Relationship of gut microbiota and immunological response in obesity-related non-alcoholic fatty liver disease in children

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In the development of NAFLD plays an important role the intestinal microflora. Our aim was to characterize role microbiota in children. Distinctive gut microbiota composition was observed in children, characterized and short-chain fatty acid producing bacteria. For the treatment of NAFLD it is possible by therapeutic manipulations with prebiotics and probiotics to modulate the gut microbiota and maintain the integrity of the intestinal barrier are potential agents.

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is now recognized as one of the most common disorders in the proper functioning of the liver, both in the pediatric and adult population. It is estimated that it affects up to 10% of the entire pediatric population, including 26–41% children with obesity (Anderson *et al.,* 2015; Schwimmer *et al.,* 2006). In the adult population, this percentage ranges from 25 to 40% (Estes *et al.,* 2018). Younossi and others (Younossi *et al.,* 2016) in a systematic review estimated that the global prevalence of NAFLD reached 25%, which resulted in a significant increase in the interest of scientists, who are trying to find out the causes leading to its development. The global increase in NAFLD prevalence among children constitutes a poor prognosis for the future (Obesity Collaborators The GBD 2015). NAFLD by itself is defined as a metabolic dysfunction-associated liver disease caused by excessive accumulation of fat in hepatocytes, where it is possible to rule out secondary factors leading to accumulation of fat in liver cells, such as alcohol consumption, hereditary disorders, and drug intake (Chalasani *et al.,* 2012). Within this disease, we can distinguish a number of liver conditions that vary in severity degree, from steatosis to non-alcoholic steatohepatitis (NASH) with or without fibrosis, to cirrhosis (Buzzetti *et al.,* 2015). In the long term, NASH might progress to hepatocellular carcinoma (HCC), a condition that increases liver-related morbidity and mortality. NAFLD is also related to metabolic syndrome (MS), including insulin resistance, dyslipidemia and visceral obesity (Boyraz *et al.,* 2013). The pathological mechanism leading to the onset and development of NAFLD is not fully known. According to the multiple-hit hypothesis we do know, that there are many factors leading to NAFLD. Among them, we can set apart genetic and epigenetic factors, lipotoxicity, insulin resistance, dietary determinants proinflammatory factors, and gut microbiome, which might be crucial due to the production of large amounts of molecular mediators that affect liver cells (Clemente *et al.,* 2012).

GUT MICROBIOME AND IMMUNOLOGICAL RESPONSE

The gut microbiota affects host physiology at least in part, through the release of metabolites and complex bacterial structural components such as lipopolysaccharides (LPS), peptidoglycans and flagellins. Metabolites derived from the gut microbiota include short-chain fatty acids (SCFAs) and branched-chain fatty acids (BCFAs), aromatic acid metabolites, vitamins, amino acids and other metabolites. Recent studies have shown that gut microbiome (GM) has a significant impact on the development of NAFLD. The gut microbiota includes microorganisms belonging to different kingdoms, such as bacteria, viruses, archaea and fungi. As a whole, they form a complex ecosystem that lives in symbiosis with its host. Human GM is dominated by four bacteria phyla: Actinobacteria, Bacteroidetes, Firmicutes and Proteobacteria, where Firmicutes and Bacteroidetes are most common (Tremaroli *et al.,* 2012). Bacteria are involved in many metabolic processes in the gut. They participate in energy harvest, regulation level of bile-acid, fermentation of polysaccharides, and lastly choline metabolism (Dumas *et al.,* 2006). Intestinal bacteria can also produce substantial amounts of endogenous ethanol, which is transported via the portal vein to the liver, where it might trigger inflammation. Disturbances in the composition of the intestinal microbiota are associated with chronic metabolic syndromes such as obesity, NAFLD and type 2 diabetes mellitus (T2DM) (Turnbaugh *et al.,* 2009; Loomba *et al.,* 2013; Donaldson *et al.,* 2016). Diet is one of the main factors that modulate the composition of gut microbiota – it has an influence on species richness. When the number of species decreases, then it comes to a condition known as dysbiosis, which has been associated with the pathogenesis of inflammatory diseases (Jennison *et al.,* 2021). Gut microbiota is responsible for maintaining of the intestinal epithelial barrier, thus preventing the entry of bacteria and their metabolites into the blood (Fig. 1). It is also known that bacterial metabolites play
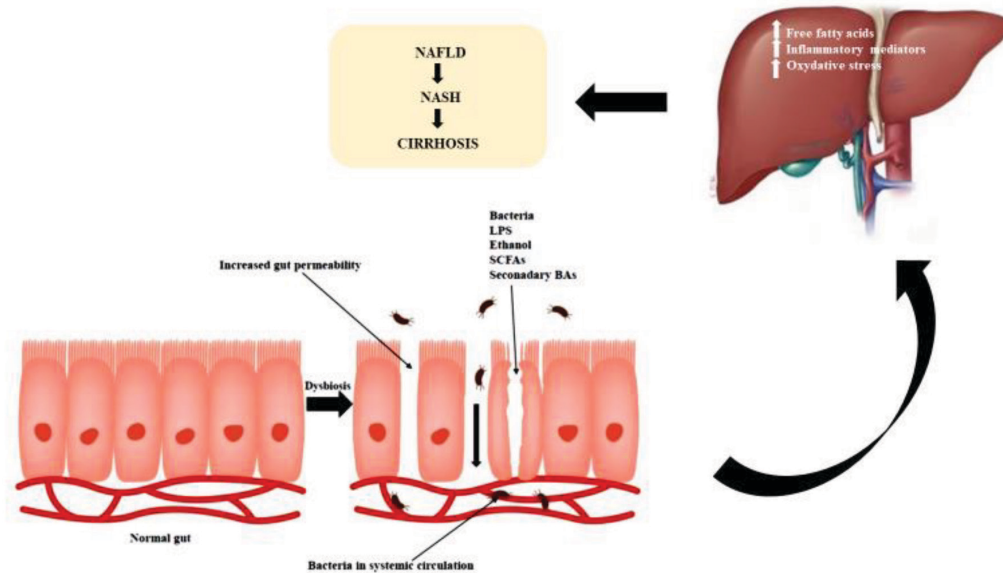
Figure 1. Gut microbiota imbalance contributes to the development of NAFLD, may lead to the nutrition imbalance and change the gut microbiota composition and its metabolites, such as SCFAs, and bile acids.
Microbiota disorders promote the intestinal endocrine L cells to secrete GLP-1 to act on the vagus nerve to activate the gut–brain liver nerve pathway and regulate insulin sensitivity, glucose production, and fatty acid oxidation.

an important role in regulating immune system activity and metabolic functions of the human body, for example, they produce intestinal hormones such as glucagon-like peptide-1 (SvegliatiBaroni *et al.,* 2011). Symbiotic organisms are also responsible for the digestion of polysaccharides, the production of vitamins and the absorption of nutrients. Bäckhed and others (Bäckhed *et al.,* 2004) in his research found out that gut microbiota is involved in adipose tissue growth, due to modulation of energy absorption and stimulation of increased triglycerides synthesis in the liver. Bacteria overgrowth may cause intestinal permeability to increase the amount of endotoxins in the systematic circulation and that will allow for the development of endotoxemia with inflammation in the liver. Some other commensal metabolites may also influence the metabolism of the host and that impact can lead to the development and progression of NAFLD (Aron-Wisnewsky *et al.,* 2020).The human intestine contains at minimum 100 trillion microbes, which belong to at least 1000 different species with 150 times more genes than the human genome has (MetaHIT Consortium *et al.,* 2010; Chassaing *et al.,* 2014). The most common phyla in the human gut are Firmicutes and Bacteroidetes, which are followed by frequently identified Actinobacteria, Proteobacteria, Verrucomicrobia and Fusobacteria (Kamada *et al.,* 2014). There are many factors that influence the composition of GM such as gender, age, diet, hormonal changes, drug intake, travel and pregnancy (Rodríguez *et al.,* 2015; Uchiyama *et al.,* 2019). It is important to keep in mind internal factors that affect the composition of the intestinal microbiota, such as the state of the intestinal mucosa, which affects the permeability of the intestinal barrier and the state of the host immune system (Adams *et al.,* 2008). These factors can lead to unfavorable conditions for some bacteria, leading to lower microbial diversity – dysbiosis. Maintaining homeostasis of GM is crucial for keeping the human body (especially the liver) in good condition. It is linked with beneficial metabolites produced by commensal bacteria. Dysbiosis may occur due to an imbalance in the gut, which will manifest itself through excessive proliferation and overproduction of

harmful substances that affect the liver, due to the fact that the portal vein transports about 70% of the total amount of blood to the liver. This is the main reason why it is exposed to endotoxins or peptidoglycans first – they are transported with blood to the liver, where they will be neutralized. The continuous influx of microbial-derived products may cause disturbances in liver functioning and that might end up with inflammation in liver cells. That generates a response from receptors located on certain types of liver cells such as Kupffer cells, sinusoidal cells, biliary epithelial cells and hepatocytes (Chen *et al.,* 2019). Gut microbiota produce many metabolites and part of them have a negative impact on the liver causing inflammation. Among them, we can highlight bile acids, short-chain fatty acids (SCFAs), choline and ethanol (Chiang *et al.,* 2020). All of them are involved in the development of NAFLD. In this study, not all microbiome metabolites were included due to the character and purpose of this study.

SHORT-CHAIN FATTY ACIDS (SCFAs) ON NAFLD

Another important group of bacterial metabolites are short-chain fatty acids (SCFAs). SCFAs are mainly produced as a result of dietary fiber fermentation in the colon. Then they might be absorbed in the intestine (Liu *et al.,* 2021; Rau *et al.,* 2018). The most common SCFAs are acetate, propionate and butyrate, which are absorbed *via* the portal vein during lipid digestion. SCFAs are binding to G-protein coupled receptor (GPCR), which occurs in many organs, such as the liver, skeletal muscles, adipose tissue, and enteroendocrine L cells, exerting influence on their metabolic and immunological functions (Li *et al.,* 2022). The short-chain fatty acids, mostly acetate and propionate can stimulate gluconeogenesis and lipogenesis, leading to fat accumulation in liver cells (Bastos *et al.,* 2022). Butyrate is responsible for the suppression of insulin resistance and obesity while high fatty diet (HFD) intake in mice model (Gao *et al.,* 2009). Butyrate also has anti-inflammatory potential – it can reduce inflammation in the intestines and reduce inflammatory response due

to activation of regulatory T cells, which will inhibit T cells and Th17 cells (Arpaia *et al.*, 2013). Moreover, it reduces levels of proinflammatory cytokines, such as tumor necrosis factor-alpha (TNFα) or monocyte chemotactic protein-1, while increasing levels of prostaglandin E2, which has an anti-inflammatory effect (Smith *et al.*, 2013; Usami *et al.*, 2008; Mouzaki *et al.*, 2019). Butyrate is the most potent anti-inflammatory mediator and can reduce local inflammation in the gut and prevent the progression of inflammatory responses into the systemic circulation. Butyrate can also promote tight junction function and intestinal integrity. Another beneficial effect of butyrate is to provide colonocytes with an energy source to maintain intestinal health. Dysbiosis leads to a decrease in butyrate levels which may cause increased gut permeability, and translocation of bacteria or lipopolysaccharides (LPS) into the systemic circulation, which can lead to the development of NALFD (butyrate promotes the regeneration of enterocytes). T-cells are responsible for releasing Glucagon-like peptides, that stimulate fatty acids β-oxidation in hepatocytes. They also increase insulin sensitivity in liver cells, thus they are important to NAFLD development (SvegliatiBaroni *et al.*, 2011]. The gut microbiota regulates bile acid homeostasis through biotransformations such as deconjugation, dehydroxylation, oxidation and desulfation. In terms of deconjugation, Bacteroides, Lactobacillus, Bifidobacterium, Clostridium and Listeria produce bile salt hydrolases (BSH) that deconjugate the taurine and glycine groups in the primary bile acids produced in the liver (Ticho *et al.*, 2019). In experimental animals with dysbiosis or treated with antibiotics, taurine deconjugation by intestinal bacteria was blocked, shifting the balance toward almost exclusively taurine-conjugated bile acids, resulting in increased taurine-conjugated bile acids in the liver, heart and kidneys (Arab *et al.*, 2017). For dehydroxylation, Clostridium and Eubacterium of the Firmicutes tribe produce 7α-dehydroxylase of bile acid, which converts primary bile acids (cholic acid and chenodeoxycholic acid) into secondary bile acids (deoxycholic acid, lithocholic acid and ursodeoxycholic acid) (Lund *et al.*, 2018). 7α- dehydroxylation occurs after deconjugation and is the most physiologically relevant conversion of bile acids in humans. Regarding oxidation, bacteria from genera such as Bacteroides, Clostridium, Eubacterium, Escherichia, Eggerthella, Peptostreptococcus and Ruminococcus produce hydroxysteroid bile acid dehydrogenases (HSDH), which convert toxic bile acids into ursodeoxycholic acid, which is less toxic to human cells and more water-soluble (Ni *et al.*, 2022). Finally, several gut bacteria, such as Clostridium sp. Strain S2, produce sulfatases that are able to enhance the desulfation of bile acids (Jackson *et al.*, 2022). Desulfation of bile acids by intestinal bacteria facilitates the reabsorption of bile acids (Song *et al.*, 2019) and is essential for the homeostasis of the bile acid pool (Moszak *et al.*, 2021). In the liver are synthesized bile acids by the rate-limiting enzyme cytochrome P450 7A1 (CYP7A1) and secreted into the intestine. Intestinal epithelial cells (IECs) are reabsorbed bile acids by the distal ileum via apical sodium-dependent bile acid transporter (ASBT) where they activate farnesoid X receptor (FXR), inducing the expression and secretion of fibroblast growth factor 15/19 (FGF15/19) to inhibit hepatic bile acid synthesis. Luminal and basolateral bile acids activate Takega G protein-coupled receptor 5 (TGR5) in enteroendocrine L-cells, resulting in the release of the incretin glucagon-like peptide-1 (GLP-1), promoting glucose tolerance (Chiang *et al.*, 2020; Zhou *et al.*, 2014). Overproduction of bile acids can promote di-

arrhea by several distinct mechanisms. In the colon, bile acids disrupt barrier integrity allowing bile acids to reach the basolateral membrane of epithelial cells and induce chloride secretion, though the receptors underlying this phenomenon are unknown. Colonic FXR and TGR5 activation inhibits the secretion of chloride and other electrolytes, which may be a compensatory mechanism. Colonic bile acids activate TGR5 on enterochromaffin cells (ECs), promoting 5-hydroxytryptamine (5-HT) release and motility. Bile acids activate neuronal TGR5, which either stimulates or inhibits motility, depending on the type of neuron and region of the GI tract. Overproduction of bile acids induces colonic secretion and enhances motility, producing a diarrheal phenotype (Wang *et al.*, 2021; Krishnan *et al.*, 2018).

MICROBIAL SYNTHESIS OF ETHANOL

Some bacteria have the ability to produce ethanol in the intestine as a result of polysaccharide fermentation. Normally, the amount of produced ethanol is small, but if dysbiosis occurs its amount may be significantly bigger than that in healthy controls (de Medeiros *et al.*, 2015). The health effect of a high concentration of ethanol is an increase in the permeability of the intestinal barrier, which may result in the development of NAFLD and liver steatosis (Chen *et al.*, 2020). Recent studies have shown that increased ethanol levels in the human body are associated with a high abundance of genera Escherichia, Bacteroides, Bifidobacterium, Clostridium and *Klebsiella pneumoniae* (Engstler *et al.*, 2016; Mir *et al.*, 2016). Zhu *et al.*, in their study have observed that obese NASH patients have higher levels of endogenous ethanol than non-obese NASH patients or healthy controls. In turn, Yuan and others (Yuan J *et al.*, 2019) proved that the presence of Klebsiella pneumoniae K14 was common for patients with NAFLD – after transfer, isolates of bacteria cause NAFLD-like changes in mice. Also in children with NAFLD who were on the same diet as healthy children noticed that ethanol levels were significantly higher. Gut microbiota have enzymes which are involved in the metabolism of ethanol, e.g. aldehyde dehydrogenase. That enzyme converts ethanol into acetaldehyde and acetate. Acetaldehyde takes part in the translocation of microbiota metabolites due to the weakening tight junctions of the intestinal barrier (Schwimme *et al.*, 2020).

OBESITY AND NON-ALCOHOLIC FATTY LIVER DISEASE

Obesity is associated with impaired intestinal barrier function and dysbiosis, resulting in metabolites and other bacterial products such as LPS and other molecular patterns associated with the microbiota, gaining access to the lamina propria in higher concentrations. Diet-induced increases in blood LPS concentrations are known as metabolic endotoxemia. Changes in the intestinal microbiota suggest that changes in diet and intestinal barrier function underlie elevated blood LPS concentrations. Alteration of intestinal TJ proteins, mainly zonula occludens-1 (ZO-1) and occludin, is a major molecular mechanism contributing to increased intestinal permeability (Lin *et al.*, 2022). High-fat diets can lead to intestinal inflammation which in turn can result in TJ changes and increased intestinal permeability. Glucagon-like peptide 2 (GLP2), has been identified as a regulator of TJ protein expression and localization in obese mice. Although the expression pattern of metabolite receptors on

specific tissue-resident leukocytes, such as those in the lamina propria of the gastrointestinal tract, is still poorly understood, manipulation of immune cell metabolism by the gut microbiota may be a powerful approach. Gut-derived metabolites may be a powerful approach to direct the immune response. As is well known, obesity is a risk factor for the development of NAFLD, as well as metabolic syndrome (Rau *et al.,,* 2018). The prevalence of overweight and obesity has increased in children and adolescents worldwide over the past 30 years. Gut bacteria produce metabolites by modifying host metabolites, including secondary bile acids. In addition to the classic role of metabolites in bioenergetics and biosynthesis, metabolites can function as signaling molecules, much like hormones and neurotransmitters. Signaling metabolites generated from nutrients or by the intestinal microbiota primarily target the enteroendocrine, neuronal and immune cells in the lamina propria of the intestinal mucosa and liver, and through these tissues to the rest of the body. Metabolites from intermediary metabolism act as metabolic stressors in adipose tissue, liver and pancreas, being effective regulators of pro- and anti-inflammatory regulators of key immune cells, which function as important drivers of low-level inflammation associated with obesity. A large number of metabolite receptors are expressed on immune cells of both the innate and adaptive immune systems. and many different types of immune cells are found in the intestinal mucosa. In lean individuals, the immune cells of the lamina propria are dominated by anti-inflammatory subtypes of innate immune cells, including tolerogenic macrophages and dendritic cells, eosinophils, IL-22-producing innate lymphoid cells and resident regulatory T cells (Tregs) (Miura *et al.,* 2014). Lean but not obese fat is enriched with a unique population of regulatory T cells that influence metabolic parameters. Diet and a sedentary lifestyle are determinants of obesity in children, but obesity in infants is influenced by the gut microbiota profile. As indicated by recently published studies Bacteroides and Lacto Bacillus spp. are the most common microbes involved in overweight in infants. However, to date, there are no certain species or genera identified as causing childhood obesity *per se* (Clemente *et al.,* 2016). Early gut microbiota in infants can shape weight gain in childhood and controlling factors affecting early infant gut microbiota may prevent later overweight and obesity in children. Exposure to antibiotics changes the diversity of the gut microbiota. Infant exposure to antibiotics is strongly associated with childhood obesity. In children under <6 months of age, repeated antibiotic exposure puts boys at higher risk than girls of developing obesity.

CONCLUSION

In conclusion, bacterial metabolites mediate the communication between the commensal microbiota and the immune system, affecting the balance between pro- and anti-inflammatory mechanisms. Gut dysbiosis has been associated with NAFLD because the effects of an altered gut microbiota in abundance and diversity are mediated by many bacterial metabolites including bile acids, butyrate, choline, amino acids and ethanol. Modulation of the intestinal microbiota and supplementation of some bacterial metabolites may have a therapeutic benefit. The heterogeneous clinical features of NAFLD can be explained by consumption of various dietary factors, interpersonal gut microbiome variability and genetics. In summary, the interplay of dietary factors, gut microbiota

and intestinal barrier integrity plays an important role in the development of obesity and obesity-related NAFLD. Alteration of the gut microbiota is a result of diet, which can promote metabolic endotoxemia and inflammation contributing to the development of obesity and NAFLD. Understanding the role of the gut microbiome, and the mechanism of dietary factor influence gut microbiota, with the identification of components of the microbiome's metabolic activity which significantly affect host metabolism and possibly contribute to obesity and obesity-related NAFLD, might also be an important consideration for NAFLD treatment. Altogether, opportunities and challenges provided by microbiome research lead to future studies, which will hopefully elucidate the more specific role of gut microbiome in NAFLD and establish microbiota-targeted treatment approaches.

Highlights/Perceived strengths
• Intestinal microbiota plays an important role in the development of NAFLD.
• Impaired intestinal barrier integrity, metabolic endotoxemia and inflammation are closely associated with obesity and NAFLD.
• The gut microbiota and intestinal barrier function, promoting metabolic endotoxemia and inflammation can alter the absorption and metabolism of energy-reach foods and their ingredients (e.g., fat and fructose)
• Therapeutic manipulations with prebiotics and probiotics to modulate gut microbiota and maintain integrity of the intestinal barrier are potential efficient agents that should be further tested in NAFLD

Declarations

Author Contributions. AWR, PC and PS drafted the manuscript. AWR and PS contributed to writing the manuscript and critical revision. All authors have read and approved the final manuscript.

Conflicts of Interest. All other authors report no conflict of interest.

REFERENCES:

Adams DH, Eksteen B, Curbishley SM (2008) Immunology of the gut and liver: a love/hate relationship. Gut 57: 838–848. https://doi.org/10.1136/gut.2007.122168

Anderson EL, Howe LD, Jones HE, Higgins JPT, Lawlor DA, Fraser A (2015) The Prevalence of non-alcoholic fatty liver disease in children and adolescents: a systematic review and meta-analysis. *Plos One* 10: e0140908. https://doi.org/10.1371/journal.pone.0140908

Arab JP Karpen SJ, Dawson PA, Arrese M, Trauner M (2017) Bile acids and nonalcoholic fatty liver disease: molecular insights and therapeutic perspectives. *Hepatology* 65: 350–362. https://doi.org/10.1002/hep.28709

Arpaia N, Campbell1 C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, Rudensky AY (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504: 451–455. https://doi.org/10.1038/nature12726

Backhed F, Ding H, Wang T, Hooper LV, Koh GJ, Nagy A, Semenkovich CF, Gordon JI (2004) The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A* 101: 15718–15723. https://doi.org/10.1073/pnas.0407076101

Bastos RMC, Rangel EB (2022) Gut microbiota-derived metabolites are novel targets for improving insulin resistance. *World J Diabetes* 13: 65–69. https://doi.org/10.4239/wjd.v13.i1.65

Boyraz M, Hatipoğlu N, Sarı E, Akçay A, Taşkın N, Ulucan K, Akçay T (2014) Non-alcoholic fatty liver disease in obese children and the relationship between metabolic syndrome criteria. *Obes Res Clin Pract* 8: e356–e363. https://doi.org/10.1016/j.orcp.2013.08.003

Buzzetti E, Pinzani M, Tsochatzis EA (2016) The multiple-hit pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Metabolism* 65: 1038–1048. https://doi.org/10.1016/j.metabol.2015.12.012

Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ (2012) The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of

Gastroenterology, and the American Gastroenterological Association. *Hepatology* **55**: 2005–2023. https://doi.org/10.1002/hep.25762

Chassaing B, Étienne-Mesmin L, Gewirtz AT (2014) Microbiota-liver axis in hepatic disease. *Hepatology* **59**: 328–339. https://doi.org/10.1002/hep.26494

Chen J, Thomsen M, Vitetta L (2019) Interaction of gut microbiota with dysregulation of bile acids in the pathogenesis of nonalcoholic fatty liver disease and potential therapeutic implications of probiotics. *J Cell Biochem* **120**: 2713–2720. https://doi.org/10.1002/jcb.27635

Chen X, Zhang Z, Li H, Zhao J, Wei X, Lin W, Zhao X, Jiang A, Yuan J (2020) Endogenous ethanol produced by intestinal bacteria induces mitochondrial dysfunction in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* **35**: 2009–2019. https://doi.org/10.1111/jgh.15027

Chiang JYL, Ferrell JM (2020) Bile acid receptors FXR and TGR5 signaling in fatty liver diseases and therapy. *Am J Physiol-Gastrointest Liver Physiol* **318**: G554–G573. https://doi.org/10.1152/ajpgi.00223.2019

Chiang JYL, Pathak P, Liu H, Donepudi A, Ferrell J, Boehme S (2017) Intestinal farnesoid X receptor and Takeda G protein couple receptor 5 signaling in metabolic regulation. *Dig Dis* **35**: 241–245. https://doi.org/0.1159/000450981

Clemente JC, Ursell LK, Parfrey LW, Knight R (2012) The impact of the gut microbiota on human health: an integrative view. *Cell* **148**: 1258–1270. https://doi.org/10.1016/j.cell.2012.01.035

Clemente MG, Mandato C, Poeta M, Vajro P (2016) Pediatric non-alcoholic fatty liver disease: Recent solutions, unresolved issues, and future research directions. *World J Gastroenterol* **22**: 8078–8093. https://doi.org/10.3748/wjg.v22.i36.8078

de Medeiros IC, de Lima JG (2015) Is nonalcoholic fatty liver disease an endogenous alcoholic fatty liver disease? – A mechanistic hypothesis. *Med Hypotheses* **85**: 148–152. https://doi.org/10.1016/j.mehy.2015.04.021

Donaldson GP, Lee SM, Mazmanian SK (2016) Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol* **14**: 20–32. https://doi.org/10.1038/nrmicro3552

Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, Fearnside J, Tatoud R, Blanc V, Lindon JC, Mitchell SC, Holmes E, McCarthy MI, Scott J, Gauguier D, Nicholson JK (2006) Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. *Proc Natl Acad Sci U S A* **103**: 12511–12516. https://doi.org/10.1073/pnas.0601056103

Engstler AJ, Aumiller T, Degen C, Dürr M, Weiss E, Maier IB, Schattenberg JM, Jin CJ, Sellmann C, Bergheim I (2016) Insulin resistance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. *Gut* **65**: 1564–1571. https://doi.org/10.1136/gutjnl-2014-308379

Estes C, Razavi H, Loomba R, Younossi Z, Sanyal AJ (2018) Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. *Hepatology* **67**: 123–133. https://doi.org/10.1002/hep.29466

Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J (2009) Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**: 1509–1517. https://doi.org/10.2337/db08-1637

Jackson KG, Way GW, Zhou H (2022) Bile acids and sphingolipids in non-alcoholic fatty liver disease. *Chin Med J* **135**: 1163–1171. https://doi.org/10.1097/CM9.0000000000002156

Jennison E, Byrne CD (2021) The role of the gut microbiome and diet in the pathogenesis of nonalcoholic fatty liver disease. *Clin Mol Hepatol* **27**: 22–43. https://doi.org/10.3350/cmh.2020.0129

Kamada N, Núñez G (2014) Regulation of the immune system by the resident intestinal bacteria. *Gastroenterology* **146**: 1477–1488. https://doi.org/10.1053/j.gastro.2014.01.060

Krishnan S, Ding Y, Saedi N, Choi M, Sridharan GV, Sherr DH, Yarmush ML, Alaniz RC, Jayaraman A, Lee K (2018) Gut microbiota-derived tryptophan metabolites modulate inflammatory response in hepatocytes and macrophages. *Cell Rep* **23**: 1099–1111. https://doi.org/10.1016/j.celrep.2018.03.109

Li D, Li Y, Yang S, Lu J, Jin X, Wu M (2022) Diet-gut microbiota-epigenetics in metabolic diseases: From mechanisms to therapeutics. *Biomed Pharmacother* **153**: 113290. https://doi.org/10.1016/j.biopha.2022.113290

Lin YC, Lin HF, Wu CC, Chen CL, Ni YH (2022) Pathogenic effects of Desulfovibrio in the gut on fatty liver in diet-induced obese mice and children with obesity. *J Gastroenterol* **57**: 913–925. https://doi.org/10.1007/s00535-022-01909-0

Liu P, Wang Y, Yang G, Zhang Q, Meng L, Xin Y, Jiang X (2021) The role of short-chain fatty acids in intestinal barrier function, inflammation, oxidative stress, and colonic carcinogenesis. *Pharmacol Res* **165**: 105420. https://doi.org/10.1016/j.phrs.2021.105420

Loomba R, Sanyal AJ (2013) The global NAFLD epidemic. *Nat Rev Gastroenterol Hepatol* **10**: 686–690. https://doi.org/10.1038/nrgastro.2013.171

Lund ML, Egerod KL, Engelstoft MS, Dmytriyeva O, Theodorsson E, Patel BA, Schwartz TW (2018) Enterochromaffin 5-HT cells – A major target for GLP-1 and gut microbial metabolites. *Mol Metab* **11**: 70–83. https://doi.org/10.1016/j.molmet.2018.03.004

MetaHIT Consortium (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**: 59–65. https://doi.org/10.1038/nature 08821

Mir H, Meena AS, Chaudhry KK, Shukla PK, Gangwar R, Manda B, Padala MK, Shen L, Turner JR, Dietrich P, Dragatsis I, Rao R (2016) Occludin deficiency promotes ethanol-induced disruption of colonic epithelial junctions, gut barrier dysfunction and liver damage in mice. *Biochim Biophys Acta BBA – Gen Subj* **1860**: 765–774. https://doi.org/10.1016/j.bbagen.2015.12.013

Miura K, Ohnishi H (2014) Role of gut microbiota and Toll-like receptors in nonalcoholic fatty liver disease. *World J Gastroenterol* **20**: 7381–7391. https://doi.org/10. 3748/wjg. v20.i23.7381

Moszak M, Szulińska M, Walczak-Gałęzewska M, Bogdański P (2021) Nutritional approach targeting gut microbiota in NAFLD – to date. *Int J Environ Res Public Health* **18**: 1616. https://doi.org/10.3390/ijerph18041616

Mouzaki M, Loomba R (2019) Insights into the evolving role of the gut microbiome in nonalcoholic fatty liver disease: rationale and prospects for therapeutic intervention. *Ther Adv Gastroenterol* **12**: 175628481985847. https://doi.org/10.1177/175628481985 8470

Ni Y, Lu M, Xu, Y Wang Q, Gu X, Li Y, Zhuang T, Xia C, Zhang T, Gou X, Zhou M (2022) The role of gut microbiota – bile acids axis in the progression of non-alcoholic fatty liver disease. *Front Microbiol* **13**: 908011. https://doi.org/10.3389/fmicb. 2022.908011

Rau M, Rehman A, Dittrich M, Groen AK, Hermanns HM, Seyfried F, Beyersdorf N, Dandekar T, Rosenstiel P, Geier A (2018) Fecal SCFAs and SCFA-producing bacteria in gut microbiome of human NAFLD as a putative link to systemic T-cell activation and advanced disease. *United Eur Gastroenterol J* **6**: 1496–1507. https://doi.org/10.1177/2050640618804444

Rinninella E, Raoul P, Cintoni M, Franceschi F, Miggiano GAD, Gasbarrini A, Mele MC (2019) What is the healthy gut microbiota composition? A changing ecosystem across age, environment, diet, and diseases. *Microorganisms* **7**: 14. https://doi.org/10.3390/microorganisms7010014

Rodríguez JM Murphy K, Stanton C, Ross RP, Kober OI, Juge N, Avershina E, Rudi K, Narbad A, Jenmalm MC, Marchesi JR, Collado MC (2015) The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis* **26**. https://doi.org/10.3402/mehd.v26.26050

Schwimmer JB, Johnson JS, Angeles JE, Behling C, Belt PH, Borecki I, Bross C, Durelle J, Goyal NP, Hamilton G, Holtz ML, Lavine JE, Mitreva M, Newton KP, Pan A, Simpson PM, Sirlin CB, Sodergren E, Tyagi R, Yates KP, Weinstock GM, Salzman NH (2019) Microbiome signatures associated with steatohepatitis and moderate to severe fibrosis in children with nonalcoholic fatty liver disease. *Gastroenterology* **157**: 1109–1122. https://doi.org/10.1053/j.gastro.2019.06.028

Schwimmer JB, Deutsch R, Kahen T, Lavine JE, Stanley C, Behling C (2006) Prevalence of fatty liver in children and adolescents. *Pediatrics* **118**: 1388–1393. https://doi.org/10.1542/peds.2006-1212

Smith PM, Howitt MR, Panikov N, Michaud M, Gallini CA, Bohloolyy M, Glickman JN, Garrett WS (2013) The microbial metabolites, short-chain fatty acids, regulate colonic T reg cell homeostasis. *Science* **341**: 569–573. https://doi.org/10.1126/science.1241165

Song Z, Cai Y, Lao X, Wang X, Lin X, Cui Y, Kalavagunta PK, Liao J, Jin L, Shang J, Li J (2019) Taxonomic profiling and populational patterns of bacterial bile salt hydrolase (BSH) genes based on worldwide human gut microbiome. *Microbiome* **7**: 9. https://doi.org/10.1186/s40168-019 -0628-3

Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, Faraci G, Pacetti D, Vivarelli M, Nicolini D, Garelli P, Casini A, Manco M, Mingrone G, Risaliti A, Frega GN, Benedetti A, Gastaldelli A (2011) Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis: Effect of exenatide on the liver. *Liver Int* **31**: 1285–1297. https://doi.org/10.1111/j.1478-3231.2011.0246

The GBD 2015 Obesity Collaborators (2017) Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* **377**: 13–27. https://doi.org/10.1056/NEJMoa1614362

Ticho AL, P Malhotra, PK. Dudeja, RK. Gill, WA (2019) Alrefai bile acid receptors and gastrointestinal functions. *Liver Res* **3**: 31–39. https://doi.org/10.1016/j.livres.2019.01.001

Tokuhara D (2021) Role of the gut microbiota in regulating non-alcoholic fatty liver disease in children and adolescents. *Front Nutr* **8**: 700058. https://doi.org/10.3389/fnut.2021.700058

Tremaroli V, Bäckhed F (2012) Functional interactions between the gut microbiota and host metabolism. *Nature* **489**: 242–249. https://doi.org/10.1038/nature11552

Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI (2009) A core gut

microbiome in obese and lean twins. *Nature* **457**: 480–484. https://doi.org/10.1038/nature07540

Uchiyama K, Naito Y, Takagi T (2019) Intestinal microbiome as a novel therapeutic target for local and systemic inflammation. *Pharmacol Ther* **199**: 164–172. https://doi.org/10.1016/j.pharmthera.2019.03.006

Usami M, Kishimoto K, Ohata A, Miyoshi M, Aoyama M, Fueda Y, Kotani J (2008) Butyrate and trichostatin A attenuate nuclear factor ϰB activation and tumor necrosis factor α secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells. *Nutr Res* **28**: 321–328. https://doi.org/10.1016/j.nutres.2008.02.012

Wang R, Tang R, Li B, Ma X, Schnabl B, Tilg H (2021) Gut microbiome, liver immunology, and liver diseases. *Cell Mol Immunol* **18**: 4–17. https://doi.org/10.1038/s41423-02-00592-6

Wisnewsky JA, Vigliotti C, Witjes J, Le P, Holleboom AG , Verheij J, Nieuwdorp M, Clément K (2020) Gut microbiota and human NAFLD: disentangling microbial signatures from metabolic disorders. *Nat Rev Gastroenterol Hepatol* **17**: 279–297. https://doi.org/10.1038/s41575-020-0269-9

Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M (2016) Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology* **64**: 73–84. https://doi.org/10.1002/hep.28431

Yuan J, Chen C, Cui J, Lu J, Yan C, Wei X, Zhao X, Li N, Li S, Xue G, Cheng W, Li B, Li H, Lin W, Tian C, Zhao J, Han J, An D, Zhang Q, Wei H, Zheng M, Ma X, Li W, Chen X, Zhang Z, Zeng H, Ying S, Wu J, Yang R, Liu D (2019) Fatty liver disease caused by high alcohol-producing *Klebsiella pneumoniae*. *Cell Metab* **30**: 675–688. https://doi.org/10.1016/j.cmet.2019.08.018

Zhou X, Cao L, Jiang C, Xie Y, Cheng X, Krausz KW, Oi Y, Sun L, Shah YM, Gonzalez FJ, Wang G, Hao H (2014) PPARα-UGT axis activation represses intestinal FXR-FGF15 feedback signalling and exacerbates experimental colitis. *Nat Commun* **5**: 4573. https://doi.org/10.1038/ncomms5573

Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR (2013) Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: A connection between endogenous alcohol and NASH. *Hepatology* **57**: 601–609. https://doi.org/10.1002/hep.26093