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Can cirrhosis secondary to NASH be related to the alteration of bile acids caused by bacterial dysbiosis?

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Abstract
Non-alcoholic fatty liver disease (NAFLD) has become the most common chronic liver disease worldwide. It begins as non-alcoholic steatohepatitis (NASH), as a benign accumulation of fat, commonly known as "fatty liver", and progresses to inflammation, and fibrosis, leading to cirrhosis and possible hepatocellular carcinoma (HCC). Many factors, including diet and genetics, have been proposed for the development of NAFLD; however, recent studies have suggested gut dysbiosis and bile acids as possible contributing factors, yet much of this remains non-confirmatory. While the intestinal flora contributes to the metabolism, synthesis, and reabsorption of bile acids, bile acids are also known to affect the growth and diversity of intestinal flora. Studies have shown that a high-fat diet and conditions like Type 2 Diabetes Mellitus (T2DM) can induce dysbiosis in the gut microbiome, leading to changes in bile acid levels and compositions in the liver. Further, these changes to the intestinal microflora can cause increased expression of inflammatory genes, which are known to contribute to HCC. Here, we describe how specific microbial changes in the gut can affect the bile acid pool within the human body and review how these bile acid changes may contribute to cirrhosis secondary to NASH.

Keywords
NAFLD, NASH, Bile acids, Microbiome, Metabolic disease, Hepatocellular carcinoma, Dysbiosis, Fatty liver, Cirrhosis, Diet

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Introduction

The liver is the central hub of metabolism within the body. Lipids are a macronutrient that include fats and oils; among others. Lipids are processed within the liver, specifically in cells called hepatocytes. Non-alcoholic fatty liver disease (NAFLD) is defined as more than 5% fat accumulation in the hepatocytes (1). Patients with NAFLD have fat stored within their cells, a condition called hepatic steatosis. In addition, some patients experience inflammation resulting in liver fibrosis. NAFLD is subdivided into nonalcoholic fatty liver (NAFLD) and nonalcoholic steatohepatitis (NASH). In NAFLD, steatosis is present without inflammation, whereas NASH is defined as hepatic steatosis associated with inflammation. When inflammation persists, it leads to excessive scar tissue called fibrosis. Cirrhosis, the most severe stage of fibrosis, occurs after years of inflammation resulting in a nodular liver and permanent damage where the liver stops working. Patients with cirrhosis are at a risk for developing hepatocellular carcinoma (HCC) (Figure 2). Treatment at this stage is limited to liver transplants. The estimated annual incidence rate of HCC in patients with NASH cirrhosis was ~ 2 percent (2). In a large cohort study of patients with NASH cirrhosis, the incidence of HCC was 1 per 100 person-years of follow-up (3).

Currently, in the United States, about 1 in 4 adults suffers from NAFLD. 75% of Americans with obesity and 90% with severe obesity are diagnosed with NAFLD. Since NASH is secondary to NAFLD, about 1.5-6.5% of adults in the United States present with it (4). A follow-up condition from NASH could be cirrhosis, which is prevalent in 3-15% of the adult population. The final condition of this liver disease progression is HCC, which about 4-27% of adults with cirrhosis secondary to NASH are diagnosed with (5).

To the best of our knowledge, there are no probiotics that can ameliorate or reverse the symptoms of NAFLD *via* modulating the gut microbiome. Since NAFLD is a build-up of fat, as long as there is no celular damage, there is a possibility for it to be reversed. The obvious method is to lose 3%-10% of body mass (6). by eating healthier and exercising. Lastly, anecdotal evidence suggests that a moderate intake of caffeine can decrease liver inflammation (7).

Another key aspect of liver disease progression involves bile acids. Bile acids are a group of steroid acids that are synthesized by the liver and stored in the gallbladder, playing a critical role in the digestion and absorption of fats in the small intestine. The primary bile acids (BAs) are synthesized from cholesterol in the liver, conjugated to glycine or taurine, secreted into bile, concentrated in the gallbladder during fasting, and expelled in the intestine in response to dietary fat, biotransformed in the colon to the secondary BAs by the gut microbiota, reabsorbed in the ileum (part of small intestine) and colon back to the liver, and minimally lost in the feces (8). When fats enter the small intestine, BAs help emulsify them into smaller droplets, increasing the surface area available for digestion by lipase enzymes which are secreted by the pancreas. Bile acids also act as detergents, breaking down large fat globules into smaller ones, making it easier for the enzymes to break down the fat molecules into fatty acids and glycerol (which allows ATP production) (8).

In addition, they also help to absorb the products of fat digestion, including fatty acids, cholesterol, and fat-soluble vitamins. They do this by forming micelles, which are small droplets of bile acids that surround the products of fat digestion and make them water soluble, allowing them to be absorbed in the bloodstream *via* the intestinal lining. They also have a feedback mechanism that helps regulate their production. When the small intestine detects the presence of fatty acids and other products of fat digestion, it signals the liver to produce more bile acids (8).

A possible contributing factor to liver disease is the overall gut bacteria composition of the patient. The gut microbiome plays a critical role in digestion by breaking down complex carbohydrates, fats, and proteins that the human body cannot digest on its own. It also produces important compounds, such as short-chain fatty acids (SCFAs) and butyrate, that help maintain the health of the intestinal lining and regulate inflammation. Four major bacterial genera are thought to play a critical role in metabolism: Bacteroides, firmicutes, proteobacteria and actinobacteria. Bacteroidetes are responsible for breaking down complex carbohydrates and fiber that are not digestible by the human body. They produce SCFAs which maintain intestinal health and regulate inflammation. Firmicutes break down dietary fats and carbohydrates and produce butyrate, a type of SCFA that helps nourish the cells lining and colon and prevent inflammation. Proteobacteria usually break down proteins and produce enzymes that help digest food, maintain the balance of the gut microbiome, and prevent the overgrowth of harmful bacteria. Actinobacteria produce enzymes that break down complex carbohydrates, like starches and cellulose. They also produce antibacterial compounds that help control the growth of harmful bacteria (9).

Dysbiosis refers to an imbalance in the composition and function of the gut microbiota which has been associated with various health conditions, including liver disease. Dysbiosis can lead to changes in the production and metabolism of bile acids which affect liver function. Bile acids can regulate gut microbial composition both directly and indirectly by activation of innate immune response genes in the small intestine. Therefore, host metabolism can be affected by both microbial modifications of bile acids, which leads to altered signaling via bile acid receptors, and by alterations in the composition of the microbiota (10).

Dysbiosis can alter the composition and function of the gut microbiota, leading to changes in bile acid metabolism and signaling, which can contribute to the development of liver disease including non-alcoholic fatty liver disease (NAFLD), cirrhosis, and hepatocellular carcinoma (11). In this review, we aim to highlight the current research on how variations in the human microbiome may affect the levels of bile acids present and could therefore contribute to the development of liver disease.

Results

Gut dysbiosis leads to changes in human lipid metabolism.

Lipid metabolism includes the biosynthesis and breakdown of lipids such as fatty acids, triglycerides, and cholesterol. Specialized lipoproteins facilitate the transport of lipids from the gut to the liver and between the liver

and peripheral tissues. Obesity is related to the dysregulation of lipid metabolism, which may result in abnormal levels of blood lipids, increased lipid deposition, and associated metabolic diseases, such as non-alcoholic fatty liver disease (NAFLD) (12). The gut microbiota can perform many processes that cannot be carried out by the host, giving rise to microbial-produced or modulated metabolites that function as substrates and signaling molecules. Diet is important to the metabolic output of the gut microbiota since it affects the composition and levels of individual microbes within the gut microbiota. Different species within the gut microbiota process various nutrients into metabolites (13,14). This review aims to summarize the current literature on how gut dysbiosis leads to changes in bile acids and how these changes may result in downstream liver cirrhosis (Figure 1). Further, we aim to determine the importance of gut dysbiosis in the development of liver damage in multiple disease contexts, such as in type II diabetes.

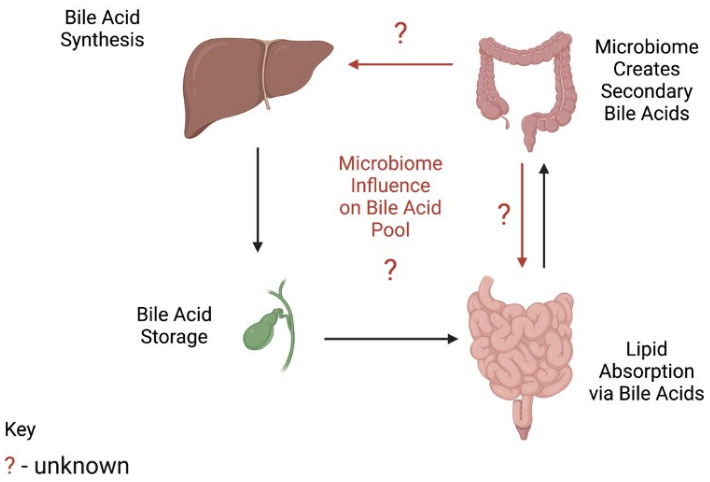

Figure 1. Bile acid progression in humans is likely influenced by the microbiome: Bile acid synthesis occurs in the liver (left). Bile acids are stored in the gallbladder (bottom left). Lipids are absorbed via bile acids in the small intestine (bottom right). Microbiomes create secondary bile acids that may aid in lipid absorption in the large intesting (top right). Secondary bile acids may be recycled back into the liver. Red text and arrows represent current inconsistencies in the literature. Image made using biorender.com

Studies in mice have shown differences based on diet. The gut microbiota differed between mice that were fed diets that were high or low in fat and between diets that contained equal amounts of fat, but from different sources (15-18). Further, a comparison of mice on a variety of diets showed that diets with saturated fat or n-6 polyunsaturated fatty acids (PUFA) as the main lipid source induced weight gain, but only the saturated fat diet increased insulin resistance, colonic permeability, and mesenteric fat inflammation (13). Other studies compared diets containing high levels of lard to high fish oil levels. Beneficial bacteria *Akkermansia muciniphila, Lactobacillus*, and *Bifidobacterium* were found to be lower in the mice on a lard diet (14). Not only may fat sources with major differences in lipid

composition, such as lard and fish oil, produce different gut microbial compositions with different functions, but fat sources that are not as dissimilar, may have significantly varying effects as well. There have been new developments in mice with the CYP2C70 and CYP2A12 genes knocked out, which represents a more "humanized" bile acid pool. Once these mice have progressed to NAFLD, changes in their bile acids, may be more useful and accurate for use in clinical studies (19).

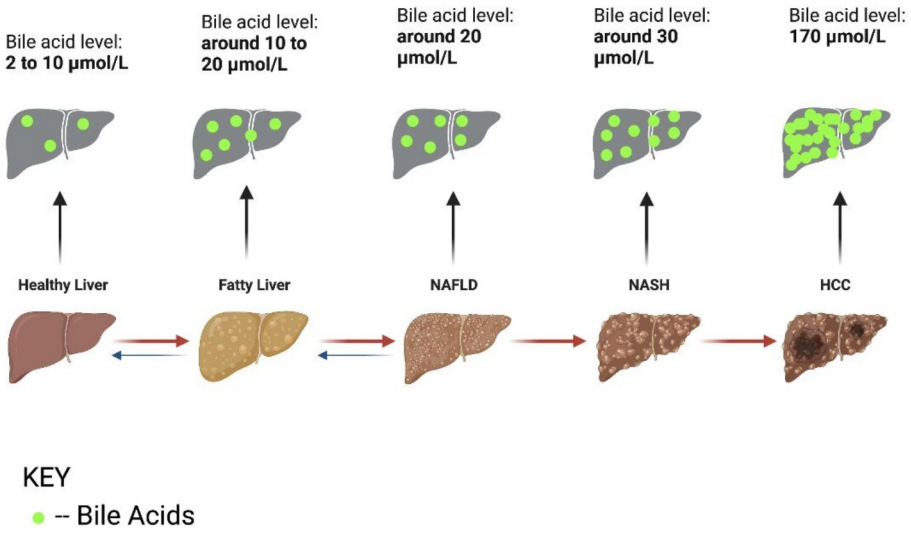

Figure 2. Levels of bile acids in humans at each stage of NAFLD: NAFLD progresses from a healthy liver to a fatty liver to NAFLD (all prior stages are reversible) to NASH to HCC (irreversible; bottom row). Healthy liver contains approximately 2-10 micromol/L of bile acids (green circles, 1st on top row). Fatty liver contains approximately 10-20 micromol/L of bile acids (2nd on top row). NAFLD liver contains approximately 20 micromol/L of bile acids (3rd on top row). NASH contains approximately 30 micromol/L of bile acids (4th on top row). HCC contains approximately 170 micromol/L of bile acids (5th on top row) (52). Image made using biorender.com

More research needs to be done to dissect the mechanisms of how fat sources affect the gut microbiome and downstream liver pathology. Some studies show how the gut microbiome affects lipid metabolism in the liver. For example, studies on gnotobiotic mice (born and raised in sterile conditions) demonstrated that the gut microbiota affects host lipid metabolism. More specifically, germ-free (GF) mice are protected against diet-induced obesity through a combination of several mechanisms including increased fatty acid oxidation and decreased deposition of triglycerides in adipocytes, when compared to conventionally raised mice (CONV-R). Furthermore, analysis of GF and CONV-R mice fed a standard rodent diet showed that the gut microbiota affected lipid composition in host tissues and serum and increased the clearance of triglyceride from the circulation (20). In contrast, circulating triglycerides, high-density lipoproteins (HDL), and total cholesterol levels were increased by the gut microbiota in mice on a high-fat diet (21). Taken together, these studies in mouse models show that the gut microbiota, in conjunction with diet, regulates host lipid metabolism and lipid levels in the serum and in tissues. In addition, specific bacteriophages are

reported to lyse cytolysin-producing *Enterococcus faecalis*, which is a major possible cause of non-alcoholic hepatitis. The amount of bacteriophages in the stool is directly correlated to a MELD score and signifies an improvement in cirrhotic patients (22).

Fecal microbiota composition has also been linked to lipid metabolism in humans. Several studies have investigated the association between the gut microbiota and dyslipidemia (imbalance of lipids). Analysis of data from obese individuals found that reduced total microbial gene diversity was associated with increased total serum cholesterol and triglycerides in obese patients. Changes in fecal microbiota composition are also present in individuals with dyslipidemia and ectopic fat deposition such as atherosclerosis and fatty liver. Several studies have found that the fecal microbiota composition in subjects with NAFLD differs from that of healthy controls and obese patients without fatty liver disease (23-25). Hoyles *et al.* recently found that patients with steatosis have decreased microbial gene diversity and altered genetic potential for several functions, including the processing of dietary lipids (11). Consequently, patients with steatosis have a different genetic makeup in their bacteria for processing dietary lipids. Changes in fecal microbiota composition are associated with the severity of NAFLD and its progression to fibrosis in non-alcoholic hepatitis (NASH) (26,27), with an increased abundance of Bacteroidetes being the most consistent findings in these studies. Bacteroidetes help the body digest its nutrients. Hence having an excess may cause an imbalance in fecal composition which, in turn, contributes to a gut microbial imbalance (27).

A common similarity between the development of NAFLD and NASH is the disruption of bile acid (BA) homeostasis. BAs are known for their role in fat absorption (28); however, they also act as signaling molecules involved in a variety of pathways that regulate BA, glucose, and lipid homeostasis (29). Therefore, pathways linked to BAs have been implicated in the pathogenesis of NAFLD and NASH, as well as in metabolic diseases (30) .

BAs are synthesized from cholesterol in the liver and are a key component of bile, the fluid the body uses to absorb fat. Bile is stored in the gallbladder and, upon consumption of food, is released into the duodenum within the small intestine *via* cholecystokinin (CCK) stimulation. In the small intestine, bile acids emulsify fat globules into smaller lipid particles (Figure 1). BAs are synthesized *via* one of two pathways: the classic pathway (*aka* neutral pathway) or the alternative pathway (*aka* acidic pathway) (31). The majority of BAs are synthesized using the classical pathway in hepatocytes, or liver cells (32). The first step in this pathway, which is the rate-limiting step, is catalyzed by the enzyme cholesterol 7α-hydroxylase (CYP7A1) to produce 7α-hydroxycholesterol (21), which is found exclusively in the liver. The major BA produced *via* this pathway in humans is colic acid (CA). The enzyme sterol 27-hydroxylase (CYP27A1) initiates the first step in the alternative pathway. CYP27A1 is a mitochondrial enzyme more widely distributed and found in macrophages and various tissues. The end products of this pathway include 25-hydroxycholesterol and 27 hydroxycholesterol. Chenodeoxycholic acid (CDCA) is the major BA synthesized via the alternative pathway. CA and CDCA are termed primary BAs. These

are often conjugated with either glycine or taurine in humans or taurine in rodents *via* the enzyme bile acid - CoA: amino acid N-acyltransferase (BAAT) (33,34). It is important to note, that glycine conjugation predominates over taurine conjugation in humans (33).

BA recycling is key to the maintenance of function. Once BAs are synthesized and exported in the duodenum, the majority are recirculated back to the liver from the terminal ileum. The remainder enter the colon where some are reabsorbed back into the liver while others are excreted, noted as enterohepatic circulation of BAs. This involves multiple transporters which include canalicular BA transporters which help the transfer from hepatocytes into the bile duct, and the bile salt export pump (BSEP), which is the main BA efflux transporter. Once they reach the terminal ileum, the majority are reabsorbed *via* apical sodium-dependent bile acid transporter (ASBT), and they are exported by organic solute transporters (OST alpha and OST beta), to return to the liver *via* the portal circulation. Once in the portal vein, they are actively transported into the hepatocytes. The remaining BAs enter the colon where bacteria deconjugate and dehydroxylate them to form the main secondary BAs, deoxycholic acid (DCA) and lithocholic acid (LCA). LCA is excreated in the stool, whereas DCA can be reabsorbed from the colon and recycled (35). The importance of a healthy microbiome is hence essential in the maintenance and proper functioning of the bile acid pathway.

In addition to their role in lipid digestion, BAs act as signaling molecules that regulate host metabolism by binding to the farnesoid X receptor (FXR) and the Takeda G-protein coupled bile acid receptor TGR5. Both the primary bile acids and the secondary bile acids are FXR agonists albeit with different affinities (36). In humans, CDCA can be transformed into UDCA, which is an FXR antagonist (37). FXR is involved in the regulation of lipid metabolism, especially triglyceride trafficking, synthesis, and utilization (38).

Recent animal studies have placed the gut microbiota as a potentially important player in the pathogenesis of NAFLD (11, 26). However, data linking gut dysbiosis with the severity of NAFLD remains poorly documented in humans. A study by Boursier J, et al., investigated whether the severity of NAFLD lesions, i.e. NASH and fibrosis, was associated with gut dysbiosis. The group enrolled 57 patients with biopsy-proven NAFLD. The taxonomic composition of gut microbiota was determined using 16S ribosomal RNA gene sequencing of stool samples. The results from 30 patients showed that they had F0/1 fibrosis stage at liver biopsy (10 with NASH), and 27 patients had significant F≥2 fibrosis (25 with NASH). Bacteroides abundance was significantly increased in NASH and F≥2 patients, whereas Prevotella abundance was decreased**.** Further, Ruminococcus abundance was significantly higher in F≥2 patients. Bacteroides abundance was independently associated with NASH and Ruminococcus abundance with F≥2 fibrosis. Stratification according to the abundance of these 2 bacteria generated 3 patient subgroups with increasing severity of NAFLD lesions. This suggested that the Bacteroides genera was independently associated with NASH and Ruminococcus genera was associated with significant fibrosis (11). Thus, gut microbiota analysis adds information to classical predictors of NAFLD severity (9). Therefore,

perhaps the microbiome could be used as a tool in providing care to patients with liver disease.

Bile acids have been implicated in the pathogenesis of fatty liver disease. Patients with NASH have been shown to have altered fecal bile acid composition (39). In addition, an inverse relation between fibroblast growth factor 19 (FGF19), an FXR-regulated hormone produced in the ileum, and NASH has been reported (40-42). The importance of understanding the interplay between gut microbiota, bile acids, and lipid homeostasis is highlighted by efforts to use microbiome modifications as a treatment for NAFLD and NASH. Additional metabolic studies suggest that the plasma levels of BAs are elevated in patients with NAFLD compared to healthy controls, however, it remains unclear if specific BAs are relevant to the transition from simple steatosis to NASH, or enhanced progression of hepatic fibrosis (42). A study by Nimer et al. on bile acid profiling and progression of NAFLD found that circulating bile acids were associated with histopathological and genetic determinants of the transition from simple hepatic steatosis and NASH. The study recruited 102 subjects who underwent liver biopsy and examined a broad panel of BAs with distinct histopathological features of NAFLD, the presence of NASH, and their association with genetic variants of NASH and NAFLD. Plasma alterations were observed through the entire spectrum of NAFLD, with several glycine-conjugated forms of BA's demonstrating significant associations with higher grades of inflammation and fibrosis. Plasma 7-Keto-DCA levels showed the strongest associations with advanced stages of hepatic fibrosis. Plasma 7-Keto-LCA levels were associated with NASH (11) (Figure 3). Taken together, these observations indicate the possibility of microbial changes that induced downstream changes in bile acids resulting in overall metabolic shifts, which, in turn, contributed to liver disease.

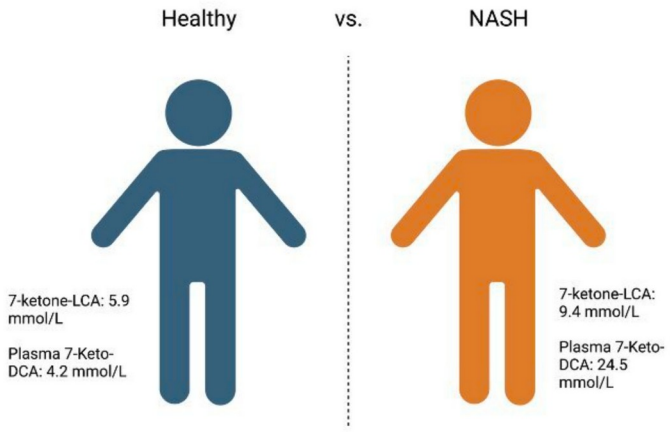

Figure 3. Levels of the two prominent bile acids in a healthy person and a patient with NASH. The level of BA in a healthy individual, specifically 7-ketone-LCA is 5.9 mmol/L and the plasma 7-keto-OCA is 4.2 mmol/L (left). The level of BA in a healthy individual, specifically 7-ketone-LCA is 9.4 mmol/L and the plasma 7-keto-DCA is 24.5 mmol/L (right) (42). Image made using biorender.com

Discussion

There are millions of different kinds of bacteria in the human body, each genera and species having a unique set of functions, growth conditions, and metabolic outputs. Certain bacteria can act on specific lipids, such as PUFA's or saturated fats, which in turn results in each person processing lipids differently based on the composition of their microbiomes (13). This variability in gut microbiomes can be explained by differences in the way that each person metabolizes food, the environment that they live in, genetics, and diet. There are also notable differences in the way liver diseases, such as NAFLD and NASH, present both in patient symptoms and pathology (Figure 4). Yet, in many people with metabolic diseases such as liver disease, it remains unclear whether initial microbial differences drive disease or whether disease results in changes to an individual's microbiome. The classic and alternative pathways in the liver maintain the production of primary BAs. Yet, BAs must be conjugated and then made into secondary bile acids by bacteria by the microbiome. Not only do individual's microbiomes differ, but there are also large variations in microbial levels and compositions throughout each person's life. It is tantalizing to speculate that levels of specific microbial species during infancy and childhood may predict a person's likelihood of developing a metabolic disease in adulthood.

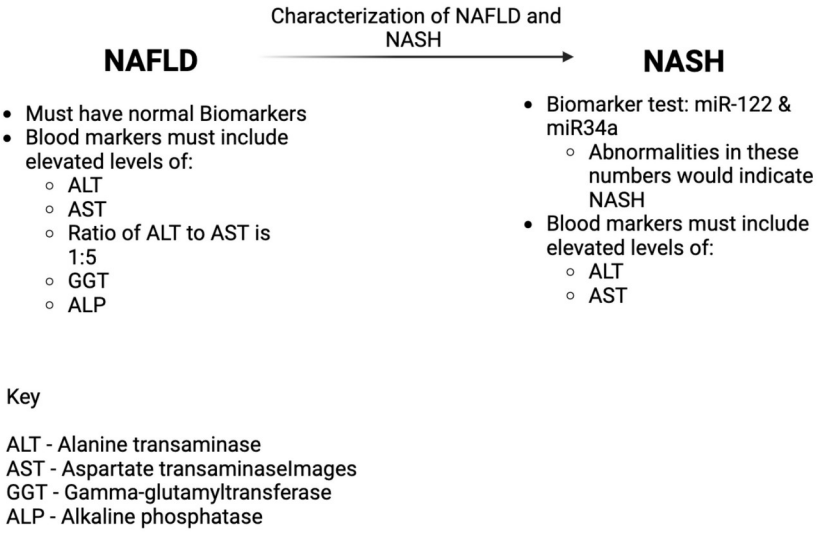

Figure 4. Characterization of NAFLD and NASH pathology in humans (53, 54)

Apart from aiding in the absorption of lipids, BA levels affect key signaling pathways. They regulate lipid metabolism through FXR. This pathway is an example of a feedback loop that keeps the body in ideal lipid homeostasis (37). We speculate that when BAs are acted on incorrectly by an individual's microbiome, this may disrupt this feedback signaling and cause abnormal lipid metabolism and possible disease. While drugs targeting FXR have been studied and produced, perhaps manipulating a person's microbiome may be a significantly non-invasive, affordable, and lasting treatment to combat metabolic diseases such as NAFLD

and NASH (31). Probiotics that contain beneficial commensal genera to correct dysbiosis may be the most natural and minimally invasive method to affect this change. A fecal transplant is another.

Conclusion

In conclusion, NAFLD and NASH are complex diseases resulting from a combination of genetic predisposition, dietary, and metabolic factors that likely influence the composition of the gut microbiome. Rodent models on a high-fat diet supported an association between altered gut health, obesity, and its metabolic sequelae (43,44). It is evident that fat from different sources has different effects on the gut microbiota. An isocaloric high-fat diet (HFD) model with the same macronutrient composition but different dietary fat profiles, found a connection between diet-induced obesity and alterations in microbiota patterns and barrier functions, the two gut parameters that are associated with metabolic outcomes. The observation that a high-fat diet and a high-fat diet with polyunsaturated fatty acids led to different microbial profiles despite similar weight gain suggests that diet induced dysbiosis in animal models is more of a consequence of specific dietary factors than of energy intake either by increased food quantity or fat consumption (18). In addition, HFD-fed mice presented with reduced barriers to resistance in the colon, increased infiltration of inflammatory immune cells, and tended toward higher bacterial DNA content in the mesenteric fat, as evidence for bacterial translocation (45, 46). These findings are consistent with a "leaky gut" hypothesis which postulates that an impaired intestinal barrier leads to a specific local proinflammation in the mesenteric fat, but not in depots more remote from the gut. In contrast, mice fed with a polyunsaturated fatty acid diet represented a diet-induced obese rodent with a normal gut barrier. This observation excluded macronutrient distribution and adiposity as specific causes of increased gut permeability. Consistent with this notion, genetically obese rodents, when fed a standard diet, had a similar gut barrier function as their lean counterparts (47,48). In addition to this, it is commonly understood that mice that are fed Western diets have very similar genes that are regulated in humans as well. ~ 40% of the genes expressed in NASH or NAFLD are also expressed in obese mice. This makes mice, representative candidates for studying liver diseases (49).

In addition to dietary factors, changes in fecal microbiota have also been suggested to play a role in the development of NAFLD. More specifically, an increase in Bacteroidetes and Ruminococcus in fecal microbiota are linked to a possible etiology and progression to NASH (9,11). Microbial processing of bile acids influences the synthesis and trafficking of triglycerides, by regulating host metabolism through the binding of FXR and TGR5. Several studies indicate that the inhibition of intestinal FXR improves metabolic phenotypes, decreasing triglyceride processing; but the underlying mechanism remains unknown (50,51). Clinical studies targeting the gut microbiota to specifically modify FXR signaling in NAFLD and/or NASH, have yet to be performed. There has been a study that compares the use of Obeticholic Acid, which is known as an FXR antagonist, on patients with pre-existing NAFLD. Over the course of 72 weeks, patients saw no increase in fibrosis and most saw their NAFLD score decrease by at least 2 points (55).

Although some of these clinical studies have not yet currently been completed, there have been others that implicate gut microbiome composition in a better prognosis for liver disease. Leung et al. (56) tested mice which had cholestasis induced by bile duct ligation (BDL), to mirror the effects of a failed bile duct. They found that impaired function of bile ducts caused an increase in disease-associated bacteria and a decrease in beneficial bacteria (56). Cholestasis induced inhibition of bile salt hydrolase (BSH) in the bile duct caused decreased levels of secondary bile acids, more specifically, a decrease in DCA and LCA (57,58). Both these bile acids are crucial to maintaining a healthy and balanced microbiota in the GI tract.

Patients with both HCC and cirrhosis have a similar intestinal permeability, which could be related to similar bile acid composition. Plasma levels of IL8, IL13, CCL3, CCL4 & CCL5 were significantly higher in patients with HCC (59). Both IL8 and IL13 have a greater effect on liver disease since the role of both is to control inflammation. With increased levels of both, it becomes increasingly difficult for the liver to manage inflammation; one of the hallmarks of NAFLD. Persistently higher IL8 or IL13 levels may signal early liver disease, especially in obese patients. If detected early, changes in diet and lifestyle may delay progression. Ghandian et al., achieved an NAFLD prediction accuracy of 80% by comparing data from 4 years prior with the current statistics of the same patients 4 years later (60). HDL levels were the major contributors to prediction accuracy, indicating consumption of a high-fat diet and a dysbiotic microbacteria composition in the gut. HDL is a protein that slowly increases over time, so regularly testing for increases will allow a patient to alter their diet as appropriate, especially since an increased HDL does not immediately indicate signs of liver disease.

There is also evidence that bile acid sequestrants can decrease the intestinal re-absorption of bile acids which would in turn decrease their circulating levels. Treatment with bile acid sequestrants could potentially change the gut bile acid levels; thereby modulating gut microbial composition; which may reverse liver damage and prevent further progression of liver disease (61). On the other hand, though, there is also evidence that lowering the bile acid circulation can worsen diet-induced obesity and diabetes (62). If bile acid sequestrants can be invented that bind to *specific* bile acids (as opposed to the universal electrostatic binding mechanism of sequestrants available now), the *composition* – rather than the *abundance* – of the bile acid pool (and hence the gut microbiota) can be modulated. This represents a gap in existing knowledge that can be utilized to prevent the progression of liver disease.

It has been found that higher levels of ammonia in the bloodstream can be associated with NASH. This could be because the liver does not have the right composition of bile acids and bacteria to fully process the ammonia input, thereby excreting unmetabolized ammonia into the bloodstream. The bacteria *Escherichia coli* strain SYNB1020 has been found to convert the ammonia in the blood into L. arginine. The role of this amino acid is to rebuild tissue, thereby decreasing NASH associated liver fibrosis. Fish, red meat, poultry, soy, whole grains, beans, and dairy (63,64) are good sources of L. arginine; as are nutritional

supplements. *Lactobacillus plantarum* was found to ameliorate NASH-related inflammation by upregulating L-arginine production (65), thereby confirming this mechanism of action. Probiotic supplements containing *Lactobacillus plantarum* may hence be reasonably assumed to decrease NASH related inflammation; again attesting to the importance of the intestinal microbial composition in preventing liver disease. Interestingly, *Lactobacillus plantarum* has BSH activity and modulates bile acid (66); but no literature exists on whether the corollary is also true.

Lastly, there are similarities between Inflammatory Bowel Disease (IBD) and NASH which can be further researched. Both IBD and NASH, present with a perturbation of choline metabolism. In IBD, choline plays a role in the development and treatment of mood disorders, while in NASH, it helps prevent the increase in harmful methylamines which causes hepatic inflammation. In many cases, IBD patients are twice more likely to present with NASH (67). Contributory factors that link the pathogenesis of both diseases have been suggested to be an altered intestinal permeability, gut dysbiosis and chronic inflammatory response (68). In cases of IBS, a bile sequestrant such as cholestyramine is used to bind with excess bile acids to reduce damage to intestinal mucosa. We propose that treatment protocols for IBD patients should consider the inclusion of obeticholic acid with cholestyramine to simultaneously prevent the progression of liver disease.

Factors aside from the microbiome may lead to liver disease and/or affect the gut microbiome. These may include environmental factors that result from people living in different geographical regions having different compositions of gut microbiomes. In addition, since there remains a large amount of research to be done on this topic, there is a limited amount of information regarding gut microbiome profiles in different people because of gender-bias, selectivity with animal studies (i.e. mostly done in mice), and differences in patient ages, location, and diet. Although gut microbiome composition significantly affects the progression of NAFLD in animal models, the microbiota itself is affected by multiple factors such as the presence of metabolic disease, genetics and diet, among others. However, if the composition of the gut microbiome is sufficiently downstream of, and is the focal point of multiple causative signaling pathway convergence, then modulating it *via* bile acid composition may significantly decrease the progression of NAFLD.

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