disease. *Contemp Clin Trials* **71**, 113–123.
95. Vrieze A, Van Nood E, Holleman F *et al.* (2012) Transfer of intestinal microbiota from lean donors increases insulin sensitivity in individuals with metabolic syndrome. *Gastroenterology* **143**, 913–916. e917.
96. Kootte RS, Levin E, Salojarvi J *et al.* (2017) Improvement of insulin sensitivity after lean donor feces in metabolic syndrome is driven by baseline intestinal microbiota composition. *Cell Metab* **26**, 611–619.

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