MAFLD under the lens: the role of gut microbiota

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Abstract

Obesity, the metabolic syndrome, and metabolic dysfunction-associated fatty liver disease (MAFLD) can be portrayed as transmissible diseases. Indeed, they can be induced, in animal models, by cohabitation or by transplantation of fecal microbiota from other animals or humans with those diseases. As such, to get a 10,000-foot view, we need to see under the lens the microbes that populate our gut. Gut microbiota participates in the harvesting of energy from nutrients, it allows the digestion of otherwise indigestible nutrients such as fibers, and it also produces short chain fatty acids and some vitamins while emitting different compounds that can regulate whole-body metabolism and elicit proinflammatory responses. The metabolic syndrome and MAFLD share physiopathology and also patterns of gut dysbiota. Moreover, MAFLD also correlates with dysbiota patterns that are associated with direct steatogenic or fibrogenic effects. In the last decade, a tremendous effort has allowed a fair understanding of the dysbiota patterns associated with MAFLD. More recently, research is moving towards the delineation of microbiota-targeted therapies to manage metabolic dysfunction and MAFLD. This review provides in-depth insight into the state-of-the-art of gut dysbiosis in MAFLD, targeting clinical hepatologists.

Keywords: MAFLD, gut microbiota, obesity, diabetes mellitus

INTRODUCTION

The steady increase in the prevalence of metabolic-associated fatty liver disease (MAFLD) since its description in the 1980s has turned it into a major health threat³⁴. Indeed, MAFLD was the fastest growing...
indication for liver transplantation over the last 20 years and is already the leading cause for liver transplantation among women and patients over 54 years in the US[2].

MAFLD is the hepatic expression of metabolic dysfunction and goes hand in hand with obesity and insulin resistance (IR)/type 2 diabetes mellitus (T2DM)[3]. Its pathogenesis is complex and dependent on additional individual susceptibility, whether genetic, behavioral, or acquired[4]. The gut microbiota is one such factor that helps explain individual susceptibility to MAFLD and liver disease progression[5]. In the last decade, we witnessed a research boom on the role of gut dysbiota in MAFLD, regarding its pathogenesis, diagnostic ability, and role as a therapeutic target.

The way the gut microbiota is assessed has been changing since the first studies before the 1990s, when only culture-based methods were available[6]. Even though culture-based methods are cheap, less than 30% of the gut microbiota has been cultured. With the development of culture-independent techniques, there has been an exponential growth in the knowledge of gut microbiota. These techniques have shown the diversity of the gut microbiota, allowing quantitative and/or qualitative information about bacterial species and in-depth comparison of the microbiota from healthy and MAFLD patients[7].

Most studies used culture-independent, biomarker-based profiling techniques that involve sequencing a ubiquitous bacterial gene - small subunit ribosomal RNA (16S rRNA) - which is highly conserved but divergent enough to allow resolution at a genus level and, in some cases, at the level of species or even strains[8]. This provides a relatively accurate fingerprint of microbial community composition but cannot accurately identify bacteria on a strain level, nor does it reflect the microbiota functional properties, even though it is possible, through algorithms, to infer in silico metagenomic analyses from 16S RNA data[9].

With recent advances in computational biology and high-throughput sequencing technology (shotgun sequencing or pyrosequencing), research is moving to untargeted whole genome sequencing and “omics” approaches[6]. They include metagenomics (determination of the functional genes encoded), metatranscriptomics (determination of the functional genes expressed), metaproteomics (identification of proteins), and metabolomics (identification of bioactive small molecules)[8,10,11]. Shotgun sequencing metagenomic data allow the characterization of the DNA library from a microbial community with an accurate portrayal of the potential microbial functional properties. However, it does not inform the activity or directionality of a certain present pathway. Those are better inferred by metatranscriptomics and metabolomics[12].

The interpretation and extrapolation of the studies of gut microbiota are challenging. First, the microbiota is as specific to an individual as a fingerprint[13]; that is, each person has a unique collection of bacterial strains and species, although there is the controversial concept of the existence of a core functional microbiome[14,15]. There is also appreciable intraindividual variability with age, medication, and changes of lifestyle[4]. At a population level, the gut microbiota is modulated by several factors, such as geographic localization, different dietary patterns, host genetics, and environmental factors. Most studies did not account for confounding factors that are known to influence the microbiota composition, which may influence the conclusions and the extrapolation of the results to all patients with the same condition. Besides bacteria, gut microbiota also has other communities, such as viral and fungal, but their role in the crosstalk between microbes and host is even less known[14,12]. Sampling may represent another weakness. To start, the choice of the sample will render different results, since the microbiota from stool (representing luminal content) will be different from those from mucosal biopsy samples. The way stool is collected, stored, and processed can have a major impact on the quality of the samples, since it may allow contamination and a
variable degree of proliferation or death of different bacteria that will change the relative proportions of bacterial species. Taking everything into consideration, it is understandable why there are striking differences between studies, which hamper the generalization of the results.

We present an in-depth review of the state-of-the-art of gut microbiota in MAFLD with basilar concepts directed to clinical hepatologists.

**OVERVIEW OF THE GUT MICROBIOTA**

The term gut microbiota refers to all microorganisms found in the digestive tract. It is considered an essential invisible organ, an example of a dynamic and complex symbiotic relationship between host and microbiota that mutually influence each other. The gut microbiota is composed of bacteria, archaea, fungi, and viruses. The most represented bacteria phyla are Bacteroidetes, Firmicutes, Actinobacteria, and Proteobacteria. Three enterotypes are described according to the bacterial genus predominance: *Bacteroides* in type 1, *Prevotella* in type 2, and *Ruminococcus* in type 3.

The term microbiome refers to the combination of genomes and genes from the members of a microbiota. The microbiome acts as a massive functional booster of the host genome, harboring more than 100-fold the number of human genes. Indeed, these extra genes encode enzymes that are not encoded by the host, adding functions that would otherwise be absent in humans, such as the breakdown of complex polysaccharides and the synthesis of polyphenols, essential amino acids, and vitamins.

Dysbiosis refers to the permanent disruption of the symbiotic relationship between host and microbiota, with changes in the composition and function of the microbiota. Dysbiosis has a profound impact on host physiology and may promote the development of diseases such as obesity, T2DM, and MAFLD. A ubiquitous feature of dysbiosis is the loss of species diversity, which is counterbalanced by the overgrowth of proinflammatory species, promoting intestinal inflammation. Alpha-diversity refers to the bacterial richness in one sample and beta-diversity to the variation in a group of samples.

The gut microbiota has several functions with potential benefit for the host homeostasis: maintenance of mucosal barrier integrity; defense against pathogenic microbes; and nutrients, bile acids, and drug metabolism.

The interaction between enterocytes and gut microbes regulates epithelial permeability. For example, the commensal *Akkermansia muciniphila* enhances tight junctions’ function. Consequently, dysbiosis is frequently associated with a leaky gut. Gut microbiota further contributes to defense by constraining pathogens colonization through the competition for attachment sites and the production of bacteriocins that inhibit their competitor’s growth. Consequently, in dysbiosis, there is a shift in this balance allowing pathogenic strains to overgrow. Importantly, the gut microbiota is also a key regulator of innate and adaptive immunity, acting on gut-associated lymphoid tissues, regulatory T cells, IgA producing plasma cells, innate lymphoid cells, resident macrophages, and dendritic cells in the lamina propria.

Regarding nutrient metabolism, the gut microbiota acts on the metabolism of carbohydrates, vitamins, and amino acids. Colonic microbes ferment complex carbohydrates producing volatile organic compounds and short-chain fatty acids (SCFA), mostly propionate, butyrate, and acetate. The critical physiological functions of SCFA are as follows: maintenance of intestinal mucosa integrity, a source of energy for the...
host, modulation of glucose and lipid metabolism, control of energy expenditure, and immune regulation\cite{34-36}. Butyrate is the most crucial SCFA for human health, being the major source of energy for colonocytes, presenting anti-colon cancer activity, and potentially decreasing gut permeability\cite{37,38}. Propionate may abrogate hepatic gluconeogenesis and promote satiety, with the potential to reduce adiposity. Acetate is the most abundant SCFA and modulates gut microbiota, for example, being an essential metabolite for \textit{Faecalibacterium prausnitzii} growth\cite{39}.

The gut microbiota is responsible for the synthesis of vitamins K and B (including biotin, cobalamin, folates, nicotinic acid, panthotenic acid, pyridoxine, riboflavin, and thiamine)\cite{39,40}. It can also extensively degrade proteins from diet, host enzymes, mucin, and sloughed-off intestinal cells, producing amino acids and their derivatives, including IR-linked branched-chain amino acids (BCAA)\cite{41,42}.

The metabolism of bile acids results from the interactive crosstalk between the host and the gut microbiome. Primary bile acids (cholic acid (CA) and chenodeoxycholic acid (CDCA)) are cholesterol-derived metabolites, synthesized and conjugated with glycine or taurine in hepatocytes\cite{43}. They are mostly absorbed by active transport in the terminal ileum, entering the enterohepatic circulation. The gut microbiota metabolizes (deconjugation, 7-dehydroxylation, and 7-dehydrogenation) them into secondary bile acids [deoxycholic acid (DCA) and lithocholic acid (LCA)], changing their structure and function\cite{44}. There is a bidirectional relationship between gut microbiota and bile acids. Bile acids have selective antimicrobial characteristics that modulate the gut microbiota composition\cite{45}. Besides the role of bile acids in fat absorption, they act through different receptors [e.g., farnesoid-X receptor (FXR) and Takeda-G-protein receptor (TGR5)] as well as receptor-independent mechanisms such as modulating membrane dynamics\cite{45}. Bile acids modulate glucose metabolism (e.g., promote hepatic glycogen synthesis and insulin sensitivity and increase pancreatic insulin secretion) and energy homeostasis (facilitate energy expenditure, favor thermogenesis, and mediate satiety in the brain)\cite{46}, but they can also induce hepatic lipogenesis, cell injury, and proinflammatory and profibrogenic responses\cite{22}.

The microbiota composition varies along the digestive tract, with different strains in the mouth, small intestine, and different colorectal localizations, as well as transversal variations (gut lumen, mucous layer, and intestine villous/cripta)\cite{47}.

Several factors regulate the microbiota composition, such as environmental factors (delivery mode, diet, antibiotics, and prebiotics/probiotics), host factors (genetic), environmental factors, and inter-microbial interaction (competition between strains).

Acute perturbations of the gut microbiota, such as acute diarrhea, short-term diet manipulations, or short-term antibiotic use, induce only transient modifications that tend to return to baseline over time, a characteristic known as resilience. However, if the perturbation perpetuates in time (e.g., long-term dietary manipulations), it can overcome microbiota resilience and lead to sustained changes in the gut microbiota\cite{40,46,49}.

The newborn microbiota is transferred vertically and is influenced by the delivery route: either colonized by microbes from the maternal vagina or from maternal skin flora with cesarean section birth\cite{50,51}. Microbiota changes with age, increasing its diversity from childhood to adulthood and then decreasing in the elderly\cite{23}.

Diet seems to shape gut microbiota in infants (breast milk or formula) and adulthood. For example, preclinical and epidemiological studies showed an association between high-fiber diets rich in fruits and
vegetables and higher gut microbiota diversity and richness\cite{32}, as well as decreased Firmicutes/Bacteroidetes ratio, increased levels of Prevotella and Xylanibacter, and a decrease in proinflammatory pathogens Enterobacteriaceae, Shigella, and Escherichia. On the contrary, the high-fat, high-protein Western diet is associated with a decrease in diversity and an increase in Firmicutes\cite{48,52-54}.

Geographic localization is also a strong determiner of gut microbiota composition. Indeed, a study evaluating individuals from four districts within one province of China found that host location showed the strongest association with microbiota variation\cite{55}. Furthermore, in developed countries, there is a trend of a loss of microbiota diversity. Geographic variations might be the result of different modulators of the gut microbiota, such as diet, lifestyle, sanitary conditions, proportion of infants delivered by cesarean sections, use of antibiotics, and genetics\cite{56,57}.

Studies with twins showed that host genetics is significantly associated with the abundance of specific gut microbial taxa, dubbed hereditable taxons. For example, there seems to be an association between Bifidobacteriaceae and the LCT locus (higher abundance of Bifidobacterium in lactose-intolerant subjects that maintain a regular intake of dairy products)\cite{58-60}.

Longitudinal studies showed that the microbial composition of a unique host may change in function of diet, drug intake, lifestyle (smoking, travelling, and physical activity), co-morbidities, and colonic transit time\cite{10,69}.

**GUT MICROBIOTA AND OBESITY**

The pathophysiology of obesity is complex and does not reduce to a simple arithmetic between the energy consumed and expended. Behavioral, genetic, and environmental factors concur.

The first evidence for the role of gut microbiota in obesity and the concept of obesity as a transmissible disease comes from seminal preclinical studies from the beginning of the century\cite{61}. Those studies showed that rodents devoid of microbiota were resistant to diet-induced obesity and would adopt an obese phenotype when submitted to fecal microbiota transplantation (FMT) from genetically or diet-induced obese rodents but not from lean donors\cite{62-64}. Furthermore, the FMT-acquired obese phenotype could be reverted by cohousing with lean rodents as a result of invasion by specific strains of Bacteroidetes, but only in mice fed a low-fat diet, clearly illustrating the interplay between diet and microbiota\cite{65}. The potential obesogenic gut microbiota is also evident in humans, with reports of new-onset obesity in patients with recurrent Clostridium difficile after FMT from overweight donors\cite{66}.

Gut microbiota has a paramount role in the regulation of whole-body energy homeostasis and may induce obesity through direct host interactions or indirectly via microbial metabolites.

The gut microbiota is able to ferment indigestible carbohydrates into SCFA, which accounts for roughly 10% of harvested energy from diet\cite{37}. Acetate is the main source of energy from SCFA and the most obesogenic one, being able to induce hyperphagia (through induction of ghrelin and glucose-stimulated insulin secretion), hypertriglycerideridemia, IR, hepatic and adipocyte lipogenesis, and ectopic lipid deposition in the liver and skeletal muscle\cite{67,68}. Conversely, butyrate and propionate are anti-obesogenic, both increasing the expression of the anorexigenic adipokine leptin\cite{69,70} and gut hormones peptide tyrosine-tyrosine (PYY) and glucagon-like peptide-1 (GLP-1)\cite{71}. Butyrate enhances energy expenditure by promoting mitochondrial activity and upregulating genes for lipolysis and fatty acid oxidation\cite{72}. Propionate directly induces intestinal gluconeogenesis, which abrogates adiposity and weight gain,
independently of food intake, through the activation of gut–brain neural circuits mediated by portal vein glucose sensors. Butyrate is mainly produced by the Firmicutes *Faecalibacterium prausnitzii*, *Eubacterium rectale*, and *Rosedberia intestinalis*. Acetate is also produced by Bacteroidetes such as *Bacteroides* and *Prevotella*, as well as *Lactobacillus*, *Bifidobacterium*, and *Akkermansia*.

Gut microbiota downregulates the intestinal expression of fasting-induced adipose factor (FIAF). FIAF is a lipoprotein lipase inhibitor that inhibits adipose tissue and hepatic cellular uptake of fatty acids from circulating lipoproteins. As such, FIAF inhibition by the gut microbiota promotes liver steatogenesis and expansion of the adipose tissue.

The interplay between dietary tryptophan and gut microbiota may also contribute to obesity. In the gut, the main metabolic pathway of tryptophan is the kynurenine pathway, which results in different bioactive metabolites. Several gut bacteria have the potential to shunt tryptophan into two minor pathways: the aryl hydrocarbon receptor (AhR) pathway directly by metabolizing it into indoles (e.g., *Lactobacillus*) and the serotonin pathway indirectly by SCFA and secondary bile acids regulation of serotonin synthesis (e.g., *Prevotella*). The AhR pathway culminates in the production of GLP-1 and IL-22, the latter being able to decrease mucosal inflammation and permeability by inducing resistance to *Candida albicans* colonization. Serotonin also acts on energy homeostasis by acting in brain reward centers and increasing lipolysis in white adipose tissue while decreasing thermogenesis in brown adipose tissue.

Dysbiota may also modulate the effects of the gut microbiota on bile acids, which may impact metabolism and energy homeostasis. For example, an impairment in the production of secondary bile acids leads to the accumulation of primary bile acids with proinflammatory and leaky gut-inducing effects.

The gut microbiota is a main regulator of the intestinal mucosal barrier integrity, and gut dysbiota can induce a leaky gut through direct mucosal inflammation or indirectly via microbe metabolites. The increased permeability to microbial products such as lipopolysaccharides (LPS) has been dubbed metabolic endotoxemia, which contributes to the inflammatory state that characterizes obesity, but it also has profound metabolic effects, inducing IR, leptin resistance, adipose tissue expansion, and hepatic steatosis. Furthermore, gut inflammation increases its vascularization and enhances nutrient absorption.

Recent research is gathering information to characterize obesity-associated gut microbiota. Obesity is characterized by decreased gut microbiota richness and diversity, with a corresponding decrease in microbiome gene count.

Obesity-associated gut microbiota seems not only quantitatively but also qualitatively different. Most, but not all, studies have described an increased Firmicutes/Bacteroidetes ratio. The huge variability between studies suggests that more than a taxonomic core, there is a microbiome core for obesity that is associated with higher efficiency in energy harvesting from diet and proinflammatory states. However, some taxonomic associations have been fairly consistent, with obesity being associated with decreased relative abundance in the families Rikenellaceae and Christensenellaceae and the genera *Bifidobacterium*, *Oscillospira*, *Faecalibacterium*, and *Akkermansia*, all of which are involved in SCFA metabolism, mucosal integrity, and protection against a leaky gut. Conversely, obesity has been associated with increased relative abundance in the families Prevotellaceae and Coriobacteriaceae and the genera *Roseburia* and *Eubacterium*. H2-oxidizing methanogenic Archaea with the potential for higher carbohydrate fermentation efficiency also seem to be increased, as well as pathogenic proinflammatory Gram-negative bacteria.
such as *Escherichia* or *Shigella*.

The diet seems to have a major role in the acquisition of an obese-associated gut microbiota, with high-fat, high-sucrose Western diets promoting a shifting microbiota composition, depleting “favorable” bacteria (e.g., *Bifidobacterium* and *Faecalibacterium*) while replenishing “unfavorable” bacteria (e.g., *Blautia* and *Acinetobacter*).

**GUT MICROBIOTA AND DIABETES MELLITUS**

Type 2 diabetes mellitus (T2DM) shares behavioral risk factors with obesity, as well as similar dysbiota profiles.

Human observational studies have shown a lower-but not-diversity in T2DM patients. Furthermore, a recent metaanalysis showed that T2DM is associated with an increased abundance of Firmicutes (e.g., Veillonellacea) and decreased Bacteroidetes. At the genus level, the most striking associations were an increased relative abundance of *Ruminococcus*, *Coprococcus*, *Eubacteria*, *Blautia*, and *Prevotella*, whereas potentially protective bacteria were *Dorea*, *Bifidobacterium*, *Bacteroides*, *Faecalibacterium*, *Akkermansia*, and *Roseburia*.

Collectively, T2DM patients have a decrease in butyrate-producing bacteria and increased expression of microbiota genes related to inflammation and oxidative stress, leading to a leaky gut.

Increased intestinal permeability leading to endotoxemia and a continuous state of low-grade inflammation can induce IR. For example, LPS binding to TLR4 induces IR by direct phosphorylation of insulin receptor. Indeed, *Roseburia*, *Bacteroides*, and *Akkermansia*, which seem to be depleted in T2DM, either induce anti-inflammatory or inhibit proinflammatory cytokines. *Bacteroides* and *Akkermansia muciniphila* further protect from the leaky gut by directly strengthening epithelial tight junctions.

Gut dysbiosis may also have a profound effect on glucose metabolism and overall energy homeostasis. For example, the potentially anti-diabetogenic *Bifidobacterium* induces gut secretion of GLP-1 and PYY, promoting insulin sensitivity and beta cell function. Microbial metabolites also modulate glucose homeostasis. For example, regarding SCFA, propionate reduces insulin sensitivity, leading to compensatory hyperinsulinemia; acetate increases glucose-stimulated insulin secretion and weight gain; and butyrate has insulin-sensitizing actions by indirectly promoting the gut barrier abrogating metabolic endotoxemia and through the induction of insulin-sensitizing gut hormones GLP-1, PYY, and GLP-2.

*Prevotella copri* is associated with increased risk for T2DM, and the link may be the production of BCAAs, which are able to induce mTORC1-dependent IR.

Trimethylamine-N-oxide (TMAO) may be another link between gut dysbiota and T2DM. TMAO induces IR through ER stress-induced FoxO1 expression. Dietary choline can be used for phosphatidylcholine synthesis, oxidized into betaine or metabolized into trimethylamine (TMA) by gut microbiota, mainly Firmicutes and Proteobacteria such as *Escherichia coli*. TMA is further oxidized in the liver to TMAO by hepatic flavin-containing monoxygenases (FMAO3). TMAO has been consistently associated with T2DM and an increased cardiovascular risk.
Lastly, T2DM patients have lower levels of secondary biliary acids, decreasing the contribution of the latter to insulin sensitivity via TGR5 induction of GLP-1 secretion\(^{123}\).

Further supporting the role of gut dysbiosis in T2DM pathogenesis, a small study with FMT in obese patients improved insulin sensitivity and glucose tolerance after 12 weeks, independent of weight loss\(^{124}\), even though this was not consistently replicated by others\(^{125-127}\).

**GUT MICROBIOTA AND MAFLD**

MAFLD is the hepatic manifestation of metabolic syndrome with strong associations with obesity and T2DM\(^4\). Unsurprisingly, it shares common core pathogenesis, which extends to sharing the gut microbiota\(^6\). However, not all MAFLD patients are obese or overweight\(^{128}\), and one third of T2DM patients will not present MAFLD\(^{129}\). Predictably, specific microbiota features may be associated with MAFLD beyond the associations shared with metabolic dysfunction.

There is strong indirect evidence that MAFLD is associated with gut dysbiosis. Similar to obesity, MAFLD can be a transmissible disease in rodents by cohousing or direct FMT of microbiota-devoid mice from mice with genetically or diet-induced hepatic steatosis or from patients with nonalcoholic steatohepatitis (NASH), even when those mice were fed a healthy isocaloric diet\(^{41,130-134}\). Furthermore, antibiotic treatment prevents the development of hepatic steatosis in dietary and genetically induced obesity in animal models\(^{135,136}\), as well as in obese patients submitted to intestinal bypass bariatric surgery\(^{137}\). There is also strong preclinical and clinical evidence of an association among small intestine bacterial overgrowth\(^{138,139}\), leaky gut\(^{140}\), and MAFLD. Although only up to 40% of patients with MAFLD present an increase in intestinal permeability, this is six folds higher than the level expected in healthy subjects\(^{141-143}\).

Studies comparing gut microbiota from healthy subjects, obese patients, and patients with MAFLD found greater differences between obese and non-obese phenotypes and greater similarities between obese and MAFLD phenotypes\(^{144}\). Both obesity and MAFLD seem to be associated with decreased bacterial species diversity\(^{145}\), although the diversity is even lower in patients with MAFLD\(^{146}\). In obesity, at the phylum level, an increased Firmicutes/Bacteroidetes ratio has been consistently described. In MAFLD, the relative abundance of Firmicutes and Bacteroidetes was variable\(^{144,147-150}\); however, when direct comparisons of the microbiota between obese patients and MAFLD patients were made, their ratio was similar in some studies\(^{146,148}\) or reversed with decreased Firmicutes and increased Bacteroidetes in MAFLD patients, particularly in patients with steatohepatitis\(^{151}\). At the family level, both obesity and MAFLD are associated with a relative decrease in Lachnospiraceae and Ruminococcaceae\(^{152}\), and, at the genus level, there was a decrease in *Faecalibacterium* as well as an increase in *Prevotella* in some but not all cohorts\(^{6,143,152,154}\).

*Faecalibacterium, Eubacterium, Rousuberia, and Coprococcus* are all butyrate-producing bacteria that consistently were shown to be decreased in MAFLD\(^{147,152}\). *Faecalibacterium prausnitzii*, in particular, belongs to the Ruminococcaceae family\(^{155}\), accounts for over 5% of the total gut microbiota in healthy humans\(^{156}\), and regulates hepatic fat content, upregulating fatty acids oxidation and increasing hepatic sensitivity to adiponectin\(^{157}\). It also secretes anti-inflammatory compounds such as salicylic acid\(^{158}\) and microbial anti-inflammatory molecule (MAM) protein\(^{159}\), which abrogate adipose tissue inflammation\(^{155}\).

*Akkermansia* depletion is associated with disruption of gut epithelial barrier, is characteristic of obesity and T2DM, and was also reported in MAFLD\(^{147}\).
Similar to T2DM and the metabolic syndrome, TMAO levels increase (with correspondent decreases in betaine) in parallel with the severity of hepatic steatosis\cite{160}. \textit{Prevotella} gut colonization seems to be associated with increased levels of TMAO\cite{161}, and several studies described increased \textit{Prevotella} abundance in MAFLD patients\cite{143}. The increase in TMAO in MAFLD may result in metabolic dysfunction-independent steatogenic effects, since TMAO synthesis shunts choline away from the synthesis of betaine (a methyl donor with antioxidant and anti-inflammatory properties\cite{162}) and phosphatidylcholine (necessary for lipoprotein export from the liver\cite{160}). Furthermore, TMAO has direct steatogenic effects in the liver by suppressing bile acid-mediated hepatic FXR signaling, which results in SREBP-1c-mediated induction of lipogenesis. This was matched with a shift in the composition of the bile acid pool, with a decrease in potent FXR agonists tauroCDCA and glycolithocholic acids and an increase in FXR antagonist taurocholic acid\cite{164}. Different bacteria belonging to Firmicutes and Proteobacteria are able to produce TMA, such as some \textit{Clostridium} species, \textit{Escherichia fergusonii}, and \textit{Proteus penner}\cite{165}. Some of those bacteria have been associated with MAFLD. For example, \textit{Escherichia fergusonii} was associated with non-obese hepatic steatosis in animal models and humans\cite{166}.

Gut dysbiota may have steatogenic effects independent of the ones leading to obesity and IR/T2DM, mainly through bile acid metabolism and microbiota end-products/metabolites. Indeed, when comparing morbid non-diabetic obese women with and without steatosis, a 75% shared variation between the gut microbiome and the molecular phenomics was found (i.e., the sum of hepatic transcriptome and plasma and urine metabolome), which was associated with steatosis, suggesting a causal effect\cite{174}.

Some bacteria produce ethanol, which is directly steatogenic\cite{144}. Accordingly, several studies, including on pediatric populations, showed MAFLD/NASH to be associated with increased endogenous ethanol levels\cite{144,147}. Examples of ethanol-producing bacteria are Proteobacteria such as \textit{Escherichia coli}\cite{166}. Interestingly, an increase in Proteobacteria relative abundance is one of the most consistent findings in MAFLD patients\cite{134,135,153,154}. An increase in Proteobacteria, and particularly \textit{Escherichia coli}, is a distinguishing gut microbiota trait between obese and MAFLD/NASH patients\cite{143,144,146,147,149}. Of note, an overgrowth of \textit{Escherichia coli} in the gut microbiota has been linked to subtherapeutic doses of antibiotics that may be a contaminant of Western diets\cite{170}. Additionally, in a Chinese cohort of MAFLD patients, 60% presented an increased relative abundance of another Proteobacteria: high alcohol-producing \textit{Klebsiella pneumoniae}\cite{171}. From that cohort, one patient presented severe NASH and auto-brewing syndrome; that is, he developed ultra-high alcohol blood concentration after an alcohol-free, high-carbohydrate diet\cite{172}. Transplantation of isolates of high alcohol producing \textit{Klebsiella pneumonia} from that patient into a rodent recipient induced hepatic steatosis\cite{171,170}. An increased relative abundance of \textit{Klebsiella} was corroborated by other groups\cite{145}.

Proteobacteria are also relevant as a source of LPS\cite{154}. Indeed, the microbiome of patients with MAFLD/steatohepatitis is characteristically enriched in genes for LPS synthesis\cite{151}.

T2DM is associated with decreased \textit{Lactobacillus}\cite{174}; however, in MAFLD, different groups described an actual increase in \textit{Lactobacillus} relative abundance\cite{143,151,153}. The increased abundance of \textit{Lactobacillus} in MAFLD is counterintuitive since it is a known probiotic that competitively inhibits pathogens, heightens the epithelial barrier function, and has immunomodulatory properties\cite{175}. However, \textit{Lactobacillus} also produces volatile organ compounds and ethanol, which are known potentiators of steatogenesis and steatohepatitis\cite{176}. Indeed, the \textit{Lactobacillus} genus comprises more than 180 species with different sugar fermentation properties, the end result of some species being predominantly lactic acid (\textit{L. acidophilus} and \textit{L. salivarius}) and others ethanol (\textit{L. casei}, \textit{L. brevis}, and \textit{L. plantarum})\cite{19}.
Gut microbiota can modulate the development of MAFLD through other microbial byproducts. MAFLD-associated microbiota also showed an increased genetic potential for hepatic inflammation and deregulation of AAA (tryptophan, tyrosine, and phenylalanine) and BCAA (valine, leucin, and isoleucine)\[^{[134]}\]. BCAAs are associated with obesity and IR\[^{[146]}\]. Steatohepatitis has been associated with an increase in the genus *Bacteroides* and *Prevotella copri* which have the ability to produce BCAA\[^{[144,154,177]}\]. Another microbiota-associated metabolite that was strongly associated with MAFLD is the AAA-derived phenylacetic acid, which showed direct steatogenic effects on hepatocytes *in vitro*, promoting hepatic lipids uptake (by induction of lipoprotein lipase), *de novo* lipogenesis (by inducing fatty acids synthase), and inhibiting the insulin receptor pathway\[^{[130]}\]. 2-butanone seems to decrease in NAFLD\[^{[131]}\] but increase in steatohepatitis, which may be the result of increased *Streptococcus pneumoniae*\[^{[143,146,154]}\]. 2-butanone is a strong inducer of CYP, and even though it is not hepatotoxic *per se*, it potentiates hepatic lesions from toxic-associated steatohepatitis\[^{[178,179]}\].

The effects of the gut microbiota on bile acid metabolism also have a crucial role in the development of MAFLD. MAFLD is associated with an increased abundance of bacteria that produce bile salt hydrolases and hence deconjugate bile acids (e.g., *Clostridium*, *Lactobacillus*, *Escherichia*, and *Bacteroides*), as well as bacteria that facilitate the conversion of primary to secondary bile acids (predominantly *Clostridium* species\[^{[142,148,160]}\]). Primary bile acids activate, whereas secondary bile acids inhibit FXR\[^{[142]}\]. FXR activity is known to be decreased in MAFLD, which is associated with an increased synthesis of hepatic bile acids, metabolic deregulation, steatogenesis, inflammation, and possibly fibrogenesis in the liver\[^{[181]}\].

Non-obese MAFLD shares microbiota signatures with obese MAFLD. In fact, some studies even found an obese-associated microbiota in lean patients with MAFLD, such as an increased Firmicutes/Bacterioidetes ratio\[^{[183]}\] and a decrease in Lachnospiraceae (e.g., *Coprococcus*) and Ruminococcaceae (e.g., *Ruminococcus*). However, other microbiota associations with lean MAFLD suggest a different microbiota profile that could induce steatogenesis by mechanisms unrelated to the metabolic syndrome\[^{[125]}\]. For example, lean versus obese NAFLD is associated with decreased *Lactobacillus* abundance\[^{[182,183]}\]. Furthermore, non-obese steatohepatitis is associated with a different mycobiome, with a lower fungal richness, increased abundance of *Candida albicans* and *Mucor* spp, and decreased *Saccharomyces cerevisiae*\[^{[184]}\]. *C. albicans* is known to induce a Th17 proinflammatory response\[^{[185]}\], and its richness is associated more with hepatic inflammation than steatosis\[^{[184]}\].

**GUT MICROBIOTA AND PROGRESSION OF MAFLD**

The presence and severity of liver fibrosis dictate the risk for progression to liver cirrhosis and morbidity\[^{[186]}\]. The gut microbiota changes according to the severity of liver disease, from mild to significant fibrosis, compensated cirrhosis, and decompensated cirrhosis. The differences found in the gut microbiota allowed the proposal of gut microbiome-based metagenomic signatures for MAFLD with advanced fibrosis and cirrhosis\[^{[169,147,180]}\], with apparent great diagnostic accuracy.

Interestingly, while - and -diversity seem to decrease with mild disease, they paradoxically increase with severe disease, with increased dispersion in cirrhosis\[^{[169]}\].

Advanced fibrosis, as compared to absence or mild fibrosis, has been associated with increased anaerobic bacteria (e.g., *Bacteroides vulgatus*, *Holdemanella*, and *Prevotella copri*, even though an increase in the latter was not consensual\[^{[22,177]}\]) and Gram-negative bacteria (e.g., the Proteobacteria *Escherichia coli* and *Shigella*)\[^{[151,147,149,191]}\]. Interestingly, *Prevotella* abundance is associated with alcohol consumption, even when moderate\[^{[190]}\], which may explain an additive effect in metabolic dysfunction and alcohol-associated liver
disease. *Prevotella* has antioxidant properties, such as the production of superoxide dismutase. However, that can have an adverse effect by creating a more permissive milieu to other bacteria less resistant to oxidative stress, hence promoting intestinal inflammation\(^{[192]}\). Similarly, *Holdemanna l bifomes* is another immunogenic commensal that has been associated with gastrointestinal inflammatory diseases and host lipid metabolism\(^{[193]}\). Lastly, *B. vulgatus* has been linked to metabolic dysfunction, with relative abundance variation correlating with body mass index, poor glucose control, and inflammation\(^{[114,194]}\).

Several reports showed an association between advanced fibrosis and decreased *Ruminococcus obeum*\(^{[22,155,177]}\) and *Eubacterium rectale*\(^{[187]}\). Both species are fiber-fermenting bacteria with the ability to produce SCFA and have also been negatively associated with the development of primary sclerosing cholangitis in patients with inflammatory bowel disease\(^{[195]}\). Indeed, SCFAs inhibit hepatic inflammation (a major driver of fibrogenesis) via promoting intestinal barrier integrity and directly with anti-inflammatory actions on Kupffer cells\(^{[196]}\).

The metagenome associated with advanced fibrosis is correlated with bacterial changes. For example, advanced fibrosis was associated with the anaerobic bacteria derived 3-phenylpropanoate\(^{[187]}\). Increased abundance of the anaerobic bacteria *Prevotella* and *Holdemanna l* is also associated with oxidative stress and a proinflammatory environment, as a consequence of changes in metabolic pathways such as urea cycle and vitamin B biosynthesis\(^{[190,197]}\).

Besides bacteria, the virome in patients with advanced fibrosis is different, with a decreased variability and a decreased relative abundance of bacteriophages, which may have a direct impact on modulating the taxonomic composition of the bacterial microbiota\(^{[198]}\). In addition, regarding mycobiome, *Candida albicans* relative abundance and immunogenicity seem to increase in patients with advanced fibrosis\(^{[184]}\).

Cirrhosis seems to be associated with invasion of the gut by oral commensals, such as *Prevotella*, *Veillonella*, and *Streptococcus*, possibly through altered bile acid metabolism\(^{[199,200]}\). Conversely, there is a further decrease in beneficial commensals such as *F. prausnitzii* and *Coprococcus*\(^{[188,199]}\).

Decompensated cirrhosis is associated with a further dramatic shift in gut microbiota, with an increased abundance of pathogenic Enterococcaceae and Enterobacteriaceae and decreased Bacteroidaceae and the Lachnospiraceae Clostridiales IV\(^{[201,202]}\), which is correlated with endotoxemia\(^{[203]}\). Again, when cirrhosis decompensates, there is a decrease in the synthesis of bile acids and their fecal concentrations, which abrogates bile acids’ negative effect on Gram-negative bacteria, increasing the ratio of Gram-negative to Gram-positive bacteria (e.g., Lachnospiraceae)\(^{[199]}\).

MAFLD-associated hepatocellular carcinoma is associated with intestinal inflammation, as evidenced by increased fecal calprotectin and increased cytokines IL-8, IL-13, and CCL-3/4/5, which is correlated with a decrease in *Akkermansia* and *Bifidobacterium*\(^{[200]}\). Furthermore, there is also an increase in pathogenic Enterobacteriaceae compared to MAFLD cirrhosis without hepatocellular carcinoma. In contrast to the increased intestinal inflammation, MAFLD-HCC microbiota induces a peripheral T cell immunosuppressive phenotype, expansion of regulatory T cells, and attenuation of effector CD8+ T cells, which may be responsible for a decreased tumoral immunosurveillance\(^{[204]}\).

MAFLD-associated hepatocellular carcinoma is also associated with increased total bile acids and serum FGF-19, which is correlated with an increase in *Lactobacilli*\(^{[205]}\). A gut dysbiota-dependent increase in DCA has been described in MAFLD cirrhosis and may have pro-tumoral effects. Indeed, DCA induces a senescence-associated secretory phenotype in hepatic stellate cells, which elicits the secretion of
proinflammatory and tumor-promoting factors. Furthermore, DCA further inhibits tumoral immunosurveillance by inhibiting the expression of CXCL16 in hepatic sinusoidal cells, hence recruiting fewer anti-tumoral CRCR6+ NKT cells [Figure 1].

INTERVENTION IN GUT MICROBIOTA FOR THE TREATMENT OF MAFLD

As of today, no treatment has been approved for MAFLD, and gut dysbiosis is a highly appealing treatment target. Microbiome-targeted therapies include different strategies, such as probiotics and prebiotics, antibiotics, FMT, and bacteriophages. Furthermore, treatment strategies applied today, for example, diet and exercise, bariatric surgery, and some anti-diabetic agents such as metformin and liraglutide, act through modulation of the microbiota. Metformin is able to alter the gut microbiota composition in a way that leads to an improvement in glucose intolerance. Indeed, metformin has a dose-dependent antimicrobial effect with a decrease of specific bacteria such as Bacteroides fragilis, and depletion of Bacterioides fragilis is associated with inhibited FXR signaling mediated by glycocholic acid. Metformin also promotes the growth of specific bacteria such as Akkermansia muciniphila, which is known to have beneficial metabolic and anti-inflammatory effects. There is also scarce preclinical evidence of treatment of Chinese herbs such as Jiangzi granules and Ixeris chinensis showing beneficial effects on MAFLD through the modulation of the gut microbiota. Furthermore, alcohol consumption promotes Prevotella growth, high protein intake decreases Bacteroides abundance, and exercise increases SCFA-producing bacteria and anti-inflammatory F. prausnitzii and A. muciniphila.

Probiotics are live non-pathogenic microorganisms that induce health benefits in humans when in appropriate quantities. The most used probiotics are Lactobacilli and Bifidobacteria. Prebiotics are fermentable dietary fibers (such as inulin and fructo-oligosaccharides) that selectively promote the growth of probiotics. The combination of probiotics and prebiotics is dubbed synbiotics. Small short-term clinical trials have evaluated the effect of probiotics on MAFLD. Different metaanalyses showed probiotic therapy to result in biochemical improvement of aminotransferases and lipid profile, as well as of steatosis and fibrosis by non-invasive techniques, while there are no data on liver histology. Comparing different studies with probiotics is hampered by the fact that each study uses different formulations of probiotics. Furthermore, even though we can find some patterns of dysbiosis in MAFLD, the gut microbiota is unique for each patient, which anticipates a highly variable effect of probiotics. The effect of probiotics is also expected to be only transient, since there is no evidence of persistent probiotic engraftment with a persistent shift in the microbiota composition. The best approach would be personalized probiotics therapy, with the selection of specific commensal bacteria that would already be present in the individual baseline microbiota to allow better engraftment.

The evidence for prebiotics in the treatment of MAFLD is even scarcer, with very small trials in adults suggesting a mild effect on body mass index and aminotransferases levels.

Less is known regarding the effect of antibiotics on MAFLD. Small studies administered rifaximin, a gut-specific antimicrobial agent, to patients with NASH and showed a mild reduction in body mass index, endotoxemia, and liver enzymes at doses of 1200 mg per day, while there was no effect for lower doses.

FMT consists of collecting stools from a healthy donor and transferring it to a patient. The transference can be accomplished by colonoscopy, nasogastric tube, enema, or gastric-resistant pills. Small studies already tested the effect of FMT on the metabolic syndrome and showed benefits in glucose and lipid metabolism, albeit with no effect on body weight. Regarding MAFLD, a small pilot study performed FMT in 21
patients and did not achieve improvements in IR or liver fat content, but a reduction in small intestine permeability was achieved, suggesting a beneficial effect on the intestinal epithelial barrier\[^{229}\].

An innovative way to modulate microbiota is through bacteriophages therapy. Bacteriophages are ubiquitous viruses that can infect and destroy bacteria, often with species-level specificity. This approach has not been studied in MAFLD\[^{230}\]. However, preclinical data on alcoholic hepatitis approached with a bacteriophage that specifically targets cytolytic *Enterococcus faecalis* show promising results\[^{231}\].

**CONCLUSIONS**

Dramatizing, we are only 50% humans, since roughly as many bacteria as the human cells that compose our body colonize us. Half of the microbes have profound effects on our health and disease pathogenesis. This is the case for the development of the metabolic syndrome and its liver associated disease, MAFLD. Furthermore, gut dysbiosis has the ability to promote MAFLD by direct steatogenic metabolic syndrome-independent effects.

The study of gut microbiota has benefited from the tremendous recent advances in molecular testing, and today we can fairly accurately characterize an individual’s microbiota and microbiome. However, generalizations for a population and even more so for different populations are hampered by inter- and intra-individual differences, as well as geographic differences. As such, even though microbiota-targeted therapies for MAFLD are quite appealing, they are still far from clinical practice application. The field is moving towards a personalized microbiome targeted therapy, in which first we would characterize the individual gut microbiota, and afterwards, we would modulate the microbiota in a species-specific fashion in order to modulate the individual gut microbiota into a healthier, metabolically beneficial, and anti-steatogenic flora.

**DECLARATIONS**

**Authors’ contributions**

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Wrote the manuscript: Machado MV, Sousa P
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All authors declared that there are no conflicts of interest.

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REFERENCES

1. Le MH, Yeo YH, Li X, et al. 2019 Global NAFLD Prevalence: a systematic review and Meta-analysis. Clin Gastroenterol Hepatol 2021;1(1):2021:1. doi:10.20517/mtod.2022.15
2. Younossi ZM, Stepanova M, Ong J, et al. Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. Clin Gastroenterol Hepatol 2021;19:580-589.e5. doi:10.1016/j.cgh.2021.05.022
3. Eslam M, Newsome PN, Sarin SK, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020;73:202-9. doi:10.1016/j.jhep.2020.02.042
4. Machado MV, Diehl AM. Pathogenesis of nonalcoholic steatohepatitis. Gastroenterology 2016;150:1769-77. doi:10.1053/j.gastro.2016.03.017
5. Machado MV, Cortez-Pinto H. Diet, microbiota, obesity, and NAFLD: a dangerous quartet. Int J Mol Sci 2016;17:481. doi:10.3390/ijms17020481
6. Aron-Wisnewsky J, Vigliotti C, Witjes J, et al. Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. Nat Rev Gastroenterol Hepatol 2020;17:279-97. doi:10.1038/s41575-019-0137-1
7. Fraher MH, O’Toole PW, Quigley EM. Techniques used to characterize the gut microbiota: a guide for the clinician. Nat Rev Gastroenterol Hepatol 2012;9:312-22. doi:10.1038/nrg3162
8. Lynch SV, Pedersen O. The human intestinal microbiota in health and disease. N Engl J Med 2016;375:2369-79. doi:10.1056/NEJMra1502771
9. Langille MG, Zaneveld J, Caporaso JG, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. Nat Biotechnol 2013;31:814-21. doi:10.1038/nbt.2708
10. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Knight R. Current understanding of the human microbiome. Nat Med 2018;24:392-400. doi:10.1038/natmed.2018.5
11. Knight R, Urbanac A, Taylor BC, et al. Best practices for analysing microbiomes. Nat Rev Microbiol 2018;16:410-22. doi:10.1038/nrmicro.2018.11
12. Sharpton SR, Ajantha V, Loomba R. Emerging role of the gut microbiome in nonalcoholic fatty liver disease: from composition to function. Clin Gastroenterol Hepatol 2019;17:296-306. doi:10.1016/j.cgh.2018.10.016
13. Franzosa EA, Huang K, Meadow IF, et al. Identifying personal microbiomes using metagenomic codes. Proc Natl Acad Sci U S A 2015;112:E2930-8. doi:10.1073/pnas.1503110112
14. Lozupone CA, Stombaugh JI, Gordon JL, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489:220-30. doi:10.1038/nature11436
15. Arumugam M, Raes J, Pelletier E, et al; MetaHIT Consortium (additional members). Enterotypes of the human gut microbiome. Nature 2011;473:174-80. doi:10.1038/nature10100
16. Ding RX, Goh WR, Wu RN, et al. Revisit gut microbiota and its impact on human health and disease. J Food Drug Anal 2019;27:623-31. doi:10.1016/j.jfda.2018.11.002
17. Rodriguez-Valera F, Martin-Cuadrado AB, Rodriguez-Brito B, et al. Explaining microbial population genomics through phage predation. Nat Rev Microbiol 2009;7:828-36. doi:10.1038/nrmicro2309
18. Quigley EM. Gut microbiota and the role of probiotics in therapy. Curr Opin Pharmacol 2011;11:593-603. doi:10.1016/j.copha.2011.03.010
19. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. Science 2006;312:1355-9. doi:10.1126/science.1122308
20. Hooper LV, Gordon JL. Commensal host-bacterial relationships in the gut. Science 2001;292:1115-8. doi:10.1126/science.1059193
21. Qin J, Li R, Raes J, et al; MetaHIT Consortium. A human gut microbial gene catalogue established by metagenomic sequencing.
Sousa et al. Metab Target Organ Damage 2022:2:14 | https://dx.doi.org/10.20517/mtod.2022.15

20. Smirnova E, Muthiah MD, Narayan N, et al. Metabolic reprogramming of the intestinal microbiome with functional bile acid changes underlie the development of NAFLD. *Hepatology* 2022. DOI PubMed
21. Ramirez J, Guarnier F, Bustos Fernandez L, Maruy A, Sdpelani VL, Cohen H. Antibiotics as major disruptors of gut microbiota. *Front Cell Infect Microbiol* 2020;10:572912. DOI PubMed
22. Chen Y, Zhou J, Wang L. Role and mechanism of gut microbiota in human disease. *Front Cell Infect Microbiol* 2021;11:625913. DOI PubMed
23. Shreiner S, Kao JY, Young VB. The gut microbiome in health and in disease. *Curr Opin Gastroenterol* 2015;31:69-75. DOI PubMed
24. Walters KE, Martiny JBH. Alpha-, beta-, and gamma-diversity of bacteria varies across habitats. *PLoS One* 2020;15:e0233872. DOI PubMed
25. Allam-Ndoul B, Castonguay-Paradis S, Veilleux A. Gut microbiota and intestinal trans-epithelial permeability. *Int J Mol Sci* 2020;21:6402. DOI PubMed
26. Plovier H, Everard A, Draut C, et al. A purified membrane protein from Akkermansia muciniphila or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat Med* 2017;23:107-13. DOI PubMed
27. Chelakkot C, Choi Y, Kim DK, et al. Akkermansia muciniphila-derived extracellular vesicles increase gut permeability through the regulation of tight junctions. *Exp Mol Med* 2018;50:e450. DOI PubMed
28. McFarland LV. Antibiotic-associated diarrhea: epidemiology, trends and treatment. *Future Microbiol* 2008;3:563-78. DOI PubMed
29. Khosravi A, Yáñez A, Price JG, et al. Gut microbiota promote hematopoiesis to control bacterial infection. *Cell Host Microbe* 2014;15:374-81. DOI PubMed
30. Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. *World J Gastroenterol* 2015;21:8787-903. DOI PubMed
31. Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003;62:67-72. DOI PubMed
32. Ahmadi TR, Haeusler RA. Bile acids in glucose metabolism and insulin signalling - mechanisms and research needs. *Diabetes Metab* 2016;42:326-32. DOI PubMed
33. Brahe LK, Astrup A, Larsen LH. Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? *Obes Rev* 2011;12:950-9. DOI PubMed
34. Rowland I, Gibson G, Heinken A, et al. Gut microbiota functions: metabolism of nutrients and other food components. *Eur J Nutr* 2018;57:1-24. DOI PubMed
35. Hill MJ. Intestinal flora and endogenous vitamin synthesis. *Eur J Cancer Prev* 1997;6 Suppl 1:S43-5. DOI PubMed
36. Macfarlane GT, Cummings JH, Allison C. Protein degradation by human intestinal bacteria. *J Gen Microbiol* 1986;132:1647-56. DOI PubMed
37. Cani PD, Van Hul M, Lefort C, Depommier C, Rastelli M, Everard A. Microbial regulation of organismal energy homeostasis. *Nat Metab* 2019;1:34-46. DOI PubMed
38. Begley M, Gahan CG, Hill C. The interaction between bacteria and bile. *FEMS Microbiol Rev* 2005;29:625-51. DOI PubMed
39. García-Cañaveras JC, Donato MT, Castell JV, Lahoz A. Targeted profiling of circulating and hepatic bile acids in human, mouse, and rat using a UPLC-MRM-MS-validated method. *J Lipid Res* 2012;53:2231-41. DOI PubMed
40. Ahmad TR, Haessler RA. Bile acids in glucose metabolism and insulin signalling - mechanisms and research needs. *Nat Rev Endocrinol* 2019;15:701-12. DOI PubMed
41. Danić M, Stanimirov B, Pavlović N, et al. Pharmacological applications of bile acids and their derivatives in the treatment of metabolic syndrome. *Front Pharmacol* 2018;9:1382. DOI PubMed
42. Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* 2016;14:20-32. DOI PubMed
43. Wu GD, Chen J, Hoffmann C, et al. Linking long-term dietary patterns with gut microbial enterotypes. *Science* 2011;334:105-8. DOI PubMed
44. Allaband C, McDonald D, Vázquez-Baeza Y, et al. Microbiome 101: studying, analyzing, and interpreting gut microbiome data for clinicians. *Clin Gastroenterol Hepatol* 2019;17:218-30. DOI PubMed
45. Domínguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A* 2010;107:11971-5. DOI PubMed
46. Mackie RJ, Sghir A, Gaskins HR. Developmental microbial ecology of the neonatal gastrointestinal tract. *Am J Clin Nutr* 1999;69:635S-45S. DOI PubMed
47. Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. *Gastroenterology* 2009;137:1716-24.e1. DOI PubMed
48. De Filippo C, Cavalieri D, Di Paola M, et al. Impact of diet in shaping gut microbiota revealed by a comparative study in children.
from Europe and rural Africa. Proc Natl Acad Sci U S A 2010;107:14691-6. DOI PubMed PMC
54. Hermanson JB, Fei N, Miyoshi S, et al. Dietary cholesterol-induced gut microbes drive nonalcoholic fatty liver disease pathogenesis in a murine model. The FASEB Journal 2022;36:fasbj.2022.36.S1.0R748.
55. He Y, Wu W, Zheng HM, et al. Regional variation limits applications of healthy gut microbiome reference ranges and disease models. Nat Med 2018;24:1532-5. DOI PubMed
56. Emerson D, Wilson W. Giving microbial diversity a home. Nat Rev Microbiol 2009;7:758. DOI PubMed
57. Sonnenburg JL, Sonnenburg ED. Vulnerability of the industrialized microbiota. Science 2019;366:eaaw9255. DOI PubMed
58. Lim MY, You HJ, Yoon HS, et al. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut 2017;66:1031-8. DOI PubMed
59. Goodrich JK, Davenport ER, Beaumont M, et al. Genetic determinants of the gut microbiome in UK twins. Cell Host Microbe 2016;19:731-43. DOI PubMed PMC
60. Qin Y, Havulinna AS, Liu Y, et al. Combined effects of host genetics and diet on human gut microbiota and incident disease in a single population cohort. Nat Genet 2022;54:134-42. DOI PubMed
61. Bäckhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718-23. DOI PubMed PMC
62. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31. DOI PubMed
63. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 2007;104:9799-804. DOI PubMed PMC
64. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009;1:6ra14. DOI PubMed PMC
65. Ridaura VK, Faith JJ, Rey FE, et al. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013;341:124124. DOI PubMed PMC
66. Alang N, Kelly CR. Weight gain after fecal microbiota transplantation. Open Forum Infect Dis 2015;2:ofv004. DOI PubMed PMC
67. Perry RJ, Peng L, Barry NA, et al. Acetate mediates a microbiome-brain-β-cell axis to promote metabolic syndrome. Nature 2016;534:213-7. DOI PubMed PMC
68. Chakraborti CK. New-found link between microbiota and obesity. World J Gastrointest Pathophysiol 2015;6:110-9. DOI PubMed PMC
69. Xiong Y, Miyamoto N, Shibata K, et al. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proc Natl Acad Sci U S A 2004;101:1045-50. DOI PubMed PMC
70. Cunningham AL, Stephens JW, Harris DA. A review on gut microbiota: a central factor in the pathophysiology of obesity. Lipids Health Dis 2021;20:65. DOI PubMed PMC
71. Chambers ES, Viardot A, Psichas A, et al. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut 2015;64:1744-54. DOI PubMed PMC
72. Coppola S, Avagliano C, Calignano A, Berni Canani R. The protective role of butyrate against obesity and obesity-related diseases. Molecules 2021;26:682. DOI PubMed PMC
73. De Yadder F, Kovatcheva-Datchary P, Goncalves D, et al. Microbiota-generated metabolites promote metabolic benefits via gut-brain neural circuits. Cell 2014;156:84-96. DOI PubMed
74. Louis P, Flint HJ. Diversity, metabolism and microbial ecology of butyrate-producing bacteria from the human large intestine. FEMS Microbiol Lett 2009;294:1-8. DOI PubMed
75. Taleb S. Tryptophan dietary impacts gut barrier and metabolic diseases. Front Immunol 2019;10:2113. DOI PubMed PMC
76. Agus A, Planchas J, Sokol H. Gut microbiota regulation of tryptophan metabolism in health and disease. Cell Host Microbe 2018;23:716-24. DOI PubMed
77. Natividad JM, Agus A, Planchas J, et al. Impaired aryl hydrocarbon receptor ligand production by the gut microbiota is a key factor in metabolic syndrome. Cell Metab 2022;28:737-749.e4. DOI PubMed
78. Zelante T, Iannitti RG, Cunha C, et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013;39:372-85. DOI PubMed
79. Dong TS, Guan M, Mayer EA, et al. Obesity is associated with a distinct brain-gut microbiome signature that connects Prevotella and Bacteroides to the brain’s reward center. Gut Microbes 2022;14:2051999. DOI PubMed PMC
80. Crane JD, Palanivel R, Mottillo EP, et al. Inhibiting peripheral serotonin synthesis reduces obesity and metabolic dysfunction by promoting brown adipose tissue thermogenesis. Nat Med 2015;21:166-72. DOI PubMed PMC
81. Li F, Jiang C, Krausz KW, et al. Microbiome remodelling leads to inhibition of intestinal farnesoid X receptor signalling and decreased obesity. Nat Commun 2013;4:2384. DOI PubMed PMC
82. Parséus A, Sommer N, Sommer F, et al. Microbiota-induced obesity requires farnesoid X receptor. Gut 2017;66:429-37. DOI PubMed PMC
83. Raimondi F, Santoro P, Barone MV, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Gastrointest Liver Physiol 2008;294:G906-13. DOI PubMed
84. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091-103. DOI PubMed PMC
115. Lynch CJ, Adams SH. Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 2014;10:723-36. DOI PubMed PMC
116. Zhuang R, Ge X, Han L, et al. Gut microbe-generated metabolite trimethylamine N-oxide and the risk of diabetes: a systematic review and dose-response meta-analysis. Obes Rev 2019;20:883-94. DOI PubMed
117. Chen S, Henderson A, Petriello MC, et al. Trimethylamine N-Oxide binds and activates PERK to promote metabolic dysfunction. Cell Metab 2019;30:1141-1151.e5. DOI PubMed
118. Gao X, Liu X, Xu J, Xue C, Xue Y, Wang Y. Dietary trimethylamine N-oxide exacerbates impaired glucose tolerance in mice fed a high fat diet. J Biosci Bioeng 2014;118:476-81. DOI PubMed
119. Arias N, Arbesley S, Allison J, et al. The Relationship between choline bioavailability from diet, intestinal microbiota composition, and its modulation of human diseases. Nutrients 2020;12:2340.
120. Dumbrava M, Latkovskis G, Kuka J, et al. Diabetes is associated with higher trimethylamine N-oxide plasma levels. Exp Clin Endocrinol Diabetes 2016;124:251-6. DOI PubMed
121. Wang Z, Klipfell E, Bennett BJ, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57-63. DOI PubMed PMC
122. Tang WH, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575-84. DOI PubMed
123. Pathak P, Xie C, Nichols RG, et al. Intestine farnesoid X receptor agonist and the gut microbiota activate G-protein bile acid receptor-1 signaling to improve metabolism. Hepatology 2018;68:1574-88. DOI PubMed PMC
124. Allegretti JR, Kassam Z, Hurtado J, et al. Impact of fecal microbiota transplantation with capsules on the prevention of metabolic syndrome among patients with obesity. Hormones (Athens) 2021:20:209-11. DOI PubMed PMC
125. Wang Z, Mocanu V, Cai C, et al. Impact of fecal microbiota transplantation on obesity and metabolic syndrome—a systematic review. Nutrients 2019;11:2291. DOI PubMed PMC
126. Yu EW, Gao L, Stastka P, et al. Fecal microbiota transplantation for the improvement of metabolism in obesity: the FMT-TRIM double-blind placebo-controlled pilot trial. PLoS Med 2020;17:e1003051. DOI PubMed PMC
127. Le Roy T, Llopis M, Lepage P, et al. Intestinal microbiota determines development of non-alcoholic fatty liver disease in mice. Gut 2013;62:1787-94. DOI PubMed
128. Henao-Mejia J, Elinav E, Jin C, et al. Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 2012;482:179-85. DOI PubMed
129. De Minicis S, Rychlicki C, Agostinelli L, et al. Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. Gut 2014;59:1738-49. DOI PubMed
130. Chiu CC, Ching YH, Li YP, et al. Nonalcoholic fatty liver disease is exacerbated in high-fat diet-fed gnotobiotic mice by colonization with the gut microbiota from patients with nonalcoholic steatohepatitis. Nutrients 2013;5:1220. DOI PubMed
131. Bergheim I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008;48:983-92. DOI PubMed
132. Hooijes J, Fernández-Real JM, Federici M, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. Nat Med 2018;24:1070-80. DOI PubMed PMC
133. Membrez M, Blancher F, Jaquet M, et al. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. Gut 2008;57:601-9. DOI PubMed PMC
134. Berghem I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008;48:983-92. DOI PubMed
135. De Minicis S, Rychlicki C, Agostinelli L, et al. Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. Gut 2014;59:1738-49. DOI PubMed
136. Chiu CC, Ching YH, Li YP, et al. Nonalcoholic fatty liver disease is exacerbated in high-fat diet-fed gnotobiotic mice by colonization with the gut microbiota from patients with nonalcoholic steatohepatitis. Nutrients 2013;5:1220. DOI PubMed
137. Bergheim I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008;48:983-92. DOI PubMed
138. De Minicis S, Rychlicki C, Agostinelli L, et al. Dysbiosis contributes to fibrogenesis in the course of chronic liver injury in mice. Gut 2014;59:1738-49. DOI PubMed
139. Chiu CC, Ching YH, Li YP, et al. Nonalcoholic fatty liver disease is exacerbated in high-fat diet-fed gnotobiotic mice by colonization with the gut microbiota from patients with nonalcoholic steatohepatitis. Nutrients 2013;5:1220. DOI PubMed
140. Hoyles L, Fernández-Real JM, Federici M, et al. Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. Nat Med 2018;24:1070-80. DOI PubMed PMC
141. Berghem I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008;48:983-92. DOI PubMed
142. Bergeron I, Weber S, Vos M, et al. Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: role of endotoxin. J Hepatol 2008;48:983-92. DOI PubMed
143. Membrez M, Blancher F, Jaquet M, et al. Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 2008;22:2416-26. DOI PubMed
144. Drenick EJ, Fisler J, Johnson D. Hepatic steatosis after intestinal bypass—prevention and reversal by metronidazole, irrespective of protein-calorie malnutrition. Gastroenterology 1982;82:535-48. PubMed
145. Wijamrapeecha K, Lou S, Watthanasuntorn K, et al. Small intestinal bacterial overgrowth and nonalcoholic fatty liver disease: a systematic review and meta-analysis. Eur J Gastroenterol Hepatol 2020;32:601-8. DOI PubMed
146. Kuang L, Zhou W, Jiang Y. Association of small intestinal bacterial overgrowth with nonalcoholic fatty liver disease in children: a meta-analysis. PLoS One 2021;16:e0260479. DOI PubMed PMC
147. Kessoku T, Kobayashi T, Tanaka K, et al. The role of leaky gut in nonalcoholic fatty liver disease: a novel therapeutic target. Int J Mol Sci 2021;22:8161. DOI PubMed PMC
148. Luther J, Garber JJ, Khalili H, et al. Hepatic injury in nonalcoholic steatohepatitis contributes to altered intestinal permeability. Cell Mol Gastroenterol Hepatol 2015;1:222-32. DOI PubMed PMC
149. Lang S, Schnabl B. Microbiota and fatty liver disease—the known, the unknown, and the future. Cell Host Microbe 2020;28:233-44. DOI PubMed PMC
150. Jiang W, Wu N, Wang X, et al. Dysbiosis gut microbiota associated with inflammation and impaired mucosal immune function in intestine of humans with non-alcoholic fatty liver disease. Sci Rep 2015;5:8096. DOI PubMed PMC
151. Zhu L, Baker SS, Gill C, et al. Characterization of gut microorganisms in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601-9. DOI PubMed
Sousa et al. Metab Target Organ Damage 2022:2:14 | https://doi.org/10.20517/mtod.2022.15

145. Crovesy L, Masterson D, Rosado EL. Profile of the gut microbiota of adults with obesity: a systematic review. *Eur J Clin Nutr* 2020;74:1251-62. DOI PubMed

146. Del Chierico F, Nobili V, Vernocchi P, et al. Gut microbiota profiling of pediatric nonalcoholic fatty liver disease and obese patients unveiled by an integrated meta-omics-based approach. *Hepatology* 2017;65:451-64. DOI PubMed

147. Oh JH, Lee JK, Cho MS, et al. Characterization of gut microbiome in Korean patients with metabolic associated fatty liver disease. *Nutrients* 2021;13:1013. DOI PubMed PMC

148. Mouzaki M, Comelli EM, Arendt BM, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013;58:120-7. DOI PubMed

149. Adams LA, Wang Z, Liddle C, et al. Bile acids associate with specific gut microbiota, low-level alcohol consumption and liver fibrosis in patients with non-alcoholic fatty liver disease. *Liver Int* 2020;40:1356-65. DOI PubMed

150. Da Silva HE, Teterina A, Comelli EM, et al. Nonalcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. *Sci Rep* 2018;8:1466. DOI PubMed PMC

151. Schwimmer JB, Johnson JS, Angeles JE, et al. Microbiome signatures associated with steatohepatitis and moderate to severe fibrosis in children with nonalcoholic fatty liver disease. *Gastroenterology* 2019;157:1109-22. DOI PubMed PMC

152. Wong VW, Tse CH, Lam TT, et al. Molecular characterization of the fecal microbiota in patients with nonalcoholic steatohepatitis—a longitudinal study. *PLoS One* 2013;8:e62885.

153. Raman M, Ahmed I, Gillevet PM, et al. Fecal microbiome and volatile organic compound metabolome in obese humans with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2013;11:868-75.e1. DOI PubMed

154. Li F, Ye J, Shao C, Zhong B. Compositional alterations of gut microbiota in nonalcoholic fatty liver disease patients: a systematic review and Meta-analysis. *Lipids Health Dis* 2021;20:22. DOI PubMed PMC

155. Lee G, You HJ, Bajaj JS, et al. Distinct signatures of gut microbiome and metabolites associated with significant fibrosis in non-obese NAFLD. *Nat Commun* 2020;11:4982. DOI PubMed PMC

156. Lay C, Sutren M, Rochet V, Saunier K, Doré J, Rigottier-Gois L. Design and validation of 16S RNA probes to enumerate members of the Clostridium leptum subgroup in human faecal microbiota. *Environ Microbiol* 2005;7:933-46. DOI PubMed

157. Munukka E, Rintala A, Toivonen R, et al. Faecalibacterium prausnitzii treatment improves hepatic health and reduces adipose tissue inflammation in high-fat fed mice. *ISME J* 2017;11:1667-79. DOI PubMed PMC

158. Miquel S, Leclere M, Martin R, et al. Identification of metabolic signatures linked to anti-inflammatory effects of Faecalibacterium prausnitzii. *mBio* 2015;6:e00300-15. DOI PubMed PMC

159. Quévraën E, Maubert MA, Michon C, et al. Identification of an anti-inflammatory protein from Faecalibacterium prausnitzii, a commensal bacterium deficient in Crohn’s disease. *Gut* 2016;65:415-25. DOI PubMed PMC

160. Chen YM, Liu Y, Zhou RF, et al. Associations of gut-flora-dependent metabolite trimethylamine-N-oxide, betaine and choline with non-alcoholic fatty liver disease. *Coomassie Blue* 2021;58:120-7. DOI PubMed

161. Mouzaki M, Comelli EM, Arendt BM, et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. *Hepatology* 2013;58:120-7. DOI PubMed

162. Cole LK, Vance JE, Vance DE. Phosphatidylcholine biosynthesis and lipoprotein metabolism. *Biochim Biophys Acta* 2012;1821:754-61. DOI PubMed

163. Tan X, Liu Y, Long J, et al. Trimethylamine N-Oxide aggravates liver steatosis through modulation of bile acid metabolism and inhibition of farnesoid X receptor signaling in nonalcoholic fatty liver disease. *Mol Nutr Food Res* 2019;63:e1900257. DOI PubMed

164. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. *mBio* 2015;6:e02481. DOI PubMed PMC

165. Xin FZ, Zhao ZH, Liu XL, et al. Escherichia fergusonii promotes non-lean non-alcoholic fatty liver disease by interfering with host hepatic lipid metabolism through its own msRNA 23487. *Cell Mol Gastroenterol Hepatol* 2022;13:827-41. DOI PubMed PMC

166. Volynets V, Küper MA, Strahl S, et al. Metab Target Organ Damage (MTOD) review and meta-analysis. *Gut Microbes* 2021;13:1-18. DOI PubMed PMC
Lebeer S, Vanderleyden J, De Keersmaecker SC. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol Mol Biol Rev* 2008;72:728-64. Table of Contents. DOI PubMed PMC

Elshaghabee FM, Bockelmann W, Meske D, et al. Ethanol production by selected intestinal microorganisms and lactic acid bacteria growing under different nutritional conditions. *Front Microbiol* 2016;7:47. DOI PubMed PMC

Bourrier J, Mueller O, Barret M, et al. The severity of nonalcoholic fatty liver disease is associated with gut dysbiosis and shift in the metabolic function of the gut microbiota. *Hepatology* 2016;63:764-75. DOI PubMed PMC

Dietz FK, Traiger GI. Potentiation of CCl4 hepatotoxicity in rats by a metabolite of 2-butanone: 2,3-butanediol. *Toxicology* 1979;14:209-15. DOI PubMed PMC

Hewitt L. Modifications in rat hepatobiliary function following treatment with acetone, 2-butanone, 2-hexanone, mirex, or chloroacrolein and subsequently exposed to chloroform.1, *Toxicology and Applied Pharmacology* 1986;83:465-73. DOI PubMed

Urdaneta V, Casadesús J. Interactions between bacteria and bile salts in the gastrointestinal and hepatobiliary tracts. *Front Med (Lausanne)* 2017;4:163. DOI PubMed PMC

Machado MV, Cortez-Pinto H. Nuclear receptors: how do they position in non-alcoholic fatty liver disease treatment? *Liver Int* 2014;34:1291-4. DOI PubMed

Wang B, Jiang X, Cao M, et al. Altered fecal microbiota correlates with liver biochemistry in nonobese patients with non-alcoholic fatty liver disease. *Sci Rep* 2016;6:23002. DOI PubMed PMC

Duarte SMB, Stefano JT, Miele L, et al. 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* 2018;28:369-84. DOI PubMed

Demir M, Lang S, Hartmann P, et al. The fecal mycobiome in non-alcoholic fatty liver disease. *J Hepatol* 2022;76:788-99. DOI PubMed PMC

Fotis D, Liu J, Dalama M. Could gut mycobiome play a role in NAFLD pathogenesis? *Metabol Open* 2022;14:100178. DOI PubMed PMC

Taylor RS, Taylor RJ, Bayliss S, et al. Association between fibrosis stage and outcomes of patients with nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Gastroenterology* 2020;158:1611-1625.e12. DOI PubMed

Loomba R, Seguritan V, Li W, et al. Gut microbiome-based metagenomic signature for advanced fibrosis in patients with chronic liver disease. *Cell Metab* 2017;25:1054-1062.e5. DOI PubMed PMC

Oh TG, Kim SM, Caussy C, et al. A universal gut-microbiome-derived signature predicts cirrhosis. *Cell Metab* 2020;32:878-888.e6. DOI PubMed PMC

Lanthier N, Rodriguez J, Nachit M, et al. Microbiota analysis and transient elastography reveal new extra-hepatic components of liver steatosis and fibrosis in obese patients. *Sci Rep* 2021;11:659. DOI PubMed PMC

Kwan SY, Jiao J, Joon A, et al. Gut microbiome features associated with liver fibrosis in Hispanics, a population at high risk for fatty liver disease. *Hepatology* 2022;75:955-67. DOI PubMed PMC

Jung WS, Katzena W, Likhisethy V, et al. A microbial signature identifies advanced fibrosis in patients with chronic liver disease mainly due to NAFLD. *Sci Rep* 2020;10:2771. DOI PubMed PMC

Hofer U. Microbiome: pro-inflammatory Prevotella? *Nat Rev Microbiol* 2014;12:5. DOI PubMed

Kakoush NO. Insights into the role of erysipelotrichaeceae in the human host. *Front Cell Infect Microbiol* 2015;5:84. DOI PubMed PMC

Aron-Wisnewsky J, Pirfiti E, Belda E, et al. Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. *Gut* 2019;68:70-82. DOI PubMed PMC

Kummen M, Thingholm LB, Rühlemann MC, et al. Altered gut microbial metabolism of essential nutrients in primary sclerosing cholangitis. *Gastroenterology* 2021;160:1784-1798.e0. DOI PubMed PMC

Visekruna A, Luu M. The role of short-chain fatty acids and bile acids in intestinal and liver function, inflammation, and carcinogenesis. *Front Cell Dev Biol* 2021;9:703218. DOI PubMed PMC

Canivet CM, David N, Pailhoriés H, et al. Cross-linkage between bacterial taxonomy and gene functions; a study of metagenome-assembled genomes of gut microbiota in adult non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2021;53:722-32. DOI PubMed

Lang S, Demir M, Martin A, et al. Intestinal virome signature associated with severity of nonalcoholic fatty liver disease. *Gastroenterology* 2020;159:1839-52. DOI PubMed PMC

Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature* 2014;513:59-64. DOI PubMed

Ponziani FR, Bhoori S, Castelli C, et al. Hepatocellular carcinoma is associated with gut microbiota profile and inflammation in nonalcoholic fatty liver disease. *Hepatology* 2019;69:107-20. DOI PubMed

Bajaj JS, Betrapally NS, Hylemon PB, et al. Gut microbiota alterations can predict hospitalizations in cirrhosis independent of diabetes mellitus. *Sci Rep* 2015;5:18559. DOI PubMed PMC

Chen Y, Guo J, Qian G, et al. Gut dysbiosis in acute-on-chronic liver failure and its predictive value for mortality. *J Gastroenterol Hepatol* 2015;30:1429-37. DOI PubMed

Bajaj JS, Heuman DM, Hylemon PB, et al. Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol* 2014;60:940-7. DOI PubMed PMC

Behary J, Amorim N, Jiang XT, et al. Gut microbiota impact on the peripheral immune response in non-alcoholic fatty liver disease related hepatocellular carcinoma. *Nat Commun* 2021;12:187. DOI PubMed PMC
Sousa Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019;575:505-11. DOI PubMed PMC

205. Sydor S, Best J, Messerschmidt I, et al. Altered microbiota diversity and bile acid signaling in cirrhotic and noncirrhotic NASH-HCC. *Clin Transl Gastroenterol* 2020;11:e00131. DOI PubMed PMC

206. Yoshimoto S, Luo TM, Atarashi K, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretion. *Nature* 2013;499:97-101. DOI PubMed

207. Ma C, Han M, Heinrich B, et al. Gut microbiome-mediated bile acid metabolism regulates liver cancer via NKT cells. *Science* 2018;360:eaa35931. DOI PubMed PMC

208. Wu H, Esteve E, Tremaroli V, et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat Med* 2017;23:850-8. DOI PubMed

209. Moreira GV, Azevedo FF, Ribeiro LM, et al. Liparaglutide modulates gut microbiota and reduces NAFLD in obese mice. *J Nutr Biochem* 2018;62:143-54. DOI PubMed

210. Maier L, Putteanu M, Kuhn M, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 2018;555:623-8.

211. Sun L, Xie C, Wang G, et al. Gut microbiota and intestinal FXR mediate the clinical benefits of metformin. *Nat Med* 2018;24:1919-29. DOI PubMed PMC

212. Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. *Appl Environ Microbiol* 2014;80:5935-43. DOI PubMed PMC

213. Zhang S, Wong YT, Tang KY, Kwan HY, Su T. Chinese medicinal herbs targeting the gut-liver axis and adipose tissue-liver axis for non-alcoholic fatty liver disease treatments: the ancient wisdom and modern science. *Front Endocrinol (Lausanne)* 2020;11:572792. DOI PubMed PMC

214. Wang RR, Zhang LF, Chen LP, et al. Structural and functional modulation of gut microbiota by jiangzhizi granules during the amelioration of nonalcoholic fatty liver disease. *Oxid Med Cell Longev* 2021;2021:2234695. DOI PubMed PMC

215. Jin W, Cho S, Laxi N, et al. Hepatoprotective Effects of inxsers chinensis on nonalcoholic fatty liver disease induced by high-fat diet in mice: an integrated gut microbiota and metabolic analysis. *Molecules* 2022;27:3148. DOI PubMed

216. Lang S, Martin A, Farowski F, et al. High protein intake is associated with histological disease activity in patients with NAFLD. *Hepatol Commun* 2020;4:681-95. DOI PubMed

217. Martinez JE, Kahana DD, Ghuman S, et al. Unhealthy lifestyle and gut dysbiosis: a better understanding of the effects of poor diet and nicotine on the intestinal microbiome. *Front Endocrinol (Lausanne)* 2021;12:667066. DOI PubMed PMC

218. Khan MY, Mihali AB, Rawala MS, Aslam A, Siddiqui WJ. The promising role of probiotic and synbiotic therapy in aminotransferase levels and inflammatory markers in patients with nonalcoholic fatty liver disease - a systematic review and meta-analysis. *Eur J Gastroenterol Hepatol* 2019;31:703-15. DOI PubMed

219. Sharpton SR, Maraj B, Harding-Theobald E, Vittinghoff E, Terrault NA. Gut microbiome-targeted therapies in nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. *Am J Clin Nutr* 2019;110:139-49. DOI PubMed PMC

220. Yang R, Shang J, Zhou Y, Liu W, Tian Y, Shang H. Effects of probiotics on nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Expert Rev Gastroenterol Hepatol* 2021;15:1401-9. DOI PubMed

221. Kristensen NB, Bryrup T, Alin KH, Nielsen T, Pedersen O. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Med* 2016:8. DOI PubMed PMC

222. Maldonado-Gómez MX, Martínez I, Bottacini F, et al. Stable engraftment of bifidobacterium longum AH1206 in the human gut and 30-day supplementation in healthy adults: a systematic review of randomized controlled trials. *Oxid Med Cell Longev* 2012;2012:734582. DOI PubMed PMC

223. Proença IM, Allegretti JR, Bernardo WM, et al. Fecal microbiota transplantation improves metabolic syndrome parameters: systematic review with meta-analysis based on randomized clinical trials. *Nutr Res* 2020;83:1-14. DOI PubMed

224. Ng SC, Xu Z, Mak JWY, et al. Microbiota engraftment after faecal microbiota transplantation in obese subjects with type 2 diabetes: a 24-week, double-blind, randomised controlled trial. *Gut* 2022;71:716-23. DOI PubMed

225. Craven L, Rahman A, Nair Parvathy S, et al. Allogenic fecal microbiota transplantation in patients with nonalcoholic fatty liver disease improves abnormal small intestinal permeability: a randomized control trial. *Am J Gastroenterol* 2020;115:1055-65. DOI PubMed

226. Sharpton SR, Schnabl B, Knight R, Loomba R. Current concepts, opportunities, and challenges of gut microbiome-based personalized medicine in nonalcoholic fatty liver disease. *Cell Metab* 2021;33:21-32. DOI PubMed PMC

227. Duan Y, Llorente C, Lang S, et al. Bacteriophage targeting of gut bacterium attenuates alcoholic liver disease. *Nature* 2019;575:505-11. DOI PubMed PMC