

REVIEWS IN BASIC AND CLINICAL GASTROENTEROLOGY AND HEPATOLOGY

Nonalcoholic Fatty Liver Disease and the Gut-Liver Axis: Exploring an Undernutrition Perspective



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Nonalcoholic fatty liver disease (NAFLD) is a chronic condition affecting one quarter of the global population. Although primarily linked to obesity and metabolic syndrome, undernutrition and the altered (dysbiotic) gut microbiome influence NAFLD progression. Both undernutrition and NAFLD prevalence are predicted to considerably increase, but how the undernourished gut microbiome contributes to hepatic pathophysiology remains far less studied. Here, we present undernutrition conditions with fatty liver features, including kwashiorkor and micronutrient deficiency. We then review the gut microbiota-liver axis, highlighting key pathways linked to NAFLD progression within both overnutrition and undernutrition. To conclude, we identify challenges and collaborative possibilities of emerging multiomic research addressing the pathology and treatment of undernourished NAFLD.

Keywords: Gut Microbiome; NAFLD; Gut-Liver Axis; Malnutrition; Undernutrition.

Nonalcoholic fatty liver disease (NAFLD), defined as $\geq 5\%$ hepatic steatosis (fat buildup), affects approximately one-quarter of the global adult population. Although associated with aging, NAFLD prevalence in nonobese pediatric cohorts ranges from 3%–12% but may increase to more than 70% among obese children. NAFLD remains a leading cause of chronic liver disease in children, particularly those of Asian, Hispanic, and White descent, although less common among pediatric African American cohorts.¹ Alarmingly, global NAFLD prevalence is expected to considerably increase in both adult and pediatric populations, along with an increased prevalence of obesity and type 2 diabetes.^{1–4}

NAFLD outcomes range from simple steatosis to nonalcoholic steatohepatitis (NASH), an inflammatory condition additionally characterized by liver fibrosis and hepatocellular ballooning. Although NAFLD/NASH are considered reversible and often asymptomatic, fatty liver pathology may advance to irreversible liver damage, necessitating liver transplantation.^{5,6} Beyond steatosis and fibrotic scarring, NAFLD increases the risk of hepatocellular carcinoma, cirrhosis, and mortality.²

NAFLD pathophysiology is linked to metabolic syndrome—a cluster of aberrant features including elevated fasting plasma glucose, hypertriglyceridemia, hypertension, decreased high-density lipoprotein cholesterol levels, and obesity. Considered the hepatic expression of metabolic syndrome, NAFLD is significantly shaped by malnutrition.^{2,7} Most often linked to dietary inadequacies, malnutrition is an umbrella term encompassing both overnutrition and undernutrition. Indeed, NAFLD etiology and treatment have been extensively examined in the context of overnutrition and obesity,^{8,9} recently reviewed by Aron-Wisnewsky et al¹⁰ and Cotter and Rinella.¹¹ Beyond diet, an altered gut microbiota-liver axis has emerged as a critical factor informing metabolic disease and NAFLD treatment.^{10,12} Despite advances in gut-liver NAFLD research, the role of undernutrition-induced NAFLD and the malnourished gut microbiome remains largely ignored.

Here, we describe undernutrition pathologies with NAFLD-like features and highlight established microbiome pathways of gut-liver interactions linked to malnourished NAFLD. Finally, we examine multiomic research addressing undernutrition-induced fatty liver within a gut-liver framework.

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Abbreviations used in this paper: BMI, body mass index; DSS, dextran sulfate sodium; EED, environmental enteric dysfunction; FMT, fecal microbiota transplant; FXR, farnesoid X receptor; LMIC, low-to-mid-income country; LPS, lipopolysaccharide; MAL, a murine malnutrition model; MBG, malnutrition + gut microbiota dysbiosis model; MDCF, microbiota-directed complementary food; MND, micronutrient deficiency; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; PUFA, polyunsaturated fatty acid; ROS, reactive oxygen species; rRNA, ribosomal RNA; RUTFs, ready-to-use therapeutic foods; SCFA, short-chain fatty acid; SAM, severe acute malnutrition; SIBO, small intestinal bacterial overgrowth; TGR5, Takeda G protein-coupled receptor 5; TLR, toll-like receptor.

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Undernutrition Pathologies Promote Fatty Liver Features

Undernutrition remains a critical public health crisis, particularly among pediatric populations within low-to-middle income countries (LMICs).^{13,14} Although dietary deficiency is the primary driver of undernutrition,¹⁵ interconnected factors contributing to malnutrition include poverty, poor sanitation, sociopolitical conflicts, climate change, and, most recently, the COVID-19 pandemic.^{16–18} Although the full extent of COVID-19 on metabolic health trajectories remains unknown,^{18,19} the pandemic is projected to significantly exacerbate child mortality, potentially resulting in >150,000 additional deaths from childhood undernutrition.²⁰

Chronic undernutrition promotes stunting (reduced height-for-age) and wasting (reduced weight-for-height). Before the COVID-19 pandemic, nearly 150 million children (22%) younger than the age of 5 years exhibited stunting, whereas more than 45 million (6.7%) displayed wasting.¹⁹ Beyond growth deficits, long-term consequences include increased susceptibility to infectious disease,^{21,22} impaired cognitive development,^{13,21} decreased muscle and cardiac function,²³ nutrient malabsorption linked to gastrointestinal dysbiosis,^{14,22,24} and metabolic alterations.^{25,26} Childhood undernutrition significantly increases the risk of metabolic syndrome, visceral adipose accumulation, and fatty liver disease.^{9,27} Despite large-scale intervention efforts and progress, the global community has fallen behind on established goals to reduce undernutrition,²⁰ notably the United Nations Sustainable Goal to reach zero hunger by 2030.²⁸ The recent surge of malnutrition

will undoubtedly contribute to the predicted increase of fatty liver conditions,^{9,20} highlighting the critical need to study the pathophysiology of undernutrition-induced NAFLD.

Undernutrition manifests across distinct pathologies. Here, we highlight prevalent conditions that promote hepatic NAFLD features in clinical settings and experimental models.

Kwashiorkor

Severe acute malnutrition (SAM) describes significant, pediatric wasting (>-3 standard deviation from the median weight-for-height)²¹ associated with sustained metabolic impairments.^{29–31} SAM is further classified into distinct undernutrition pathologies—kwashiorkor and marasmus.^{21,27}

Kwashiorkor, a form of severe protein-energy malnutrition, emerges during the early-life weaning period³² (Table 1). Kwashiorkor presents with enlarged, fatty liver accompanied by abdominal and peripheral edema.^{32,33} In contrast, marasmus, a nonedematous form of SAM, occurs when a child experiences sustained deficits across all forms of nutritional intake (eg, protein and fat deficiencies) and is generally characterized as severe wasting.^{32,34}

Beyond distinctive edema, kwashiorkor features include reduced muscle mass, skin lesions, and fatigue.^{32–34} This condition is linked to altered lipoprotein synthesis (eg, very-low-density lipoprotein) and subsequent hepatic steatosis due to impaired hepatic processing and clearance of lipids.^{9,34} Patients with kwashiorkor exhibit additional metabolic alterations, including glucose intolerance and insulin resistance.^{34,35} Intervention strategies combatting

Table 1.Clinical Features of Undernutrition-Induced NAFLD

Undernutrition conditions	Clinical population	Clinical symptoms	Key NAFLD/hepatic features
Kwashiorkor	6–36 mo Highest prevalence in South-East Asia and sub-Saharan Africa	Abdominal edema Reduced muscle mass Fatigue/appetite loss Dermatitis Cognitive impairment	Impaired hepatic peroxisome function Hepatic steatosis Glucose intolerance/ insulin resistance Systemic inflammation Altered bile acid profile
Micronutrient deficiency (iron, zinc, vitamin E, copper, selenium)	2 billion people globally More common in children, pregnant women, women of childbearing age, and food insecure people Highest prevalence in LMICs	General: fatigue, muscle weakness, poor cognitive function, increased infection, loss of peptide, growth deficits, anemia (iron deficiency), and diarrhea (zinc deficiency)	Hepatic steatosis, insulin resistance, altered glucose metabolism, dysregulated lipid metabolism, increased oxidative stress, systemic inflammation, altered bile acid profile
Subclinical undernutrition (EED)	Observed in regions of poor sanitation More common in children Highest prevalence in LMICs	Small intestine villi blunting epithelial barrier Disruption intestinal inflammation High pathogen load	Hepatic steatosis Altered fatty lipid metabolism Altered bile acid profile

SAM typically involve ready-to-use therapeutic foods (RUTFs), rich in lipids, polyunsaturated fatty acids (PUFAs), and proteins.^{26,36,37} Although early childhood renutrition strategies largely mitigate long-term metabolic impairment and fatty liver, SAM significantly increases the risk of cardiometabolic disease.^{29–31}

Protein restriction also promotes hepatic steatosis within kwashiorkor rodent models.^{24,36,38–40} A protein-deficient diet (5% daily calories from protein) triggered fatty liver features in newly weaned Wistar rats, compared with counterparts fed a healthy diet (20% daily calories from protein). After 4 weeks of low-protein diet, young rats displayed hepatic steatosis and hypoalbuminemia, pathologies linked to impaired peroxisome proliferator-activated receptor function and fatty acid beta-oxidation within the liver.⁴⁰ Our laboratory explored early-life undernutrition in C57BL/6J mice fed a protein/fat-deficient, carbohydrate rich-diet (a murine malnutrition model [MAL]).^{24,38,41} This diet reflects reported macronutrient availability from an undernourished population within northeastern Brazil.^{24,42} MAL mice display increased adipose accumulation within the abdomen but reduced lean and bone mass. This model develops hepatic steatosis and exhibits elevated liver triglyceride content compared with healthy controls.^{24,38} Pathophysiologies in hepatic steatosis models likely involve broad macronutrient shifts because protein-deficient rodent diets often contain high loads of simple carbohydrates, a feature associated with hepatic lipogenesis and obesity-associated NAFLD.^{7,34,43}

Certain undernutrition models have even used designer diets matching macronutrient content and particular dietary sources from specific malnourished communities. For example, researchers in the Gordon laboratory developed a murine kwashiorkor diet composed of corn flour and mustard greens, staple foods reported in a protein-deficient Malawian pediatric cohort (≤ 3 years of age).³⁶ Metabolomic profiling of fecal and intestinal (cecal) samples revealed altered amino acid and bile acid metabolism, reflecting NAFLD-like metabolic profiles.^{12,35} Collectively, experimental findings demonstrate that protein deficiency can trigger hepatic steatosis and systemic metabolic features of NAFLD. As such, rodent models provide an attractive tool to examine undernourished hepatic pathogenesis, as well as putative gut-liver interactions driving fatty liver disease.

Micronutrient Deficiency

Micronutrients are essential vitamins and minerals required to maintain health. Micronutrients perform various functions, often synergistically, including immune regulation, DNA synthesis/repair processes, and lipid metabolism.^{44,45} Vitamins are categorized into 2 main groups: fat-soluble (A, D, E, and K) and water-soluble (C and B-complex) vitamins.⁴⁶ Although micronutrients are primarily obtained from dietary sources, commensal gut microbes synthesize fat-soluble vitamin K (K₂) and multiple members of water-soluble vitamin B (including biotin, cobalamin, folic acid, niacin, and riboflavin).⁴⁷ Regardless of origin, micronutrient deficiency (MND) triggers broad pathologies, including hepatic dysfunction.^{48,49}

Described as the “hidden hunger,” MND affects more than 2 billion people globally, primarily within LMICs.⁵⁰ Pediatric cohorts are particularly vulnerable, with half of all children between 6 and 60 months suffering from one or multiple MNDs.^{50–52} During early childhood, reduced access to nutrient-rich breastmilk and increased demands for growth can exacerbate MND.⁵³ Throughout life, MND may arise from poor diet and malabsorption conditions (eg, gastrointestinal damage from enteric pathogens).⁵⁴ Moreover, deficiency of one micronutrient may affect the use and absorption of another.⁵⁵ Consequences of chronic MND include growth faltering, increased susceptibility to infection and diarrheal disease, delayed neurocognitive development, anemia, blindness, and gut-liver disruption.⁵⁰

Dietary staples within LMICs include carbohydrate-rich grains such as wheat, rice, millet, and maize (corn), containing phytates (phosphorous storage compounds) that block gastrointestinal absorption of key minerals, notably iron and zinc.⁵⁴ The liver performs an indispensable role in metabolism, transport, and storage of micronutrients, all of which are impaired in liver disease.⁴⁸ MND, in turn, has been implicated as a driver of fatty liver pathophysiology.^{48,49}

A recent epidemiologic review reported striking MND in patients diagnosed with liver cirrhosis, including deficiencies in vitamin A (28%-35%), vitamin D (68%-86%), and zinc (>80%).⁵⁶ MND was also linked to pediatric cases of chronic liver disease. A pediatric study of 166 children who underwent liver transplantation found that 66.6%, 40.6%, and 36.3% of patients were deficient in vitamin A, vitamin E, and vitamin D plasma levels, respectively.⁵⁷ Whether these shifts reflect a cause and/or consequence of NAFLD remains disputed. Here, we review key micronutrients linked to the progression and treatment of fatty liver disease.

MNDs and NAFLD

Vitamin E

Although vitamin E deficiency is rare within high-income countries, lipid malabsorption, genetic abnormalities, advanced liver disease, and total parenteral nutrition can promote vitamin E deficiency.^{58–61} In LMICs, vitamin E deficiencies are frequently reported in vulnerable elderly or pediatric populations.⁶²

Vitamin E has 8 different isomers (α -, β -, γ -, and δ -tocopherol and α -, β -, γ -, and δ -tocotrienol). These isomers, particularly dietary α -tocopherol, exhibit potent antioxidant properties.^{58,63} Like most fat-soluble vitamins, α -tocopherol is stored in the liver.⁶⁴ Growing evidence links vitamin E deficiency with NAFLD pathology, and several clinical trials suggest that vitamin E supplementation has therapeutic benefits for fatty liver disease.^{65–68}

The landmark PIVENS (Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis) randomized control trial examined the role of 800 IU of daily vitamin E compared with 30 mg of daily pioglitazone (an antidiabetic medication stimulating peroxisome proliferator-activated

receptor) or placebo among nondiabetics. After 96 weeks, both treatment arms displayed reduced lobular inflammation, hepatic ballooning, and serum alanine aminotransferase (ALT). Compared to placebo controls, steatohepatitis histologic features were improved in 34% of subjects in the pioglitazone arm and 43% of the vitamin E cohort, with vitamin E treatment.⁶⁶ The TONIC (Treatment of NAFLD in Children) trial later assessed a daily dose of vitamin E 800 IU compared with 1000 mg of metformin (antidiabetic medication that targets hepatic gluconeogenesis) for 96 weeks. Metformin and vitamin E reduced pediatric hepatic ballooning, but not hepatic fibrosis, steatosis, or lobular inflammation.⁶⁸ As a potent antioxidant, the therapeutic effect of vitamin E in NAFLD is likely attributed to a reduction in oxidative stress and inflammation, a hepatic feature of NAFLD.⁶⁹ Following these findings, the American Association for the Study of Liver Disease and the European Association for the Study of the Liver recommend short-term pharmacologic treatment with 800 IU of vitamin E for NAFLD.^{67,68}

Iron

Iron plays a vital role in oxygen transport, energy metabolism, and DNA replication/repair.^{70,71} A regulatory mechanism of iron excretion does not exist; consequently, iron homeostasis is tightly controlled by the liver to prevent substantial daily loss.^{70,72} Iron deficiency and hemochromatosis (ie, iron overload) have been mainly studied in the context of iron deficiency anemia. Beyond anemia, aberrant iron levels contribute to hepatic injury, lipid peroxidation, and NAFLD severity.^{48,73}

Ma et al⁷⁴ reported increased iron overload in obese/overweight patients with more severe NAFLD compared with subjects with normal body mass index (BMI) or mild to moderate NAFLD. Iron levels were also significantly higher in patients with dyslipidemia, hypertension, and hyperuricemia. The researchers reported significant positive correlations between iron and BMI, ALT, serum triglycerides, fasting insulin, postprandial glucose, serum-free fatty acids, and hepatic steatosis.⁷⁴ Both impaired iron and vitamin E (α -tocopherol) levels were linked to hepatic steatosis pathology within a genetic hemochromatosis murine model.⁷⁵ Intriguingly, a genetic model of anemia promoted early-onset NAFLD, accompanied by elevated serum ALT and triglycerides,⁷⁶ suggesting a role for iron deficiencies and hepatic steatosis.

Copper

Like vitamin E, copper exhibits antioxidant functions, participates in mitochondrial redox reactions, contributes to bone and nervous maturation, and regulates lipid metabolism.⁷⁷⁻⁷⁹ Copper also regulates iron metabolism, highlighting micronutrient interdependency.⁷⁸ In addition to poor diet, nutrient malabsorption from gastrointestinal disruption (eg, after gastric bypass surgery) promotes copper deficits.^{77,80}

Copper deficiency has been extensively studied in the context of metabolic pathologies, including obesity, type 2

diabetes, and liver disease.^{77,81} Copper deficiency was recently associated with hepatic pathology in a stepwise manner. A NASH cohort of 31 biopsy-confirmed adults exhibited greater serum copper deficiency compared with a cohort ($n = 93$) with milder NALFD pathology (simple hepatic steatosis).⁸² Copper deficiency was also linked to pediatric NAFLD because serum copper levels were inversely associated with NAFLD activity score and presence of hepatic ballooning in 100 children with biopsy-confirmed NAFLD.⁸³

Moreover, the relationship between copper deficiency, fructose intake, and metabolic syndrome has been widely established, with independent rodent and human studies reporting that high-fructose diets exacerbate copper deficiency. Elevated fructose intake impairs gastrointestinal copper absorption promoting hepatic fat accumulation, hypercholesterolemia, and hypertriglyceridemia.^{81,84} Indeed, rodent studies assessing carbohydrate intake reported that even modest fructose supplementation (3% wt/vol) was sufficient to induce copper deficiency.⁸¹

Selenium

Selenium exhibits broad functionality, regulating thyroid hormone activation, antioxidant defense, and cardiovascular function.⁸⁵ Aberrant selenium levels have been linked to hepatic pathology because elevated circulating selenium was associated with increased NAFLD prevalence,⁸⁶ including a cross-sectional study of Chinese adults ($N = 8550$) that reported significant correlations between plasma selenium and ALT, circulating triglycerides, and ultrasound-diagnosed NAFLD.⁸⁷ The National Health and Nutrition Examination Survey (NHANES)-III study ($N = 33,944$ patients with NAFLD) later reported an inverse correlation between serum selenium levels and liver fibrosis (particularly in elderly, female, and Caucasian participants).⁸⁸ Intriguingly, selenium and zinc cosupplementation promote improved metabolic outcomes in rodent NAFLD models,^{89,90} suggesting that selenium and zinc deficiencies also contribute to hepatic pathology (see *Supplemental Material: Zinc*).

Despite repeated associations with MND and NAFLD, these studies are largely correlative in nature. As reported, MND and gut dysbiosis are strongly intertwined. Altered intestinal metabolism and/or gastrointestinal disruption drive malabsorption of fat-soluble vitamins.^{48,77,91} Malabsorption conditions are key undernutrition pathologies linked to NAFLD. Indeed, nondietary components—including poverty, poor sanitation, and pandemic-induced health disruptions—promote intestinal malabsorption and NAFLD-like features, further highlighting putative gut-liver interactions.^{13,18}

Malabsorption Conditions Underscores Fatty Liver

Poverty remains a key, underlying determinant of undernutrition and, alongside poor diet, an interdependent contributor to undernutrition-induced fatty liver disease.^{13,92,93} Poverty impacts access and availability of nutritionally adequate diets. Furthermore, impoverished communities often lack access to hygiene, sanitation, and

safe drinking water.^{14,94} Environmental enteric dysfunction (EED), a prevalent malabsorption condition, occurs in regions with poor sanitation.⁹⁵ Poor diet and frequent exposure to fecal-oral contaminants promote EED progression.^{95,96} EED pathology is characterized by gastrointestinal inflammation and barrier disruption due to blunting of the small intestinal villi. Beyond nutritional impairment, EED has been linked to systemic metabolic shifts and NAFLD-like features.^{25,38}

EED cohorts exhibit altered circulating metabolomic profiles, notably decreased carnitine and glycerophospholipid metabolites, metabolites involved in hepatic fatty acid oxidation and lipid transport.^{25,97,98} The hallmarks of EED, poor nutrient absorption, low-grade inflammation, and metabolic alterations, likely promote undernutrition-induced hepatic steatosis.^{24,95,96} Indeed, our laboratory observed that repeated fecal-oral contamination (from a bacterial cocktail comprised of Bacteroidales/*Escherichia coli* members) exacerbated small intestinal malabsorption and increased hepatic triglyceride levels in malnourished mice.^{24,38}

A recurrent theme of undernutrition-associated NAFLD is the significant role of gut dysbiosis. Kwashiorkor, MND, and malabsorption conditions remain inextricably linked to intestinal impairment. Fatty liver pathologies, in turn, exert a profound effect on gut function, influencing nutrient absorption, metabolism, and alteration of the gut microbiome.^{10,12} Growing evidence, largely from obesity-associated NAFLD, indicates that gut microbes contribute to fatty liver pathology.^{14,38} These pioneering studies used multiomic approaches, integrating gut microbiome (eg, metagenomic sequencing) and host (eg, liver metabolomics) data to interrogate NAFLD from a gut-liver perspective. Here, we (1) review the gut-liver axis, noting microbial-dependent pathways frequently reported in NAFLD studies, and (2) highlight emerging multiomic research exploring undernourished gut microbiota-liver interactions.

The Gut Microbiota-Liver Axis

The largest organ within the human body, the liver, orchestrates nutrient metabolism storage and transport.^{99,100} An essential hub for systemic homeostasis, the liver actively participates in bidirectional gut-liver signaling. The gut-liver axis describes collective interactions across the liver, gastrointestinal tract, and resident gut microbial communities.^{12,101} Trillions of microorganisms form the gut microbiota, with bacterial microbes alone outnumbering our cells (1.3:1) and comprising 150x more genes than the human genome.¹⁰² This dynamic and varied community exerts a significant role in host metabolic function.^{38,103}

Relatively recent studies have assessed both compositional (eg, bacterial profiling via 16S ribosomal RNA [rRNA] sequencing) and functional (eg, shotgun metagenomics) shifts in the NAFLD-associated microbiome.^{38,104,105} Early microbiome research characterized the overnutrition-induced NAFLD microbiome to (1) explore a causative role of gut microbes in fatty liver disease and (2) identify putative bacterial signatures of NAFLD.

Landmark cohousing and fecal microbiota transplant (FMT) studies from the Gordon laboratory demonstrated that gut microbes shape systemic metabolic processes in rodents.^{106–108} Bäckhed et al¹⁰⁷ first reported a pivotal role of the gut microbiota in carbohydrate absorption and subsequent hepatic gluconeogenesis, a process impaired in NAFLD.¹⁰⁹ Subsequent studies later demonstrated a causal role for gut bacteria on host adiposity profiles in germ-free models using FMT from twin pairs discordant for obesity. In addition to distinct gut microbiota signatures, mice that received an obesity-associated FMT gained increased weight compared with the “lean” FMT controls and displayed altered metabolic profiles, including reduced bacterial-derived short-chain fatty acids (SCFAs).¹⁰⁶ Independent work revealed that fecal bacteria specifically influence NAFLD-like outcomes because FMT from mice maintained on a high-fat diet promoted steatosis and impaired insulin responses in recipient mice.¹⁰⁸

Subsequent studies explored putative microbial features from clinical populations, including bacterial abundance. Small intestinal bacterial overgrowth (SIBO) is defined as increased bacterial colony-forming units within the small intestine ($\geq 10^5$ /mL luminal aspirate). SIBO is frequently accompanied by intestinal malabsorption and ensuing malnutrition and hepatic injury.¹¹⁰ A recent meta-analysis reported a significant association between NAFLD and SIBO: pooled odds ratio: 3.82; 95% confidence interval: 1.93–7.59; and 65% I^2 .¹¹¹ Beyond abundance, specific bacterial signatures have been reported within obese NAFLD cohorts.

Stool samples from NASH patients at the University Health Network in Toronto, Canada, exhibited decreased abundance of bacterial members from the Bacteroidetes phyla compared with a simple steatosis or healthy cohort.¹¹² Bacteroidetes prevalence, however, was not significantly decreased in a NAFLD cohort recruited at Peking’s University People’s Hospital.¹¹³ Within Bacteroidetes, the genus *Bacteroides* was also decreased in a liver cirrhosis cohort of Han Chinese background, whereas *Clostridium*, *Prevotella*, *Streptococcus*, and *Veillonella* were enriched, including oropharyngeal bacterial taxa, suggesting impaired compartmentalization of the gut microbiome by invasion of oral microbes.¹¹⁴ Bacteroidetes was also reduced in a pediatric Italian cohort,¹¹⁵ although Bacteroidetes member *Prevotella copri* was positively associated with liver fibrosis from pediatric participants in the NASH Clinical Research Network based in California.¹¹⁶ 16S rRNA sequencing of fecal samples from the Familial Cirrhosis cohort and Twins and Family cohort recruited at the University of San Diego, California revealed an increase in members belonging to the Enterobacteriaceae family within patients with NAFLD-cirrhosis compared with non-NAFLD controls; these controls exhibited enriched Peptostreptococcaceae and Rikenellaceae families. Researchers then developed a random forest classifier model that identified NAFLD-cirrhosis based on bacterial features (validation cohort: area under the receiver operating characteristic = 0.87).¹¹⁷ Finally, ongoing work has started to uncover potential alterations of nonbacterial components,

with a recent virome study associating NAFLD with a reduction of bacteriophage presence.¹¹⁸ How microbes of viral, fungal, and/or archaeal origin contribute to NAFLD remains largely unexplored.

Although there are broad correlations across studies, notably reduced frequency of Bacteroidetes and reduced alpha diversity,^{115–117} distinct bacterial signatures likely depend on cohort (eg, ethnicity), disease progression (eg, severity), and even study design (eg, sequencing platform). Although informative, sequencing alone cannot definitively demonstrate microbial causality in the clinical setting. For example, the obese and lean human microbiome display a high level of shared microbial organisms within familial twins, although unique signatures were identified at the gene level, rather than bacterial lineage.¹¹⁹ Moreover, genus-level signatures fail to distinguish potential strain-level differences and/or metabolic capacity (eg, bacterial fermentation) that shape host metabolism and adiposity.^{106,120} For a detailed review of NAFLD microbiome signatures and the opportunities and limitations of this approach, see Aron-Wisnewsky et al.¹⁰

Although we have noted several bacterial signatures linked to NAFLD, this review focuses on gut microbiota-liver interactions shaped by malnutrition. Indeed, emerging studies have shifted away from identity-based microbial signatures to assess the functional alterations linked to bacterial dysbiosis through multiomics-based study.^{105,114,116} This gut-liver approach has identified key microbial-dependent pathways linked to NAFLD progression, largely in the context of obesity and overnutrition.

Here, we review the NAFLD gut microbiota-liver axis (Figure 1). Next, we present multiomic studies of under-nutrition pathologies, highlighting parallel gut-liver disruptions that contribute to both overnutrition- and under-nutrition-induced NAFLD.

Microbes Shape Gut-Liver Pathways in NAFLD

Alteration of a critical microbe-host interface—the gastrointestinal epithelial barrier—has been reported across numerous NAFLD studies.^{12,121,122} The largest mammalian-microbial interface,¹²³ the gastrointestinal tract, promotes symbiotic interactions between the host immune system and intestinal microorganisms.¹²⁴

Within the large intestine, the gastrointestinal barrier is composed of (1) a mucosal layer, (2) intestinal epithelial cells, and (3) the lamina propria—the connective tissue encircling the gastrointestinal tract. Intestinal mucus serves as the first physical barrier to the host lumen environment. An organized glycoprotein matrix, intestinal mucus houses mucosa-associated bacteria while preventing mucosal-associated microorganisms from breaching the epithelial barrier.¹²⁵

Appropriate barrier function supports healthy digestive, immune, and metabolic function.^{12,125} Increased epithelial permeability, however, contributes to diet-driven NAFLD/NASH features.^{14,121,122} Clinical studies and fatty liver rodent models have reported a positive correlation between intestinal permeability and NAFLD severity.^{121,126} As

commensal microbes influence expression of epithelial tight junction proteins, shape intestinal inflammatory responses, and maintain gut mucosal lining,^{12,24,41} microbial dysbiosis undoubtedly contributes to gastrointestinal barrier disruption in hepatic disease.^{111,127} A key consequence of barrier disruption is bacterial translocation, the escape of microorganisms and/or microbial components through the gastrointestinal epithelium. Bacterial translocation promotes hepatic inflammatory and oxidative responses, exacerbating NAFLD pathology.^{116,128,129}

To experimentally address the impact of barrier integrity and bacterial translocation, researchers used dextran sulfate sodium (DSS) to induce epithelial damage in male C57BL/6 mice.¹³⁰ A combination of DSS treatment and a high-fat diet not only reduced expression of epithelial tight junction proteins but also triggered NAFLD-like features. In comparison with non-DSS treated controls, DSS elicited a striking increase of hepatic steatosis and up-regulation of proinflammatory cytokines, as well as hepatic leukocyte infiltration in mice fed a high-fat diet. Moreover, livers from DSS + high-fat diet models displayed a marked increase in expression of toll-like receptor (TLR) 4 and TLR9, which recognize bacteria/bacterial components, including lipopolysaccharide (LPS), a membrane component of gram-negative bacteria.^{130,131} Indeed, LPS entry was likely enhanced by intestinal barrier dysfunction produced by DSS + high-fat diet.

TLRs are expressed on most hepatic cells, including hepatocytes, Kupffer cells, and biliary epithelial cells. On LPS activation, TLRs promote production of proinflammatory cytokines.^{131,132} Indeed, the NAFLD liver exhibits increased expression of proinflammatory cytokines, activation of Kupffer cells (hepatic macrophages), as well as broader immune cell infiltration; for immunoinflammatory responses linked to bacterial translocation, see *Supplemental Material: Inflammation*.^{12,132,133}

As expected, liver disease increases the risk of bacterial infection.¹³⁴ Bacterial infections may occur in as many as 47% of patients hospitalized with liver cirrhosis.^{134,135} Unmitigated bacterial translocation can trigger a rare but potentially fatal inflammatory response, sepsis. A recent prospective study reported NAFLD as a key risk factor for sepsis mortality when comparing NAFLD ($n = 129$) and non-NAFLD ($n = 395$) patients (adjusted odds ratio: 2.918; 95% confidence interval: 1.693–5.03; $P < 0.001$). Significance remained when adjusted for age and sex, but not BMI ≥ 25 .¹³⁶ Murine models reported that hepatic steatosis exacerbates sepsis outcomes via impaired macrophage function and aberrant corticosterone release.^{136,137} Because bacteria and bacterial endotoxins are typically absent in the healthy liver,¹²² mitigating bacterial translocation provides a putative gut-liver therapeutic target.

In addition to bacterial translocation, oxidative stress contributes to NAFLD development and progression.^{1,138,139} Beyond oxidative stress linked to reduced micronutrient antioxidants,⁶⁵ hepatic steatosis impairs mitochondria, the primary source of intrinsic reactive oxygen species (ROS) production.¹⁴⁰ Disruption of pro-oxidant and antioxidant mechanisms, resulting from mitochondrial dysfunction and

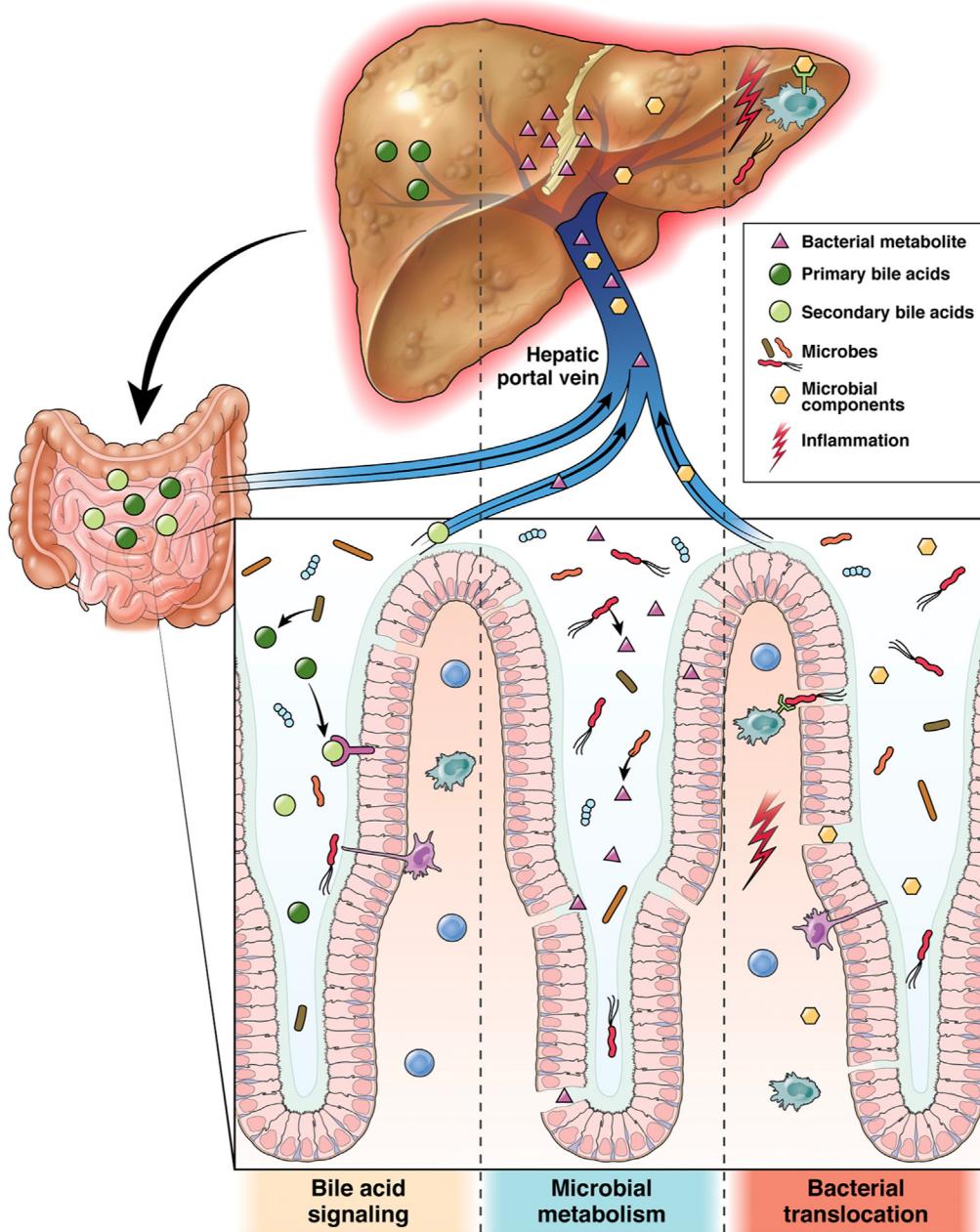


Figure 1. Key pathways of the gut-liver axis. The gut-liver axis describes bidirectional signaling between the gut/gut microbiota and the liver. Study of the obesity-associated NAFLD microbiome has revealed key gut-liver pathways contributing to NAFLD. NAFLD cohorts exhibit altered bile acid profiles. Synthesized within the liver, primary bile acids modulate nutrient processing. Within the intestinal tract, microbe-dependent reactions transform primary bile acids into secondary bile acids. Largely reabsorbed in the small intestine, bile acids are transported to the liver via the hepatic portal vein, a process known as enterohepatic circulation. Secondary bile acids can bind to receptors within the intestine and liver (eg, FXR, TGR5) shaping epithelial barrier function and hepatic metabolism. Gut microbes produce metabolites (eg, phenylacetic acid) linked to hepatic steatosis, whereas murine and clinical NAFLD cohorts display altered gastrointestinal barrier function. Reduced barrier integrity facilitates aberrant escape of enteric microorganisms and/or microbial components—bacterial translocation. LPS, a gram-negative endotoxin, triggers hepatic inflammatory responses through TLR activation.

other ROS production sources (eg, peroxisomes, xanthine oxidase, and cytochrome P450 2E1), contribute to disease progression in NAFLD.^{141–143} Excessive ROS leads to macromolecule oxidation of proteins, carbohydrates, lipids, and DNA within hepatocytes, stellate, endothelial, and Kupffer cells.¹⁴⁴ In addition to macromolecular oxidation,

excessive ROS promotes hepatic fibrosis, aberrant innate immune responses, and impaired protein kinase signaling, extensively reviewed by Mansouri et al.¹⁴⁵

Dietary shifts significantly shape commensal microbial communities,^{24,146,147} and the gut microbiota might be an important, yet poorly explored, factor in oxidative stress

Table 2. Undernutrition and Overnutrition-Induced NAFLD Pathophysiology and Treatment Guidelines

Undernutrition	Overnutrition
↓ Protein, fat, and/or ↑ carbohydrate diets	↑ Fat, carbohydrate diets
Altered micronutrients (MND, iron deficiency)	Altered micronutrients (↑ ferritin, MND)
Increased oxidative stress	Increased oxidative stress
Impaired hepatic metabolism (↓ PUFA, ↑ triglyceride)	Impaired hepatic metabolism (↓ PUFA, ↑ triglyceride)
Metabolic syndrome (increased risk)	Metabolic syndrome (increased risk)
Hepatic steatosis	Hepatic steatosis
Gut microbiota dysbiosis (↑ LPS, EED: increased pathogenic microbes)	Gut microbiota dysbiosis (↑ LPS, reduced Bacteroidetes)
Altered bile acid profile (kwashiorkor: ↑ secondary bile acids)	Altered bile acid profile (↓ secondary: primary bile acid ratio)
Treatment guidelines:	Treatment guidelines:
↑ Protein and/or caloric consumption, ↑ body weight, ↓ gut dysbiosis (Kwashiorkor: MDCF intervention)	↓ Carbohydrate and/or caloric consumption, ↓ body weight, ↓ gut dysbiosis (probiotic + dietary intervention)

Clinical and experimental model studies exploring gut-liver pathologies from a multiomic perspective suggest that both dietary excesses and deficits trigger similar pathologic features during NAFLD pathology. Here we provide several examples of altered gut-liver interactions in NAFLD.

PUFA, polyunsaturated fatty acid.

homeostasis. Indeed, specific *Lactobacillus* commensals were reported to trigger nonmitochondrial ROS production via NOX-family enzymes (ROS-generating NADPH oxidases) and promotion of iron/copper-dependent redox pathways within the intestinal epithelium.¹⁴⁸ Because malnourished diets, gut microbial dysbiosis, and bacterial translocation promote aberrant oxidation,^{38,149,150} it is likely that microbial-mediated oxidative stress and/or oxidative stress-induced gut microbial dysbiosis contributes to systemic NAFLD consequences. Microbes not only shape hepatic immune and oxidative responses but also directly contribute to systemic metabolic pathways.^{38,151}

Bacterial Metabolism and Bile Acids in NAFLD

Largely recognized for their role in lipid digestion, bile acids traverse the gut-liver axis via enterohepatic circulation. The liver synthesizes primary bile acids from cholesterol. Before excretion within the small intestine, bile acids are conjugated to amino acids (eg, glycine, taurine).^{12,152} These primary bile acids not only facilitate absorption of dietary fats and fat-soluble vitamins, but also exert antimicrobial properties curbing the expansion of small intestinal microbes.^{12,153} Intestinal gut microbes can transform primary bile acids into secondary bile acids (eg, via deconjugation reactions).^{154,155} These secondary bile acids exert broad metabolic effects via farnesoid X receptor (FXR) and Takeda G protein-coupled receptor (TGR5) signaling. FXR activation modulates hepatic bile synthesis, epithelial barrier function, and systemic gluconeogenesis, whereas TGR5 signaling shapes energy metabolism by triggering production of glucagon-like peptide-1, a hormone that regulates insulin secretion and reduces food intake.^{152,156,157} Both receptors have become potential targets for NAFLD and metabolic disease. Indeed, work in genetic murine models

supports bile-dependent modulation of fatty liver features. FXR-null mice develop severe hepatic steatosis along with insulin resistance and elevated hepatic cholesterol and triglyceride content, whereas bile acid-dependent FXR stimulation in wild-type mice reduces serum glucose levels.¹⁵⁸ Homozygous TGR5-deficient rodents also exhibit metabolic disruption on a high-fat diet, gaining significant adipose accumulation compared with wild-type controls.¹⁵⁹

Unsurprisingly, aberrant bile acid profiles have been reported across liver pathologies from metabolic syndrome to hepatocellular carcinoma.^{160,161} Researchers recently profiled circulating bile acids from an obese-associated NAFLD/NASH cohort and BMI-matched controls recruited at Virginia Commonwealth University.³⁵ The levels of total primary bile acids increased in a stepwise manner from healthy controls to NAFLD and NASH subjects. The NASH bile acid profile was further characterized by elevated primary bile acid conjugation (eg, increased glycocholate, taurocholate) with a reduction in the ratios of total secondary to primary bile acids.³⁵ Although the gut microbiota was not characterized, alteration of primary and secondary bile acid metabolism was indicative of both dietary and microbial influence.^{147,154}

Beyond bile acid modulation, microbial metabolism generates hepatomodulatory compounds from beneficial SCFAs to potentially pathogenic agents (eg, phenylacetic acid).^{12,105,162} SCFAs, end products from microbial fermentation of dietary fiber, exert broad biological activities, promoting fatty acid oxidation, resolving inflammatory responses, modifying host caloric intake, and maintaining the gastrointestinal epithelial barrier.^{162–164} Although the precise role of SCFAs in NAFLD remains uncertain, both clinical and rodent models of NAFLD report decreased SCFA abundance.¹²

In 2018 Hoyle et al¹⁰⁵ reported a link between obesity-associated NAFLD and phenylacetic acid, a bacterial product of amino acid metabolism. Researchers assessed the fecal

metagenome (shotgun sequencing), hepatic transcriptome (RNA microarray), and plasma/urine metabolomics (1H-NMR spectroscopy) from the FLORINASH study (2 independent cohorts of obese women). Metabolome-wide association analysis identified phenylacetic acid as a key microbial metabolite significantly associated with hepatic steatosis. Importantly, functional metagenomic analysis associated steatosis and insulin resistance with increased aromatic amino acid metabolism, a bacterial pathway contributing to phenylacetic acid production. Researchers then used murine models to validate a causal role of the NAFLD microbiome and microbial-produced phenylacetic acid. FMT of the NAFLD microbiome into germ-free mice rapidly triggered increased hepatic triglyceride levels, whereas oral phenylacetic acid treatment increased hepatic lipid accumulation and expression of lipid metabolism genes. Multivariate modeling to classify steatosis improved when multiomic features were integrated (area under the curve = 87.07%), whereas noninvasive clinical data yielded an area under the curve of 58.38%.

Ongoing gut-liver studies have revealed a profound impact of commensal microbes on hepatic function. Although sequencing of the fecal microbiome identified putative microbial signatures within NAFLD cohorts, emerging studies have interrogated gut-liver interactions from a multiomic approach, identifying microbial-dependent pathways contributing to NAFLD (eg, bile acid metabolism, phenylacetic acid). We now examine emerging research of the undernourished gut-liver axis. Intriguingly, multiomic techniques have revealed broad similarities in gut-liver pathways contributing to undernourished NAFLD in clinical and experimental settings (Table 2).

Assessing the Undernourished Gut Microbiota-Liver Axis

The Malnourished Gut Microbiome and Kwashiorkor

Groundbreaking work from the Gordon laboratory demonstrated that both diet and gut microbes contribute to kwashiorkor pathology.^{36,146} To examine pediatric kwashiorkor, the Gordon laboratory fed gnotobiotic mice an undernourished diet based on Malawian food staples. Mice then received an FMT from pediatric twin pairs discordant for kwashiorkor, and microbial and host metabolic signatures were assessed. Combination of the Malawian diet and the kwashiorkor FMT recapitulated growth faltering and metabolic disruption reported in pediatric kwashiorkor cases.³⁶ Notably, gnotobiotic mice that received an FMT from the healthy twin exhibited higher levels of urinary taurine, an amino acid required for bile acid conjugation. Lower taurine levels correlated with increased fecal *Bilophila wadsworthia*, gram-negative bacteria that facilitate bile acid metabolism and use taurine-conjugated bile acids as an energy source.^{36,120}

Independent, undernutrition models have reported similar bile acid trends.^{24,40} Newly weaned male Wistar rats placed on a protein-deficient diet (5% protein) also

developed hepatic steatosis, accompanied by a reduction of taurine-conjugated bile acids in plasma.⁴⁰ Our MAL model (diet: 7% protein, 5% fat) displayed robust loss of intestinal taurine-conjugated bile acids compared with healthy controls (diet: 20% protein, 15% fat). These shifts were accompanied by an expansion of bacterial members belonging to the Bacteroidetes and Proteobacteria phyla within the small intestine,²⁴ microbes linked to bile acid processing.¹⁶⁵

Altered bile acid profiles have also been reported in pediatric cohorts. Total bile acids were elevated within plasma samples of a Malawian SAM cohort (61.4% kwashiorkor), compared with healthy children.¹⁶⁶ SAM patients displayed a modest increase of glycine-conjugated bile acids within their serum, although there were no significant differences in taurine-conjugated bile acids between healthy and SAM participants. Researchers also assessed undernourished fecal bile acid profiles and reported decreased secondary bile acids after hospitalization and treatment, indicative of altered gut bacterial transformation. Moreover, levels of fecal calprotectin, a marker of gut inflammation, were reduced in SAM cohorts after hospitalization. Although the precise role of bile acid shifts was not determined, researchers speculated that microbiota-dependent shifts and bile-dependent inflammatory pathways likely contribute to SAM-induced NAFLD features.¹⁶⁶

In summary, undernutrition rodent models display a reduction in tauro-conjugated bile acids,^{24,40} whereas the Malawian SAM cohort exhibited higher glycine-conjugated bile acids.¹⁶⁶ Intriguingly, obesity-associated NAFLD has been linked to elevated taurocholate (taurine-conjugated) and glycocholate (glycine-conjugated) plasma levels.³⁵ Although these distinctions may reflect murine- or model-specific alterations, these findings suggest the possibility of biological markers specific to undernourished fatty liver. These studies highlight the impact of microbes on systemic metabolic pathologies, indicating the putative benefit of therapies targeting gut-liver dysbiosis.

Diet significantly alters the composition and functional capacity of the gut microbiome.¹⁴⁷ Indeed, researchers observed shifts in the fecal microbiome after 2 weeks of RUTF feeding in undernourished mice. More intriguingly, researchers reported that gut microbes are not only shaped by RUTF intervention but also influence RUTF outcomes because the kwashiorkor microbiome was linked to persistent weight deficits compared with undernourished mice that received a healthy donor FMT.³⁶ Gehrig et al²⁶ later reported specific dietary interventions improving early-life undernutrition in an independent Bangladeshi SAM cohort. Researchers performed metabolomic and proteomic plasma profiling, as well as fecal metagenomic analyses, identifying correlations between biomarkers of improved health and bacteria composition throughout dietary intervention. Next, researchers used gnotobiotic models to identify microbiota-directed complementary food (MDCF), dietary interventions that improved fecal microbiota shifts (ie, toward a microbiota associated with growth). MDCF treatment improved immune and metabolic features in comparison with standard dietary intervention

(ie, khichuri-halwa, a rice/lentil-based meal) in both undernourished models and in a randomized, double-blind pediatric intervention study.^{26,167} Notably, mass spectrometry of murine hepatic samples revealed increased markers of insulin-like growth factor-1 signaling after MDCF,²⁶ a growth hormone reduced in clinical NAFLD.¹⁶⁸ These findings provide a multiomic template to assess putative microbiota-directed therapies targeting undernourished fatty liver.

After birth, the gut microbiota rapidly develops and remains at a high level of plasticity before stabilizing between the ages of 3 and 5 years.¹⁶⁹ SAM emerges around this early-life timeframe,^{170,171} warranting further study of the early-life microbiome and pediatric NAFLD (see also **Supplemental Material**: Marasmus). Undernutrition pathologies, however, can occur throughout life, including the hidden hunger of MND.

MND and the Gut Microbiome

Poor diet and/or impaired bile acid processes—features of NAFLD—drive micronutrient malabsorption of essential vitamins and minerals.^{57,172,173} Fat-soluble vitamins, in particular, require appropriate bile acid metabolism. Bile salts facilitate the absorption of dietary fats through the intestinal lining.⁹¹ Deficiency of fat-soluble vitamins, in turn,

disrupts hepatic function, likely influencing bile acid processing and subsequent gut dysbiosis.^{48,91,174}

How, and to what extent, do micronutrients modulate the composition and functionality of the gut microbiome also remains largely unstudied. The Gordon laboratory reported that MND deficiencies shift the fecal microbiota of gnotobiotic mice colonized with a consortium of human-derived bacteria and placed on various micronutrient depletion diets. Microbiota shifts (Bray-Curtis dissimilarity) were not significant after 2 weeks on repletion diets (sufficient MND content), indicating that the MND microbiome is receptive to dietary intervention.¹⁷⁵ Because many micronutrients exhibit antimicrobial properties, MND likely facilitates aberrant bacterial blooms within the malnourished gut.^{172,176,177} Iron deficiency has been linked to decreased alpha diversity and increased gram-negative Enterobacteriaceae abundance in rodent models,¹⁷² whereas copper-deficient/high-fructose diets reduced *Akkermansia*,¹⁷⁸ a bacterial genus involved in glucose metabolism and inflammatory responses.^{179,180} Moreover, copper deficiency was associated with increased circulating LPS, an inflammatory feature observed in obesity-associated NAFLD.^{10,116,178}

Elevated bacterial endotoxins may also reflect disruption of the intestinal barrier. MND promotes epithelial

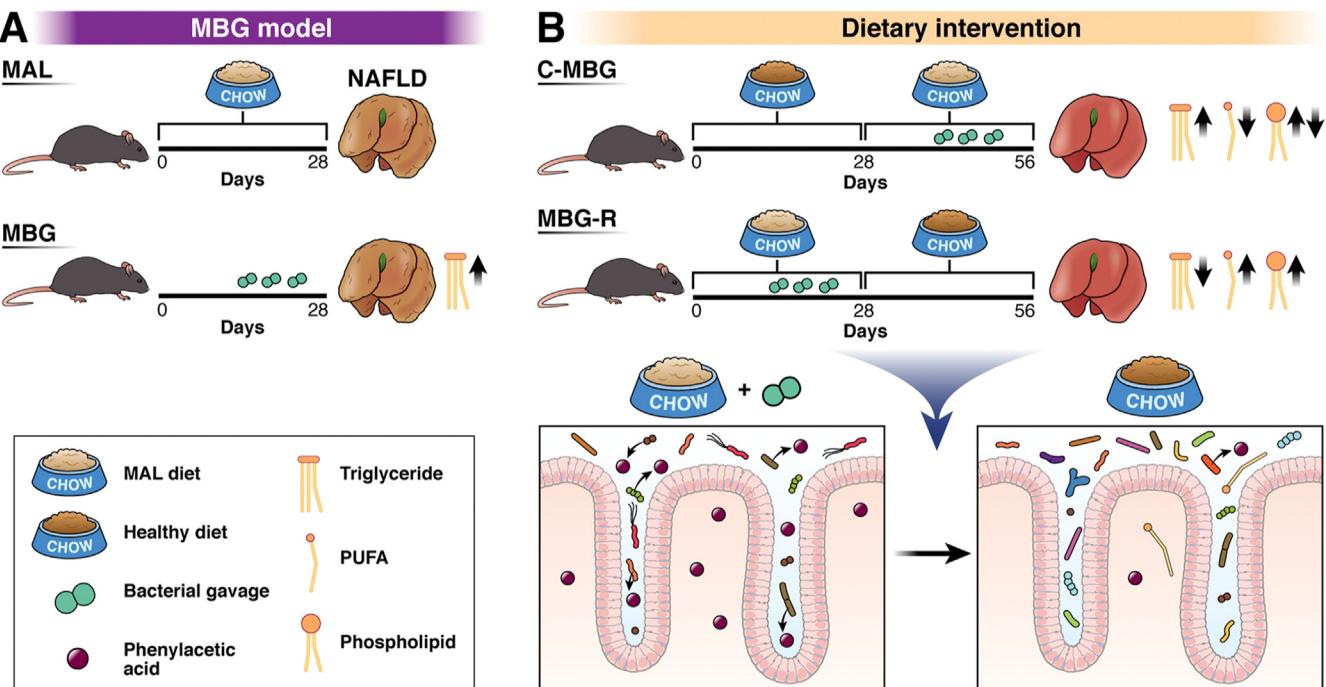


Figure 2. Dietary intervention improves gut microbiota alterations and undernutrition-induced NAFLD features. Protein/fat deficiency triggers growth faltering and NAFLD-like pathology in young mice. To model chronic fecal-oral contamination, a condition associated with undernutrition and poverty, a subset of malnourished mice received repeated exposure to a bacterial cocktail (MBG model). Compared with undernutrition-alone, MBG mice exhibit increased hepatic triglyceride levels (see Brown et al²⁴ and Bauer et al³⁸); healthy controls are not displayed. Malnutrition altered the liver metabolome, specifically lipid profiles and metabolism of phenylacetic acid, a bacterial metabolite linked to obesity-associated NAFLD.¹⁰⁵ Dietary intervention largely improved growth deficits, fatty liver features, and compositional microbiota shifts. This MBG reversal (MBG-R) model also displayed reductions in predictive phenylacetic acid pathways within the gut microbiome, compared with the nonintervention controls (not pictured). Intriguingly, hepatic steatosis was not observed in adult-onset malnutrition (control diet to MBG model, C-MBG mice).

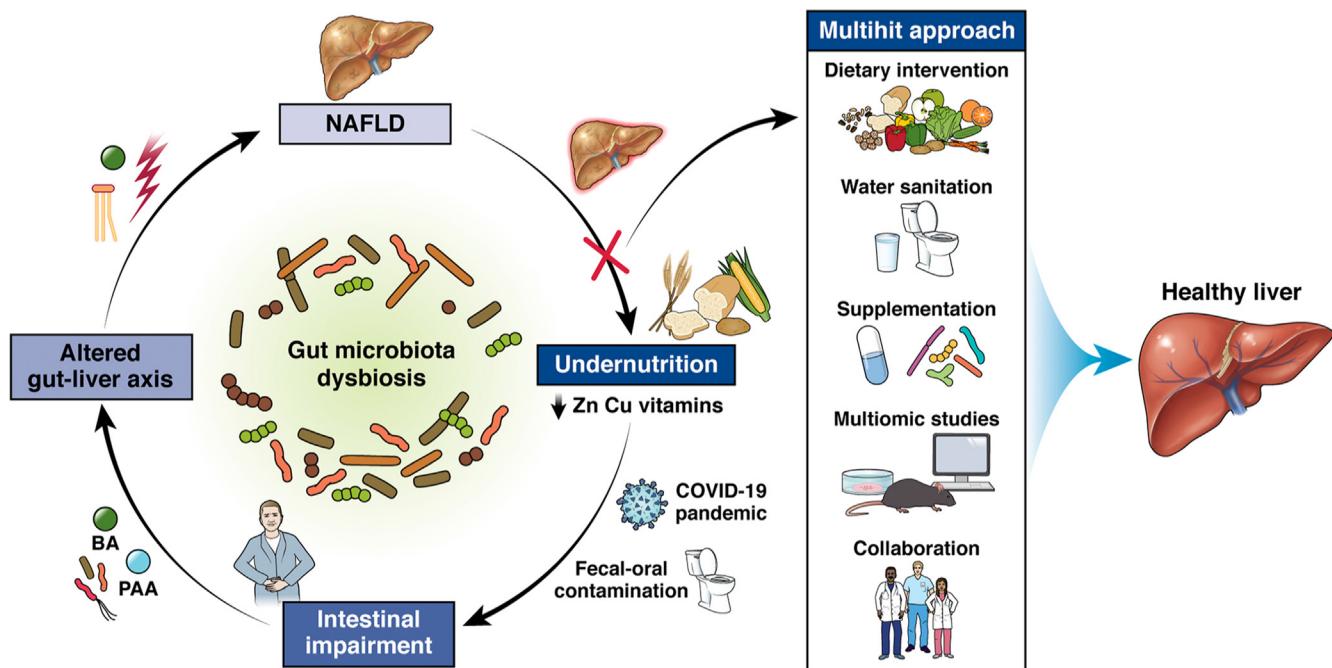


Figure 3. The vicious cycle of undernutrition-induced NAFLD. A vicious cycle of gut-liver impairment promotes malnutrition-induced NAFLD. External factors, including poor diet and gastrointestinal insult, alter gut metabolism and barrier function, contributing to gut microbiota dysbiosis. The dysbiotic gut, in turn, contributes to undernutrition and hepatic pathologies by promoting malabsorption and altering the gut-liver axis through microbial-dependent pathways. Breaking this vicious cycle will require a multihit approach that involves dietary intervention and gut microbiota-targeted therapies (eg, MDCF). Multiomic studies integrating microbial and metabolomic datasets may provide valued insight into the specific mechanisms and signaling pathways of the NAFLD gut microbiota-liver axis. Despite progress in combatting global poverty, undernourished pathologies are expected to increase as a consequence of the ongoing COVID-19 pandemic. We anticipate that recognition of the gut microbiota-liver axis will provide a valued framework to identify therapeutic targets of undernutrition-induced NAFLD. Figure concept based on Guerrant et al.¹⁴ BA, bile acids; PAA, phenylacetic acid.

permeability via inflammatory processes and disruption of tight junction proteins, facilitating subsequent bacterial translocation.^{181,182} Furthermore, because many micronutrients have antioxidative properties—including vitamin E, copper, and selenium—it is likely that MND impacts multiple gut microbiome-liver signaling pathways, involving inflammatory, oxidative, and metabolic processes.^{172,173} Whether multiomic exploration of MND and undernutrition-induced NAFLD will reveal analogous gut microbiota-liver biomarkers remains unknown.

Beyond macronutrient deficits and MND, external microbial insults also exacerbate undernutrition pathology.^{14,38}

Gut Microbes Promote Intestinal Malabsorption and NAFLD-Like Features

Recurrent exposure to fecal-oral contamination promotes intestinal inflammation and disrupts barrier integrity—key hallmarks of EED, a subclinical undernutrition pathology reported in regions with poor access to sanitation.^{14,24,95} EED has been associated with stunting, impaired neurocognitive development, and altered metabolic profiles,^{14,25,183} prompting study of fecal-oral contamination and the undernourished gut microbiota.

To assess chronic fecal-oral contamination, our laboratory developed a model combining malnutrition and

repeated bacterial gavage (MBG mice; Figure 2).^{24,38} Following weaning, C57BL/6 mice were placed on a protein/fat-deficient diet for 4 weeks (MAL model). An MBG subset received repeated bacterial *E. coli*/Bacteroidales cocktails, as noted earlier.²⁴ These fecal commensals were selected as *E. coli* and Bacteroidales exposures promoted growth stunting within an independent kwashiorkor model.^{24,146} MBG mice displayed small intestinal barrier disruption characteristic of malabsorption conditions. Epithelial permeability was further validated via intestinal *Salmonella typhimurium* infection. Compared with healthy controls, MBG livers exhibited elevated proinflammatory cytokines and *S typhimurium* burden, indicative of increased bacterial translocation and immune impairment.²⁴

Although undernutrition alone was sufficient to promote hepatic steatosis and fecal microbiome shifts, the combination of poor diet and fecal-oral contamination exacerbated hepatic triglyceride levels, indicating a link between bacterial-induced gut barrier disruption and NAFLD progression.^{24,38} Fatty liver pathology in MAL and MBG mice was accompanied by marked alteration of liver metabolomic pathways, notably PUFA and glycerophospholipid metabolism, features reported across undernutrition and obesity-associated NAFLD.^{40,97,98,105,184,185}

To explore the impact of nutritional intervention, we conducted dietary reversals (malnourished to healthy diet or healthy to malnourished diet). These experiments used a

multiomic approach, integrating microbiome (16S rRNA sequencing) and metabolomic (untargeted Fourier transform mass spectrometry) analyses. Prolonged dietary intervention largely reversed NALFD-like features and altered gut microbiota profiles, notably relative abundance of Coriobacteriaceae and Streptococcaceae members. In contrast, adult-onset undernutrition promoted an undernutrition-like liver metabolome, but not hepatic steatosis, indicating distinct NAFLD pathophysiology occurring in early-life and adult-onset undernutrition in mice.^{38,101}

To further investigate gut microbiota-liver metabolism in the MBG model, we used weighted gene co-expression network analysis,^{186,187} an approach to identify clusters (modules) of highly correlated metabolites. We subsequently correlated these modules to hepatic steatosis and triglyceride content. A metabolic module largely composed of glycerophospholipid metabolites was significantly correlated to fatty liver features, but a bile acid module (predominantly taurocholic metabolites) was not significantly correlated to either NAFLD-like features or dietary intervention, suggesting that reported shifts in tauro-conjugated bile acids may reflect a consequence of undernutrition, rather than be a driver of hepatic steatosis within the MBG model.^{24,38}

Intriguingly, decreased phenylacetic acid metabolism was associated with reduction of hepatic steatosis in the liver metabolome and predicted metagenome analyses.³⁸ As described earlier, Hoyles et al¹⁰⁵ first reported and demonstrated a causal role for bacterial-derived phenylacetic acid in obese-associated NAFLD. We anticipate that further multiomic NAFLD studies will identify shared metabolic and microbial-dependent therapeutic targets in overnutrition- and undernutrition-induced pathologies, including EED.

Conclusion: NAFLD and a Gut-Liver Perspective

A “multihit” disorder, dietary, metabolic, and microbial alterations contribute to the complex and interconnected pathophysiology of malnutrition-induced NAFLD.^{9,38} Uniquely linked to metabolic syndrome, NAFLD has been largely studied in the context of obesity, insulin resistance, and aging.^{2,7,12} Despite a strong association with overnutrition, hepatic steatosis may occur in the presence of dietary deficiencies.^{33,171} Although global undernutrition rates have decreased in the 21st century,¹⁹ malnutrition, particularly among children and vulnerable populations, is expected to increase in the wake of the COVID-19 pandemic.^{18,20} Increased malnutrition prevalence will likely contribute to the ongoing increase of pediatric NAFLD, leading to lasting metabolic and hepatic disruption among the affected populations.¹

In 2013, Guerrant et al¹⁴ outlined the “vicious cycle” of poverty, highlighting the combined influence of gastrointestinal dysbiosis, poor diet, and infectious disease in the promotion and persistence of poverty-associated pathologies. Perhaps a similar model should be applied to

malnutrition-induced NAFLD (Figure 3). NAFLD is a multifactorial disease shaped by the dysbiotic gastrointestinal environment.^{10,12} In this model, hepatic steatosis subsequently affects susceptibility to microbial insults and ensuing metabolic disruption.^{1,9,38}

The recognition of gut microbiota-liver interactions will require an expanded study of NAFLD etiology, progression, and treatment. Metagenomic analyses and murine models have revealed putative diagnostic markers of NAFLD,^{116,117} whereas multiomic approaches—integrating microbiome and hepatic datasets—have identified bacterial-dependent mechanisms linked to malnutrition-induced NAFLD.^{38,105} Whether obese-associated NAFLD and undernutrition-induced NAFLD present with unique (or consistent) biological markers remains largely unstudied.

Irrespective of the causes contributing to NAFLD, nutritional modification remains the key therapeutic strategy and first-line course for intervention, although compliance and/or ability to comply is poor.^{9,101,188} Modest weight loss of $\geq 7\%$ significantly improves histological features of obesity-associated NAFLD, including steatosis, lobular inflammation, and hepatocellular ballooning.¹⁸⁹ Although sustained weight loss significantly improves NAFLD features, these clinical guidelines fail to address prevalent undernutrition pathologies promoting NAFLD-like features and gut-liver dysbiosis.⁹ Microbiota-focused intervention (eg, MDCF) in combination with established therapeutic food intervention (eg, RUTF supplementation) reflects an innovative trend for the treatment of undernutrition-induced pathologies (see **Supplemental Material: Intervention**).

In summary, multihit disorders require an expanded “multihit” approach, including collaboration from microbiome and hepatic research teams, as well as translational studies involving medical and public health professionals. We anticipate that emerging multiomic studies will not only contribute to a better understanding of the gut-microbiota-liver axis but also identify novel dietary and microbial targets to treat or reverse malnutrition-induced NAFLD.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at <https://doi.org/10.1053/j.gastro.2022.01.058>.

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Conflicts of interest

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Supplementary Material

Zinc

Zinc contributes to numerous enzymatic and metabolic activities, including insulin storage and secretion, and mitigation of oxidative damage.^{190,191} Dietary zinc is absorbed through the intestine and transported to the liver via the portal vein.¹⁹² Appropriate levels of dietary zinc are required for micronutrient homeostasis because excessive zinc intake impairs intestinal copper absorption.⁷⁸ Zinc is necessary for insulin regulation and secretion in pancreatic beta cells,¹⁹³ and deletion of zinc transporters impairs insulin secretion and hepatic glucose metabolism.^{194,195} Unsurprisingly, zinc deficiency has been linked to insulin resistance and type 2 diabetes.¹⁹⁰ Zinc deficits have also been reported in adult and pediatric liver disease cases, from NAFLD to cirrhosis.^{56,57,196}

Independent clinical and rodent studies highlighted a role for zinc deficiency and supplementation in NAFLD. A randomized double blind clinical trial assessed weight loss diet and zinc supplementation (30 mg) in an obese NAFLD cohort: n = 29, n = 27 placebo. Compared to the placebo arm, zinc supplementation reduced fasting blood sugar and improved oxidative stress markers, highlighting the systemic metabolic impacts of micronutrients.¹⁹⁷ Sprague-Dawley rats fed a high-fat diet for 20 weeks exhibited NAFLD-like features, including hepatic steatosis and elevated fasting blood sugar compared with control counterparts. Cosupplementation with zinc and selenium during the final 8 weeks of the study significantly reduced these features.⁸⁹

Importantly, zinc deficiencies are also prevalent in pediatric undernourished cohorts,^{13,92,198} with zinc supplementation linked to modest benefits in linear growth and decreased diarrheal episodes in regions of poor sanitation.^{13,92} Whether zinc supplementation exerts beneficial effects on undernourished-induced NAFLD remains unknown.

Inflammation

Bacterial translocation and subsequent cytokine-induced inflammation contribute to NAFLD progression from simple steatosis to fibrotic scarring and cirrhosis.¹² Although the full extent of bacterial-dependent inflammation within NAFLD remains uncertain, researchers reported higher levels of circulating LPS and hepatic TLR4 expression in patients with NAFLD presenting with SIBO compared with NAFLD participants without SIBO.¹⁹⁹ Duodenal aspirates revealed gram-negative *E coli* as the predominant member of the SIBO bacterial community. A prospective, cross-sectional study identified microbial inflammatory biomarkers within a pediatric, obese cohort.¹¹⁶ Metagenomic analyses identified elevated Kyoto Encyclopedia of Genes and Genomes orthologs—fecal microbial genes involved in LPS biosynthesis and flagellar assembly—which were significantly correlated with pediatric NASH.¹¹⁶ An independent liver cirrhosis study cultured bacteria from

circulating blood, notably the hepatic portal vein, which links the gastrointestinal tract and liver.¹²⁸ Subsequent 16S rRNA sequencing showed viable bacteria (*Staphylococcus*) from the blood of patients with liver cirrhosis. Bacterial presence was associated with circulating proinflammatory cytokines, indicating that microbial organisms themselves may drive inflammatory responses during fatty liver progression.¹²⁸

Marasmus

Pham et al¹⁷⁰ recently assessed microbiome features in kwashiorkor and marasmus pediatric cohorts from Niger and Senegal. Microbiome analyses from cultured fecal samples revealed a bloom in Proteobacteria within colonies from patients with kwashiorkor. Fecal bacterial profiling (16S rRNA), however, failed to distinguish the kwashiorkor and marasmus microbiome. Whether edematous features of kwashiorkor are partially driven by specific microbes remains unclear.

Intervention

Multiomic approaches recognize that both gut and liver dysbiosis must be addressed to break the vicious circle of NAFLD pathology. We recently showed that dietary intervention (replenishing protein/fat deficits) largely mitigates hepatic steatosis and altered liver metabolome profiles in the MBG model.³⁸ Unfortunately, short-term dietary interventions are largely insufficient to fully restore health in undernourished pediatric populations.⁵³ Critical challenges, including long-term availability to appropriate dietary options and significant reduction of chronic microbial dysbiosis (eg, exposure to infectious pathogens from poor sanitation),^{13,14,183} likely reduce the probability that broad dietary guidelines will sufficiently improve early-life fatty liver features in undernourished populations.

Emerging work has also explored putative bacteria-targeted approaches. Supplementation with VSL#3, a cocktail of 8 probiotic species belonging to *Streptococcus*, *Bifidobacterium*, or *Lactobacillus* genera, improved NAFLD features in a parallel-arm, double-blind, randomized controlled trial for an obese, pediatric cohort.²⁰⁰ Gehrig et al²⁶ reported a multiomic approach to identify and assess the efficacy of microbiota-targeted treatment of undernutrition—MDCFs. An earlier study of the SAM microbiome revealed that pediatric undernutrition was associated with persistent microbiota immaturity (ie, gut microbiome composition resembling those from younger, healthy children within the same community).²⁰¹ These findings provided cohort-specific microbial signatures to classify undernutrition and healthy states.^{167,201} Subsequent multiomic research used microbial and metabolic markers to test the efficacy of various dietary interventions. Recurrent characterization of the plasma proteome and metabolome, as well as the fecal microbiome, identified MDCFs correlated to improved growth, metabolic profiles, and microbiome maturity. Experimental studies in undernourished murine models supported MDCF metabolic benefits.²⁶

Given the broad functionality of the human microbiome, microbial-targeted therapies will likely shape varied gut-liver interactions with complementary or even conflicting outcomes. We note that a microbe associated with positive metabolic functions in one context can elicit negative metabolic or immune outcomes within a different setting.¹⁸⁰ Although much work remains, multiomic study provides a context-dependent approach to assess the influence of gut microbes within specific undernourished communities.

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