Metabolic-Associated Fatty Liver Disease and the Gut Microbiota

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Invited Review Article for 'Endocrinology and Metabolism Clinics of North America'

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Word count abstract: **99**
Word count full article: **5,155**
(includes Key Points, Clinical Care Points, Table legend and References)
Number of figures: **1**
Number of tables: **1**

Conflict of Interest and Financial Disclosure: The authors have no conflicts of interest.
Funding statement: This study did not receive any funding.

Abstract

As an important sequela of the burgeoning global obesity problem, Metabolic-associated Fatty Liver Disease (MAFLD) has gained increasing prominence recently. The Gut Liver Axis (GLA) provides a direct conduit to the liver for the gut microbiota and their metabolic by-products (including secondary bile acids, ethanol, and trimethylamine). These GLA-related factors, including the host inflammatory response and integrity of the gut mucosal wall, likely contribute towards the pathogenesis of MAFLD. Accordingly, these GLA-related factors are targets for possible preventive and treatment strategies for MAFLD, and include probiotics, prebiotics, bile acids, short-chain fatty acids, faecal microbiota transplantation, carbon nanoparticles, and bacteriophages.

Keywords

Obesity; Metabolic-Associated Fatty Liver Disease; Gut Microbiota

Key Points

- Driven by the burgeoning global obesity problem, Metabolic-associated Fatty Liver Disease (MAFLD) has now assumed the commonest cause of chronic liver disease, with a global prevalence of ≥25%.
- Much 21st Century chronic ill-health likely has its pathogenic origins within the dysbiotic milieu of the gastrointestinal tract.
- The Gut Liver Axis (GLA) is a nutrient-based highway between the gut and the liver via the portal vein and provides a direct conduit for the gut microbiota and gut-derived metabolic by-products (GDMBs) to interact with the liver.
- Key GLA-related factors contribute towards the development of MAFLD, including the gut microbiota, host inflammatory response, integrity of the gut mucosal wall, and GDMBs that include secondary bile acids, ethanol, and trimethylamine.
- Possible preventive and therapeutic strategies for MAFLD that target the gut microbiota and GLA include probiotics, prebiotics, bile acids, short-chain fatty acids, faecal microbiota transplantation, carbon nanoparticles, and bacteriophages.

Clinical Care Points

- In the clinical assessment of obesity, it is important to screen for the presence of MAFLD through liver function tests and where necessary appropriate imaging studies. Other risk factors such as hypertension, dysglycaemia and dyslipidaemia should also be screened for.
- It should be noted that the development of MAFLD is often gradual and insidious, and frequently asymptomatic. In patients diagnosed with MAFLD, healthcare professionals should take the time to explain its development and clinical implications empathically and encourage the adoption of a healthy lifestyle.
- Currently, we lack specific targeted therapies for MAFLD, although novel treatment and preventive approaches to improve the healthiness of our gut microbiota and optimise proper GLA functioning are under development. Our current best advice is to avoid excessive weight gain through usual principles of healthy lifestyle (or to lose weight in the context of obesity), adopt and maintain a balanced, healthy, and high-fibre diet, avoid sedentariness and excessive alcohol intake, and optimise stress.
- In short, we need to nurture our gut microbiota to enable our gut microbiota to nurture our GLA, our liver, and us.

Introduction

Metabolic dysfunction, including most notably Type 2 Diabetes Mellitus (T2D), forms a major component of obesity-associated morbidity and mortality (1). Insulin resistance and associated hyperinsulinemia, oxidative stress and chronic inflammatory milieu underlies such obesity-related metabolic dysfunction, and indeed much obesity-related chronic disease generally (2). Obesity-associated T2D often co-exists with other dysmetabolic conditions that include hypertension, dyslipidaemia, and Obstructive Sleep Apnoea (OSA) (1). Within this obesity-associated dysmetabolic realm, Metabolic-Associated Fatty Liver Disease (MAFLD) looms prominently as a key contributor to the pathogenesis of obesity-associated metabolic dysfunction through its close association with insulin resistance and dyslipidaemia (3).

Driven by the burgeoning global obesity problem, MAFLD has now assumed the commonest cause of chronic liver disease, with a global prevalence of ≥25% and a leading cause of cirrhosis and hepatocellular carcinoma (4, 5). MAFLD is characterized by the accumulation of triglycerides within hepatocytes in non-alcohol users (6). MAFLD can progress to non-alcoholic steatohepatitis (NASH) in around 10% of patients through complex lipotoxic pathways that implicate mitochondrial and lysosomal dysfunction and stress within the endoplasmic reticulum (7). Whilst the pathogenesis of MAFLD is complex and incompletely understood, this likely implicates an array of environmental factors (including diet, lifestyle, physical fitness, sleep sufficiency and stress) interacting with a background of genetic susceptibility (8). To add to this gene-environment complexity, in recent years our understanding of the pathogenesis of MAFLD has been transformed by a renewed understanding of the role of the gut microbiota in mediating interactions within the Gut-Liver Axis (GLA). In this narrative review, we provide an overview of the gut microbiota in the development of MAFLD, mediated via the GLA. We explore the therapeutic implications for MAFLD of modifying the gut microbiota composition and targeting the GLA, and the future directions of this promising and emerging field.

The Gut Microbiota

The human microbiota consists of foreign (primarily prokaryotic) cells and occupies multiple locations including the skin and urogenital tract, and most notably the gastrointestinal tract (with around 70% of the microbiota inside the colon) (9). Compared with their eukaryotic counterparts, prokaryotic cells and viruses are much smaller. Therefore, despite their collective and relative low weight and volume, the gut microbiota out-number our own host

cells by at least an order of magnitude, with estimates of 100 trillion microbes (5, 10, 11). However, only approximately 1,000 human-based microbiota have actually been identified to date (9). Although mostly anaerobic, the precise composition of the gut microbiota is unique to each individual and manifests heterogeneity between individuals. Furthermore, the gut microbiota is influenced by multiple factors that include the site within the gut, age, and lifestyle factors such as physical activity, stress and importantly, diet (5).

Our gut microbiota, through co-evolution with us over hundreds of millions of years (10, 11), plays an essential role for normal immune development and functioning. Indeed, much 21st Century chronic ill-health likely has its pathogenic origins within the dysbiotic milieu of the gastrointestinal tract, as a harbinger of aberrant immunological development and functioning (10, 11). Most of our insights into the gut microbiota (and its potential role in human health and disease) stem from evidence derived from rodent-based studies that tend to focus on just one or a collection of gut microbes (11). However, we should exercise caution in extrapolating insights from rodent-based studies to humans, and we should be cognizant that just as with any organ-system, the gut microbiota functions as a whole and includes myriad interactions both between microbes and between microbes and their host. Future research should focus more on human-based studies and extend correlations between individual microbiota and biomarkers to a more holistic approach that considers the impact of the entire gut microbiome (as a functioning unit) on the positioning of its host within the health-disease spectrum. Such an approach will require a more refined and objective definition of the status of the gut microbiota, perhaps as a graded score of gut microbiota 'healthiness' rather than the current use of the dichotomized and rather vague umbrella terms, 'eubiosis' and 'dysbiosis'. We need to explore how the gut microbiota (in its eubiosis-dysbiosis spectral states) interact with us as its host, and vice versa. Such interactions are mediated via the GLA.

The Gut-Liver Axis (GLA)

Given the separateness of the gut microbiota from the host cells, it is important to explore the mechanisms by which these two entities interact and influence each other. Perhaps unsurprisingly, given the central regulation of appetite, metabolism, mood and overall wellbeing, there are close interlinks between the gut microbiota and the brain, mediated via the 'gut-Brain Axis' (GBA). Although beyond the scope of this review, these interactions operate through diverse mechanisms that include neural and hormonal signals, and direct effects of the microbiota and gut-derived metabolic by-products (GDMBs) (12). In addition to the GBA, the gut microbiota interacts with its host through other pathways, most notably the GLA which is particularly relevant for the pathogenesis of MAFLD.

In its broader sense, the GLA is well-characterised and understood (13). Indeed, the assimilation of nutrients (and de-toxification of ingested toxins) as a key role of the liver stems directly from the existence of the GLA. Given the central role of the liver in nutrient handling, it is hardly surprising that a majority (70%) of the liver's blood flow is supplied by the portal vein (5). However, this direct nutrient-based highway between the gut and the liver via the portal vein also provides a direct conduit for the gut microbiota and GDMBs to interact with the liver. As such, the GLA limits the systemic dissemination of microbes and toxins beyond the liver and enables its function to extend well beyond mere nutrient processing (5). Importantly, the GLA is bi-directional. In addition to the gut microbiota and GDMBs having direct access to and effects on the liver, bile acids and antibodies (each derived from the liver) also have direct access to and effects on the gut microbiota (outlined in ***Figure 1***) (13). Indeed, the liver-coordinated control of the gut microbiota is central to the overall homeostasis and proper functioning of the GLA (13), which is also influenced by myriad other factors that include the host genetic background and the host interactions with its environment (such as diet and other aspects of lifestyle) (5).

The Gut Microbiota and GLA in the pathogenesis of MAFLD

A rationale for considering an important role for the gut microbiota in the development of MAFLD (and NASH) stems from the influence of the gut microbiota on the digestion and the absorption of nutrients, and the observation that certain gut microbiota species that are transplanted faecally in rodent-based studies induce obesity (14). Given the association of MAFLD with obesity, the gut microbiota known to associate with obesity (5) may also overlap somewhat with MAFLD. Furthermore, the gut microbiota influences the development and homeostasis of immunity within the host, and the production of gut hormones (such as Glucagon-Like Peptide 1 [GLP1]) that modify overall host metabolism (14).

Compared with healthy controls, people with MAFLD appear to have higher levels of *Prevotella* and *Porphyromonas* species and a lower level of *Bacteroidetes* within their gut microbiota (5, 14). Furthermore, the development of MAFLD appears to depend on the expression within the host of tumour necrosis factor-alpha (TNF-α) receptor and Toll-like receptors (TLR) 4 or 9, that in turn likely mediate the hepatic inflammatory response to translocated gut microbiota and/or GDMBs (5, 14). Beyond the type of gut microbiota and host inflammatory response, other key GLA-related factors contribute towards the development of MAFLD, including the integrity of the gut mucosal wall (and the harmful

effects of alcohol) and specific GDMBs that include secondary bile acids, ethanol, and trimethylamine (13).

Integrity of the gut mucosal wall: The functioning of the GLA is influenced by the integrity and intactness of the gut mucosal wall and factors that influence gut mucosal integrity such as the mucus lining of the gut wall. Disruption of the gut mucosal wall can have grave implications for the GLA, with the translocation of gut microbiota into the liver. Indeed, cirrhosis associates with damage to the gut mucosal wall, including impairments of epithelial, vascular, and immune barriers, associated with profound alterations of the gut microbiota (13). In eubiosis, small numbers of gut microbiota and GDMBs enter the liver via the portal vein and are mostly eliminated by Kupffer cells. The permeability of the intestinal mucosal barrier (and therefore propensity of bacterial translocation from the gut to the liver) is influenced by multiple physiological protectors that include the mucous layer, tight junctions within the intestinal epithelium and antimicrobial peptides (15). Eubiosis promotes an intact intestinal mucosal barrier. Conversely, dysbiotic gut microbiota associates and often co-exists with a compromised and permeable intestinal mucosal barrier, resulting at least in part from reduced production of long-chain fatty acids (that in turn promote the growth of commensal Lactobacilli and maintain the gut mucosal wall) (16). Dysbiosis co-existing with a compromised gut mucosal wall can result in exposure of the hepatocytes, Kupffer and stellate cells to gut microbes and GDMBs (via the portal vein) and hepatic production of proinflammatory cytokines with subsequent development of MAFLD and NASH (5, 17).

Excessive alcohol intake disrupts the GLA at multiple levels, including the gut mucosal wall (including the intestinal epithelial cell tight junction), mucus barrier, antimicrobial peptide production, enhanced inflammatory milieu within the liver, and adverse impact on the gut microbiota itself (13). Accordingly, alcohol-induced disruption of the GLA represents an important risk factor for both the development of liver disease and dysbiosis with implications for the development of multiple chronic diseases. When the gut-mucosal barrier is compromised by intestinal inflammation (often in the context of dysbiosis), or indeed by other factors like alcohol (18), large numbers of gut microbiota translocate to the liver, with enhanced activation of Kupffer cells and hepatic stellate cells (5). Lipopolysaccharides (LPS) represent one particular ‘bacteria-derived factor’ that activates these hepatic cells via binding to TLR 4, with subsequent inflammatory reactions and liver damage (5).

Secondary bile acids: Bile acids (BAs) are produced from cholesterol within the liver and facilitate the absorption of dietary lipids and fat-soluble vitamins (15, 19). Broadly, BAs are

classified as primary (synthesised by the liver and accounting for 70-80% of the total BA pool), or secondary (primary BAs that are modified by the gut microbiota) (15). BAs are further sub-classified according to their conjugation status and hydrophobicity, with considerable potential for heterogeneity in the functioning of BAs (including digestion and nuclear receptor binding) (15). BAs play a key role in mediating interactions between the gut and the liver, including the maintenance of the enterohepatic circulation and intestinal mucosal integrity. Furthermore, dysregulation of BA signalling may contribute to chronic liver diseases such as MAFLD through inflammatory and fibrotic processes (15).

Most BAs undergo recycling via the enterohepatic circulation, with around 5% of circulating BAs lost in the faeces (15, 20). The Farnesoid X receptor (FXR) represents a key mediator of the effects of BAs on the gut-liver axis. FXR is a nuclear hormone receptor for BAs, and regulates numerous downstream signalling cascades that include the peroxisome proliferator-activated receptors (PPAR) (15). Other receptor targets of BAs include the Takeda G protein-coupled receptor-5 (TGR5) and the vitamin D receptor (15). One key mechanism whereby BAs can impact on MAFLD development and metabolic health more generally (including glucose, lipid, and cholesterol metabolism) is through the stimulated release of GLP1 from intestinal L-cells via the stimulation of TGR5 receptors as shown in a murine model (21, 22).

Ethanol: The progression of MAFLD to NASH may be influenced by ethanol-producing microbiota (5). In one study on the composition of the gut microbiota in NASH, obese and healthy children, and adolescents (determined by 16S ribosomal RNA pyrosequencing), there was increased representation of alcohol-producing gut bacteria in the NASH participants (14). A gut microbiome that is rich in ethanol-producing *Escherichia* could represent a driver for the development of MAFLD and NASH from obesity (14).

Trimethylamine: Trimethylamine (TMA) is a GDMB, produced by the gut microbiota during the metabolism of methylamine-containing nutrients, including lecithin, L-carnitine, and choline (23). Following its delivery to the liver via the portal vein, TMA is further processed to trimethylamine N-oxide (TMAO) by hepatic flavin monooxygenases (23). There appears to be a correlation between plasma levels of TMAO and both the risk of cardiovascular events and prevalence of cardiovascular disease in humans (23, 24). Furthermore, in rodent-based models, the gut microbial capacity for production of TMA appears to influence the size of aortic atherosclerotic lesions (23). Further rodent-based data supporting a possible role for TMA as a cardiovascular risk factor originates from a *Fmo3* knock-down model, in which reduced atherosclerotic lesions occur (23, 25). (FMO3 is the primary enzyme that converts TMA to TMAO, and itself is regulated by BAs via the FXR receptor) (23). However,

associations between levels of TMA and atherosclerotic lesions (derived mainly from rodent studies) are inconsistent and therefore contentious (23). Future studies should explore possible direct hepatic effects of TMA, including as a potential pathogenic factor for MAFLD, and possible interlinks between BAs and TMA metabolism in the pathogenesis of MAFLD.

MAFLD and Therapeutic Implications of the Gut Microbiota and the GLA

In this section, we explore potential preventive and therapeutic strategies for MAFLD that target the gut microbiota and GLA, including the use of probiotics, prebiotics, antibiotics, bile acids and the FXR, short-chain fatty acids, faecal microbiota transplantation (FMT), carbon nanoparticles, and bacteriophages (summarized in *table 1*).

Probiotics: Probiotics are live microorganisms intended to have health benefits when consumed or applied to the body. The ingestion of probiotics (including *Lactobacillus*, *Bifidobacterium,* and *Streptococcus*) provides a possible strategy to modify the gut microbiota, and therefore potentially modify the pathogenesis of MAFLD. Engineered probiotics could also be designed to consume toxic metabolites released by the gut microbiota, with conversion into non-toxic chemicals (13). Animal-based models of NASH using probiotics have shown promising results. Use of the probiotic, 'VSL#3' reduced fatty deposits within the liver and associated damage to the liver parenchyma and improved serum alanine aminotransferase (ALT) levels (5, 26). Human-based studies are limited by small sample sizes and potential confounding from lifestyle effects, including diet and exercise (5). However, despite these limitations, data were reported from four randomised-controlled trials (RCTs) which revealed that probiotic therapy significantly reduced serum levels of ALT (27-30), but liver steatosis improved in only two of these trials (29, 30). In a meta-analysis of these four RCTs, probiotic therapy was associated with significant improvements in insulin sensitivity (homeostasis model assessment of insulin resistance), and significant reductions in serum levels of TNF-α, aminotransferases, total cholesterol, and high-density lipoprotein (HDL) cholesterol (31). In a more recent systematic review and meta-analysis of the literature, probiotics were shown to reduce serum ALT and AST levels in people with MAFLD (16, 32, 33). Combined, these data provide proof of concept for potential therapeutic benefits of probiotics, worthy of further scientific scrutiny in larger and better powered RCTs in multiple and diverse population groups.

Prebiotics: A prebiotic is a substance that promotes the growth of beneficial intestinal microorganisms. A useful analogy here is the use of fertiliser within soil to promote the growth of plants within a garden. Dietary fibre (the non-digestible component of plant-

based foods) provides a natural form of prebiotic (34). The ingestion of prebiotic supplements such as inulin and oligofructose in people with MAFLD and T2D have been shown to improve serum transaminase activity and glycaemic and lipid profiles (35-37). Furthermore, in an RCT on the metabolic effects of synbiotics (a combination of probiotics with prebiotics) combined with lifestyle intervention resulted in significant reductions in hepatic steatosis and fibrosis, and improved markers of glycaemia, lipid profile and inflammatory mediators (38). As with probiotics, the potential metabolic benefits of prebiotics, including their combination with probiotics as synbiotics should be explored further.

Antibiotics: The role of bacterial translocation between the gut and liver in the development and progression of chronic liver conditions like MAFLD provides a rationale for antibiotic prophylaxis to reduce intestinal bacterial overgrowth, thereby protecting the liver from bacterial exposure, and its deleterious pathogenic consequences (15). However, prophylactic antibiotic administration does not usually form part of the conventional management of MAFLD due to a relative lack of supportive data. Furthermore, the burgeoning problem of antibiotic resistance globally (39) relegates the use of antibiotic use as an unlikely future treatment option for MAFLD.

Bile acids and the FXR: As mediators of the GLA, orally administered BAs (acting via the FXR receptor) provide a therapeutic opportunity for MAFLD via modulation of the GLA. In a mouse model in which cirrhosis was induced, there was gross impairment of the gut muco-epithelial barrier (promoting gut bacterial translocation) (40). However, the impaired gut mucosal wall was modulated by FXR-agonist therapies that also reduced the gut bacterial translocation to the liver via the portal-venous route (40). In a separate rodent model of cirrhosis, oral administration of BAs reduced intestinal bacterial overgrowth and bacterial translocation (15), with these effects likely mediated via the FXR receptor (15, 41). Furthermore, in a mouse model of colitis, treatment with the FXR agonist, obeticholic acid (OCA), improved intestinal barrier integrity (15, 42). However, we lack human-based data on the potential therapeutic benefits of BAs in MAFLD (15, 19).

Short-chain fatty acids: Within the human colon, the digestion of dietary fibre (including non-digestible complex carbohydrates, cellulose, and plant polysaccharides) occurs through the action of fermentation by the gut microbiota (5). This process enables the gut microbiota to yield energy to facilitate their own growth, and the release of a GDMB termed ‘short-chain fatty acids’ (SCFAs) (5, 11). These organic fatty acids have health-promoting properties for the human host that includes butyrate acting as an energy substrate for the colonic epithelium, and propionate and acetate as energy substrates for the peripheral

tissues (5). SCFAs may also have immunomodulatory and anti-inflammatory properties and influence the hepatic control of carbohydrate and lipid metabolism (5, 43). The existing data on the potential health benefits of SCFAs promote their therapeutic potential in the management and/or prevention of MAFLD. Currently, we lack human-based data to support such an approach. Furthermore, SCFAs may simply increase energy intake without playing a major role in improved metabolic health (44, 45). However, the encouragement of a high-fibre diet should form an integral part of the management and prevention of MAFLD (34).

Faecal Microbiota Transplantation (FMT): Currently within the National Health Service (NHS) of the United Kingdom, the use of FMT is reserved solely for the management of patients suffering from intractable colonic colonisation with *Clostridium difficile* (46). Based on data from rodent studies, FMT has potential as a future treatment option for MAFLD. In a high-fat diet-induced model of steatohepatitis in mice, FMT for 8-weeks corrected disturbances in the gut microbiota (including an elevated abundance of *Lactobacillus* and *Christensenellaceae*) (47). Furthermore, in mice that received FMT there was alleviation of steatohepatitis (with a significant reduction in intrahepatic lipid accumulation and pro-inflammatory cytokines), improved intestinal tight junction protein 'ZO-1' and reduced endotoxaemia (47). However, early reported human-based studies on the metabolic effects of FMT have been disappointing, with only transient improvements in insulin sensitivity and without appreciable improvements in other clinical parameters (16). A more recent assessment of the metabolic effects of a faecal capsule in obese adults showed no significant improvement in insulin sensitivity or body composition (16, 48). The conflicting data on FMT between rodents and humans may be partly explained by behavioural differences (such as the practice of coprophagia), that highlight important limitations of rodent-based models in this context (49, 50).

Carbon nanoparticles: An alternative therapeutic strategy to modification of the gut microbiota through FMT includes detoxification of the toxic byproducts of the gut microbiota, thereby limiting their pathogenic effects within the liver via the GLA. Yaq-001 is a synthetic non-absorbable carbon that adsorbs bacterial toxins. In a rodent model of Secondary Biliary Cirrhosis, Yaq-001 significantly attenuated LPS-induced production of reactive oxygen species within monocytes and altered the ratio of Firmicutes and Bacteroidetes (13). A human-based study of Yaq-001 is currently ongoing (13).

Bacteriophages: Bacteriophages are viruses that infect and kill bacteria (13). Therapeutically, bacteriophages can be used to target infectious bacteria. However, an alternate therapeutic strategy involves the development of 'phage cocktails' that target specific bacteria within the gut microbiome, to diminish and possibly eliminate those strains

that promote dysbiosis, whilst maintaining the healthy gut microbiota (13). A human-based study on the use of bacteriophage treatment for patients with Primary Sclerosing Cholangitis is ongoing (13).

Conclusions and Future Directions

MAFLD is now the commonest cause of chronic liver disease, affecting one in four of the world's population, and a leading cause of cirrhosis (4, 5). MAFLD often develops insidiously and asymptomatically in those who gain weight in the context of an underlying genetic predisposition. Based on the evidence outlined, we argue for a sea-change in our perspectives on MAFLD as a key linchpin in the complex development of metabolic dysfunction, and as an important gateway and portal between the gut microbiota and the overall metabolic status of the host, mediated via the GLA. As such, MAFLD should be considered as a condition that is influenced in its pathogenesis by the healthiness of the gut microbiota, and the status of the GLA (including the intactness of the gut mucosal wall). Outstanding human-based research questions include the role of the gut microbiota and other GLA-related factors in the development of MAFLD, and optimal preventive, treatment, and screening strategies (such as probiotics, prebiotics, synbiotics and FMT). This will require robust, powerful, and well-validated RCTs, performed in diverse populations. Novel treatment and preventive approaches for MAFLD should focus on improving the healthiness of the gut microbiota and optimising the proper functioning of the GLA, including avoidance of excessive weight gain through the usual principles of healthy lifestyle, a healthy and high-fibre diet, and avoidance of excessive alcohol intake. In essence, we need to nurture our gut microbiota to enable our gut microbiota to nurture our GLA, our liver, and us.

Table 1: Potential preventive and therapeutic strategies for MAFLD that target the gut microbiota and GLA

(ALT: Alanine Transaminase; FMT: Faecal Microbiota Transplantation; GLA: Gut Liver Axis; MAFLD: Metabolic-associated Fatty Liver Disease; RCT: Randomised Controlled Trial; ROS: Reactive Oxygen Species; SCFA: Short Chain Fatty Acid; 'VSL#3': a probiotic; 'Yaq-001': a synthetic non-absorbable carbon that adsorbs bacterial toxins)

Strategy	Rationale	Rodent-based data	Human-based data
Probiotics	Live microorganisms to modify gut microbiota	VSL#3 reduced fatty deposits within the liver & improved serum ALT	RCTs & meta-analysis: Improved serum ALT, insulin sensitivity & lipids
Prebiotics	Promotion of growth of beneficial gut microbiota	-	Improved serum transaminase activity & glycaemic/lipid profiles
Antibiotics	Reduces overgrowth of the gut microbiota	-	Microbial resistance to antibiotics precludes this as a viable option
Bile Acids	Reduces translocation of gut microbiota to liver	Reduced gut microbial overgrowth & translocation to liver	-
SCFA	Provides energy substrate for colonic epithelium	-	Immunomodulatory & anti-inflammatory properties
FMT	Transformation of the gut microbiota to eubiosis	Altered microbiota & alleviated steatohepatitis & endotoxaemia	Either transient or no improvement in insulin sensitivity
Carbon nanoparticles	Detoxifies by-products of the gut microbiota	Yaq-001 attenuated production of ROS and altered microbiota	Trial of Yaq-001 ongoing
Bacteriophages	Promotes eubiosis through 'phage cocktail'	-	Trial in Primary Sclerosing Cholangitis ongoing

References

1. Pi-Sunyer X. The medical risks of obesity. Postgraduate medicine. 2009;121(6):21-33.
2. Fruh SM. Obesity: Risk factors, complications, and strategies for sustainable long-term weight management. Journal of the American Association of Nurse Practitioners. 2017;29(S1):S3-S14.
3. Sakurai Y, Kubota N, Yamauchi T et al. Role of Insulin Resistance in MAFLD. Int J Mol Sci. 2021;22(8).
4. Powell EE, Wong VW, Rinella M. Non-alcoholic fatty liver disease. Lancet. 2021;397(10290):2212-24.
5. Minemura M, Shimizu Y. Gut microbiota and liver diseases. World J Gastroenterol. 2015;21(6):1691-702.
6. 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(1):202-9.
7. Geng Y, Faber KN, de Meijer VE et al. How does hepatic lipid accumulation lead to lipotoxicity in non-alcoholic fatty liver disease? Hepatol Int. 2021;15(1):21-35.
8. Jamialahmadi O, Mancina RM, Ciociola E et al. Exome-Wide Association Study on Alanine Aminotransferase Identifies Sequence Variants in the GPAM and APOE Associated With Fatty Liver Disease. Gastroenterology. 2021;160(5):1634-46 e7.
9. Mueller NT, Bakacs E, Combellick J et al. The infant microbiome development: mom matters. Trends in molecular medicine. 2015;21(2):109-17.
10. Qin J, Li R, Raes J et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464(7285):59-65.
11. Barber TM, Valsamakis G, Mastorakos G et al. Dietary Influences on the Microbiota-Gut-Brain Axis. Int J Mol Sci. 2021;22(7).
12. Cryan JF, O'Riordan KJ, Cowan CSM et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99(4):1877-2013.
13. Albillos A, de Gottardi A, Rescigno M. The gut-liver axis in liver disease: Pathophysiological basis for therapy. J Hepatol. 2020;72(3):558-77.
14. Zhu L, Baker SS, Gill C et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology. 2013;57(2):601-9.
15. Simbrunner B, Trauner M, Reiberger T. Review article: therapeutic aspects of bile acid signalling in the gut-liver axis. Aliment Pharmacol Ther. 2021;54(10):1243-62.
16. Malnick SDH, Fisher D, Somin M et al. Treating the Metabolic Syndrome by Fecal Transplantation-Current Status. Biology (Basel). 2021;10(5).

17. Mouries J, Brescia P, Silvestri A et al. Microbiota-driven gut vascular barrier disruption is a prerequisite for non-alcoholic steatohepatitis development. J Hepatol. 2019;71(6):1216-28.
18. Seo YS, Shah VH. The role of gut-liver axis in the pathogenesis of liver cirrhosis and portal hypertension. Clin Mol Hepatol. 2012;18(4):337-46.
19. Weickert MO, Hattersley JG, Kyrou I et al. Effects of supplemented isoenergetic diets varying in cereal fiber and protein content on the bile acid metabolic signature and relation to insulin resistance. Nutr Diabetes. 2018;8(1):11.
20. Di Ciaula A, Garruti G, Lunardi Baccetto R et al. Bile Acid Physiology. Ann Hepatol. 2017;16(Suppl. 1: s3-105.):s4-s14.
21. Katsuma S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enteroendocrine cell line STC-1. Biochem Biophys Res Commun. 2005;329(1):386-90.
22. Thomas C, Gioiello A, Noriega L et al. TGR5-mediated bile acid sensing controls glucose homeostasis. Cell Metab. 2009;10(3):167-77.
23. Schoeler M, Caesar R. Dietary lipids, gut microbiota and lipid metabolism. Rev Endocr Metab Disord. 2019;20(4):461-72.
24. Tang WH, Wang Z, Levison BS et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med. 2013;368(17):1575-84.
25. Miao J, Ling AV, Manthena PV et al. Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. Nat Commun. 2015;6:6498.
26. Fedorak RN, Madsen KL. Probiotics and prebiotics in gastrointestinal disorders. Curr Opin Gastroenterol. 2004;20(2):146-55.
27. Aller R, De Luis DA, Izaola O et al. Effect of a probiotic on liver aminotransferases in nonalcoholic fatty liver disease patients: a double blind randomized clinical trial. Eur Rev Med Pharmacol Sci. 2011;15(9):1090-5.
28. Vajro P, Mandato C, Licenziati MR et al. Effects of Lactobacillus rhamnosus strain GG in pediatric obesity-related liver disease. J Pediatr Gastroenterol Nutr. 2011;52(6):740-3.
29. Malaguarnera M, Vacante M, Antic T et al. Bifidobacterium longum with fructo-oligosaccharides in patients with non alcoholic steatohepatitis. Dig Dis Sci. 2012;57(2):545-53.
30. Wong VW, Won GL, Chim AM et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. Ann Hepatol. 2013;12(2):256-62.
31. Ma YY, Li L, Yu CH et al. Effects of probiotics on nonalcoholic fatty liver disease: a meta-analysis. World J Gastroenterol. 2013;19(40):6911-8.

32. Gilijamse PW, Hartstra AV, Levin E et al. Treatment with Anaerobutyricum soehngenii: a pilot study of safety and dose-response effects on glucose metabolism in human subjects with metabolic syndrome. NPJ Biofilms Microbiomes. 2020;6(1):16.
33. Depommier C, Everard A, Druart C et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 2019;25(7):1096-103.
34. Barber TM, Kabisch S, Pfeiffer AFH et al. The Health Benefits of Dietary Fibre. Nutrients. 2020;12(10).
35. Jeznach-Steinhagen A, Ostrowska J, Czerwonogrodzka-Senczyna A et al. Dietary and Pharmacological Treatment of Nonalcoholic Fatty Liver Disease. Medicina (Kaunas). 2019;55(5).
36. Mouzaki M, Comelli EM, Arendt BM et al. Intestinal microbiota in patients with nonalcoholic fatty liver disease. Hepatology. 2013;58(1):120-7.
37. Asemi Z, Khorrami-Rad A, Alizadeh SA et al. Effects of synbiotic food consumption on metabolic status of diabetic patients: a double-blind randomized cross-over controlled clinical trial. Clin Nutr. 2014;33(2):198-203.
38. Mofidi F, Poustchi H, Yari Z et al. Synbiotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. Br J Nutr. 2017;117(5):662-8.
39. Aslam B, Khurshid M, Arshad MI et al. Antibiotic Resistance: One Health One World Outlook. Front Cell Infect Microbiol. 2021;11:771510.
40. Sorribas M, Jakob MO, Yilmaz B et al. FXR modulates the gut-vascular barrier by regulating the entry sites for bacterial translocation in experimental cirrhosis. J Hepatol. 2019;71(6):1126-40.
41. Verbeke L, Farre R, Verbinnen B et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. Am J Pathol. 2015;185(2):409-19.
42. Gadaleta RM, van Erpecum KJ, Oldenburg B et al. Farnesoid X receptor activation inhibits inflammation and preserves the intestinal barrier in inflammatory bowel disease. Gut. 2011;60(4):463-72.
43. Maslowski KM, Vieira AT, Ng A et al. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. Nature. 2009;461(7268):1282-6.
44. Weickert MO, Arafat AM, Blaut M et al. Changes in dominant groups of the gut microbiota do not explain cereal-fiber induced improvement of whole-body insulin sensitivity. Nutr Metab (Lond). 2011;8:90.

45. Isken F, Klaus S, Osterhoff M et al. Effects of long-term soluble vs. insoluble dietary fiber intake on high-fat diet-induced obesity in C57BL/6J mice. J Nutr Biochem. 2010;21(4):278-84.
46. Kim KO, Gluck M. Fecal Microbiota Transplantation: An Update on Clinical Practice. Clinical endoscopy. 2019;52(2):137.
47. Zhou D, Pan Q, Shen F et al. Total fecal microbiota transplantation alleviates high-fat diet-induced steatohepatitis in mice via beneficial regulation of gut microbiota. Sci Rep. 2017;7(1):1529.
48. Allegretti JR, Kassam Z, Mullish BH et al. Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients. Clin Gastroenterol Hepatol. 2020;18(4):855-63 e2.
49. Weickert MO, Pfeiffer AFH. Impact of Dietary Fiber Consumption on Insulin Resistance and the Prevention of Type 2 Diabetes. J Nutr. 2018;148(1):7-12.
50. Weickert MO, Pfeiffer AF. Metabolic effects of dietary fiber consumption and prevention of diabetes. J Nutr. 2008;138(3):439-42.