Concise review

Microbiota and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH)

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ABSTRACT

Genetic predisposition, the intestinal microbiota (IM) and environmental factors, such as sedentary lifestyle and inadequate diet, should be considered as critical factors for the development of nonalcoholic fatty liver disease (NAFLD). Recently, some studies have demonstrated an association between dysbiosis and NAFLD; however, the exact mechanisms that lead to intestinal membrane damage, bacterial translocation and inflammation are not well elucidated. Due to the relevance of this theme, the IM and its metabolites have received special attention in recent years in an attempt to better understand the mechanisms related to the prevention, physiopathology, and treatment of NAFLD. In this paper, we provide a review of the human IM and its role in diet, obesity, and the development/progression of NAFLD/NASH, as well as the use of prebiotics and probiotics in the modulation of IM.

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1. Human intestinal microbiota

The human intestinal epithelium has an absorptive surface comparable to 300–400 m^2, with approximately 1014 resident bacterial species. Bacterial colonization occurs before birth and continues for life. Most of the bacteria that colonize the intestine are of the anaerobic type and comprise approximately 36,000 different species [1,2], constituting a variety of species of microorganisms that, quantitatively and qualitatively, differ between individuals [3].

The main functions of the intestinal microbiota (IM) include metabolic and immunological activities. The prominent metabolic function of the IM is the fermentation of nondigestible components of the diet. The products of this complex metabolic activity are a spectrum of organic acids, which are an important source of energy for the host. The epithelial cells of the gut participate in immunological processes. The signaling pathways of these cells are highly regulated to avert uncontrolled inflammatory responses [4]. The gut epithelium is a major barrier between the host and the environment. This barrier is formed by tight junctions that act as a highly regulated entry that open and close depending on

signals, such as those from cytokines and bacterial components from the lumen, and excessive contact with bacteria may cause an increase in the proinflammatory immune response [5]. Therefore, the IM supports immune regulation and ensures that effector immune responses are activated in response to invading pathogens [6]. Another important function of the IM is protection against exogenous microorganisms. These bacteria can inhibit the growth of pathogenic microorganisms through the synthesis of antimicrobial substances or by competing for nutrients and reducing the attachment and penetration of the enteroinvasive pathogen into epithelial cells [7].

The IM has a heterogeneous distribution, with the colon being the place with the greatest density due to the favorable conditions for the proliferation of microorganisms, characterized by slow peristalsis, absence of intestinal secretions and presence of a nutritional supply [8]. Dysbiosis generates bacterial overgrowth, toxin production and increased intestinal permeability, resulting in changes in immunological and hormonal effects. Thus, lifestyle habits such as diet, stress and the use of antibiotics, for example, cause the transient microbiota to prevail over the resident microbiota, predisposing the host to gastrointestinal disorders. The actions that the IM exerts on the various systems of the organism also reflect the maintenance of the metabolism [9].

Approximately 98% of all bacterial species that make up the IM belong to the following phyla: *Firmicutes* (64%) and *Bacteroidetes* (23%), which represent most of the IM in addition to *Proteobacteria* (8%) and *Actinobacteria* (3%) [10]. The Firmicutes phylum contains

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more than 250 genera, including *Lactobacillus* [11]. The phylum *Bacteroidetes*, similar to Firmicutes, is found in the small and large intestines, especially in the colon, and encompasses several genera, such as *Bacteroides*, *Flavobacteria* and *Sphingobacteriales*, with *Bacteroides thetaiotaomicron* being one of the most abundant [12].

Although the functions of the bacteria belonging to the Firmicutes phylum have not yet been fully elucidated, evidence suggests that some members of this phylum, such as *Clostridium butyricum* bacteria, are among the bacteria producing butyric acid from lactic acid or lactose in a chemical reaction known as butyric fermentation, and this type of fermentation increases the energy extracted from the diet [13]. On the other hand, members of the Bacteroidetes phylum participate in the metabolism of carbohydrates and perform this metabolism by expressing enzymes similar to the glycosyltransferases, which promote glycosylation, that is, to join carbohydrates to the proteins formed in the ribosomes. Members of the *Bacteroidetes* phylum also express glycoside hydrolases that degrade biomass, such as cellulose and hemicellulose, in antibacterial defense strategies. Finally, they also express the polysaccharide-lyase enzyme that catalyzes the breakdown of a carbon–oxygen bond in polysaccharides, leading to an unsaturated product and the elimination of alcohol [14].

2. Intestinal microbiota and obesity

Intestinal microorganisms influence energy production, energy homeostasis and, potentially, the development of obesity and metabolic disorders [11]. Increasing evidence supports the importance of the composition of the IM for the development of obesity and demonstrates that the type and quantity of the bacterial population can determine some important characteristics of metabolic pathways that play an important role in obesity [15,16].

Microbial signals regulate the release of fasting-induced adipose factor (FIAF) from intestinal epithelial cells, acting as a lipoprotein lipase inhibitor and regulating the storage of peripheral fat. In addition, by unknown mechanisms, the IM regulates the energy mediator in the liver and muscle via phosphorylation of AMP-activated protein kinase (Fig. 1) [17].

Microbial metabolites, such as short chain fatty acids (SCFAs), bind to conjugate receptors of G protein on intestinal epithelial cells by regulating energy from gut-derived hormones, such as the intestinal peptide hormone, and controlling the inflammatory response of the host. The activation of Toll-like receptor-5) in the epithelia or myeloid cells affects the structural composition of the intestinal microbiota, which regulates appetite, weight gain and insulin sensitivity (Fig. 1) [18].

It is suspected that the intestinal microbiota of obese individuals presents peculiarities that may induce chronic inflammation. The induction of endotoxemia in obesity, diabetes mellitus and insulin resistance causes the expression of inflammatory factors that resemble those present in a diet rich in fat [19].

Obesity and diet composition can cause sensitivity to the intestinal barrier and result in the malfunction of the intestine–liver axis, allowing the indiscriminate passage of bacterial components into circulation. The intestinal barrier is a complex functional unit composed of luminal and mucosal elements, such as the epithelial cell layer, the mucosal barrier, and innate and acquired immune components. This barrier plays a key role in protecting against enteric organisms, potentially harmful toxins and bacterial bioproducts that are closely associated with health and disease susceptibility [20,21]. The intestine–liver axis refers to the anatomical and functional relationship between the gastrointestinal (GI) tract and the liver. The interaction between the two organs, whether healthy or diseased, includes the transfer of the IM and its potential hepatotoxic products to the liver [22]. Alterations in this axis (consisting

of the intestinal barrier, the IM and the liver) may lead to changes in intestinal permeability favoring excessive pathogenic bacterial growth (dysbiosis). In particular, the excessive growth of Gram-negative bacteria increases the production of hepatotoxic products such as lipopolysaccharides (LPS), which in turn stimulate Toll-like receptor-4 and ultimately nuclear factor kappa B (NF-kβ) and proinflammatory cytokines, such as interleukin-6, transforming growth factor-beta and tumor necrosis factor-alpha (TNF-α) [23,24].
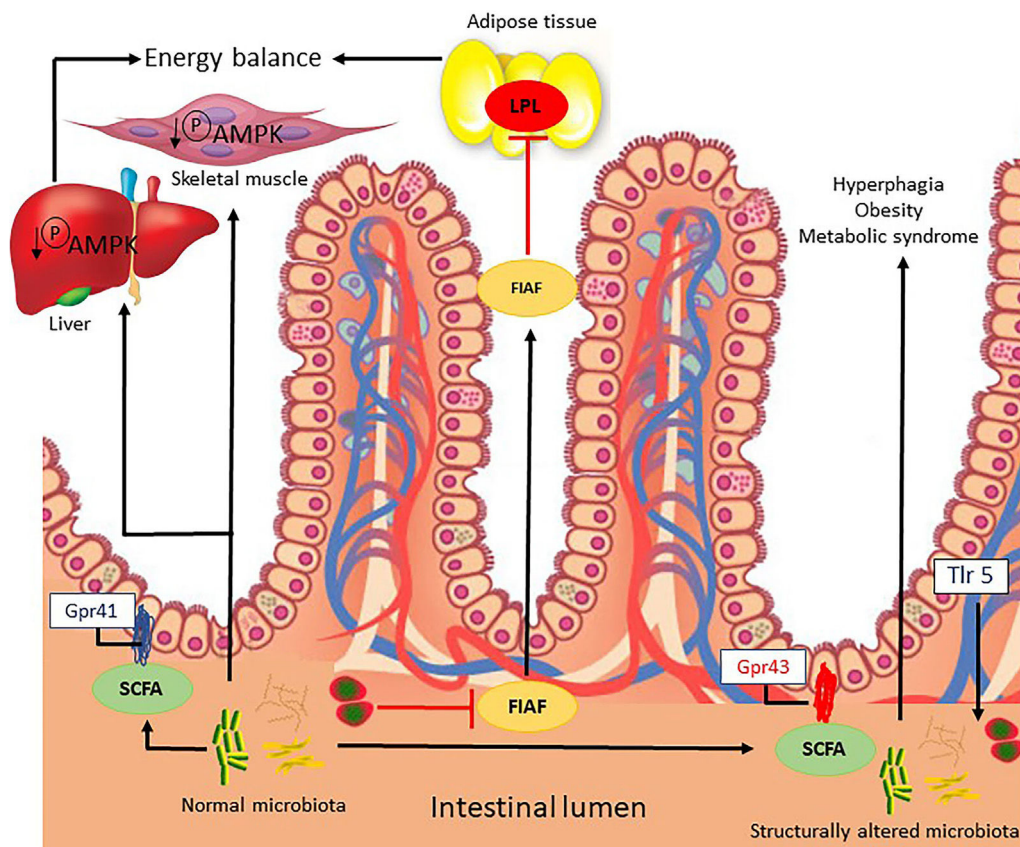
3. Intestinal microbiota and diet

The relationship between the diet and the composition and function of the IM is evident. Indeed, bacteria in the gut are responsible for the digestion and production of many essential vitamins and minerals. The dietary components provide nutrients for bacteria, which then produce metabolites involved in energy balance, metabolism, and immune response, but these metabolites can also result in obesity and metabolic diseases [25,26]. The high caloric content of the Western diet (high in fat and carbohydrates) is a major contributor to weight gain, which is associated with an altered IM [27,28]. A recent study showed that the IM promotes intestinal absorption and the transfer of monosaccharides to the portal circulation by increasing the expression of the glucose-1 transporter in the small intestine. This increased carbohydrate flow stimulates lipogenesis in the liver and adipose tissue [15]. A study comparing a control diet vs. diets high in nondigestible carbohydrates (fibers) demonstrated that nondigestible carbohydrates produce significant changes in the species composition (*E. rectale* and *Ruminococcus bromii*) of the IM within a number of days [29]. The link between diet, IM, and health has been shown in animal models that were switched from low-fat/fiber rich plant diets to high-fat/high-sugar diets and exhibited significant increases in *Bacilli* and *Erysipelotrichia* from the *Firmicutes* phylum, which were associated with a significant decrease in the abundance of members of the *Bacteroidetes* phylum [30]. The GI transit time could be affected by the IM according to Kashyap *et al.* They proposed an animal model to evaluate the interactions between diet, IM, and GI transit time in the host. Diet can independently affect both GI transit time and IM composition and function (as determined by metabolite profiles). However, diet-induced changes in microbial composition may be mediated in part by changes in GI transit time, and the effect of diet on GI transit time may be a result of altered functionality of the IM community caused by the dietary change [31].

4. Intestinal microbiota and NAFLD/NASH

Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disorders worldwide due to the epidemic increase in the prevalence of obesity in affluent societies [32]. NAFLD includes a wide spectrum of hepatic pathological characteristics ranging from simple steatosis to nonalcoholic steatohepatitis (NASH) with or without fibrosis, cirrhosis, and hepatocellular carcinoma [33]. The development and progression of NAFLD involve complex pathophysiological processes [32] that are influenced by host factors such as genetic polymorphisms, diet, and, as reported more recently, the composition of the IM [34,35].

The IM and its derived metabolites may also contribute to the development of NAFLD. Increased intestinal permeability stimulates monosaccharide absorption from the intestinal lumen, leading to bacterial translocation. The endotoxins produced by these bacteria penetrate the portal vein and decrease FIAF secretion by increasing LPL activity, promoting de novo fatty acid synthesis and triglyceride production [9] and activating inflammatory Toll-like

Fig. 1. Possible mechanisms related to the intestinal microbiota that lead to energy imbalance and consequently obesity.
FIAF, fasting-induced adipose factor; Gpr41/43, G-protein coupled receptor; LPL, lipoprotein lipase; SCFAs, short chain fatty acids; AMPK, AMP-activated protein kinase; TRL5, Toll-like receptor-5.

receptors in hepatocytes [23]. A permeable intestinal barrier and small intestine bacterial overgrowth, which is a condition in which elevated levels of small intestinal bacteria lead to abdominal distension and other common symptoms related to irritable bowel syndrome, are frequent in obese patients and induce liver injury by increasing LPS production derived from Gram-negative bacteria in the gut, which activates NF-kβ and TNF-α production. This suggests that IM increases liver exposure to endotoxins, which play an important role in the progression of NASH (Fig. 2) [36,37].
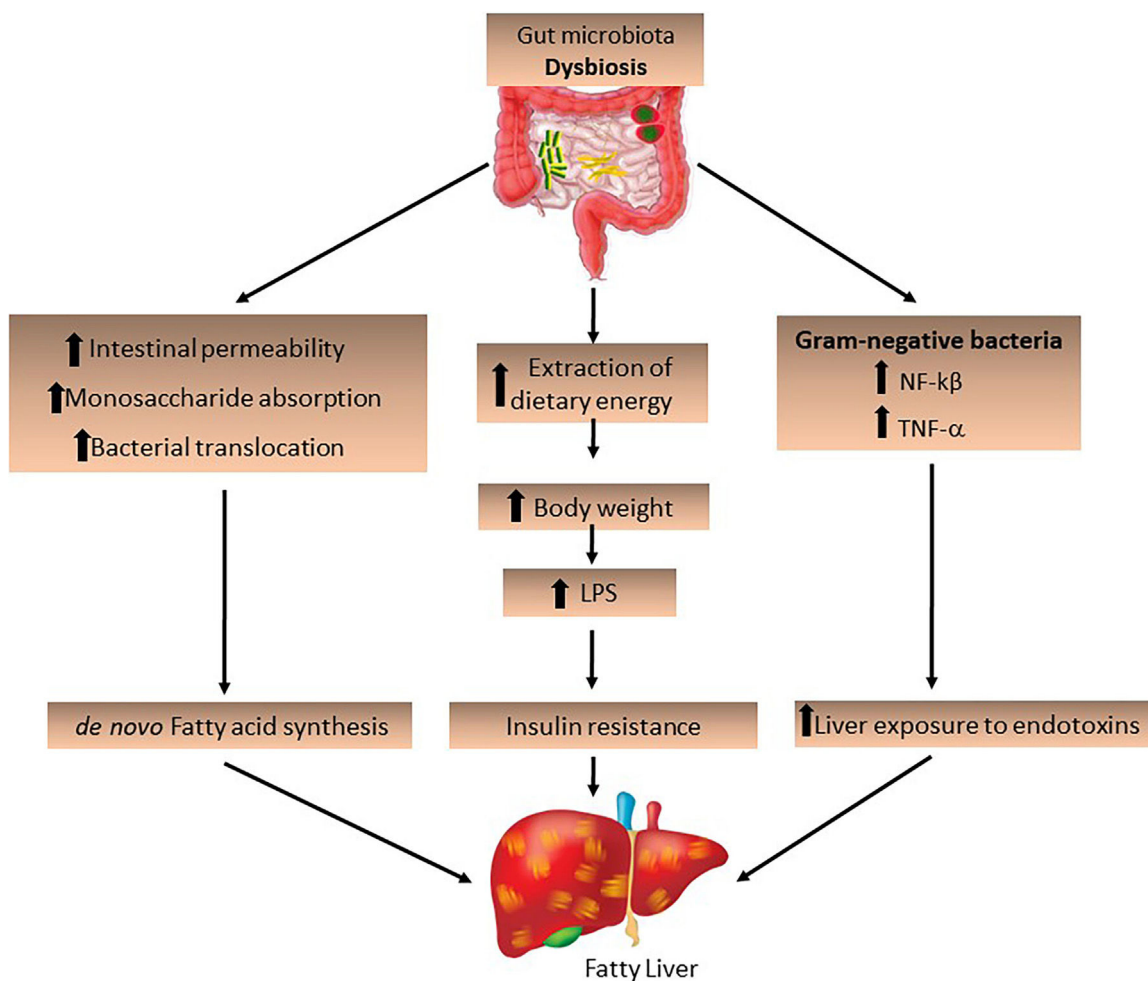
Miele *et al.* showed that NAFLD is associated with increased intestinal permeability and SIBO, and these factors are associated with the severity of hepatic steatosis. They reported evidence of a "leaky" intestine with tight junction disruption in human NAFLD with evidence of increased small intestine bacterial overgrowth [38]. Some studies in animal models and NAFLD or NASH patients showed an association with small bowel overgrowth and increased intestinal permeability [39–42]. Brun *et al.* demonstrated that leptin-deficient obese mice naturally presented increased intestinal permeability, higher circulation of inflammatory cytokines and increased serum levels of LPS [40]. Another study showed that LPS in mice simulated the effects of a high-fat diet by metabolic endotoxemia, which led to dysregulation of inflammation and triggered body weight gain, IR, and the development of NAFLD [43]. Studies involving obese and NAFLD patients showed an association between metabolic endotoxemia and hepatic and systemic inflammation [41,43,44]. Verdam *et al.* also observed an increase in plasma antibodies against LPS in patients with NASH compared to healthy controls. This increase was directly proportional to the increased severity of liver disease [45]. A recent study showed that obese patients with NASH had a decreased abundance of *Faecalibacterium* [46]. Species of

Faecalibacterium exhibited anti-inflammatory effects, partly due to secreted metabolites that block NF-kB activation and interleukin 8 (IL-8) secretion. In addition, the effects were associated with a decrease in proinflammatory colonic cytokine synthesis and with the induction of anti-inflammatory cytokine secretion [47].

Recently, our group demonstrated a decreased abundance of *Faecalibacterium* and *Ruminococcus* in lean NASH patients [48]. *Ruminococcus* belongs to the family Ruminococcaceae, which are also fermenting anaerobes that lead to the production of SCFAs, promoting the development of NAFLD [49]. Interestingly, in the same study, advanced fibrosis (stage >2) was associated with an increase in *Lactobacillus* in obese NASH patients [48]. The genus *Lactobacillus* comprises more than 180 species with important immunological functions and metabolic activities [50]. Some of these bacteria produce lactic acid from the fermentation of carbohydrates in the diet and can produce acetate and ethanol [51], which can induce liver damage, such as inflammation, leading to the progression of NASH [52].

Some studies have shown an association between *Bacteroides* and *Escherichia* and advanced fibrosis [53,54]; *Bifidobacterium* spp. and *Akkermansia muciniphila* were strongly associated with markers of lipid metabolism and negatively associated with inflammation in adipose tissue and circulating glucose, leptin, triglycerides and insulin [55]. Despite the fact that the diet is directly connected to dysbiosis, a study showed that the IM of a genetically obese human could promote the onset of liver steatosis independent of dietary factors [21].

A possible mechanism by which the MI contributes to the development of NAFLD may be related to the amount of ethanol produced by bacteria (*Escherichia coli*) [56]. The ethanol produced

Fig. 2. The role of the intestinal microbiota in the development of fatty liver. Intestinal dysbiosis may be a determining factor for the development and progression of NAFLD/NASH. The increase in pathogenic bacteria and Gram-negative bacteria increases dietary energy extraction, leading to increased intestinal permeability and bacterial translocation, stimulating de novo fatty acid synthesis and increasing LPS expression, as well as the expression of NF-kβ and TNF-α.
LPS, lipopolysaccharides; NF-kβ, nuclear factor-kappa β; TNF-α, tumor necrosis factor-alpha.

by these bacteria contributes to physiological and morphological changes in the intestinal barrier associated with small intestine bacterial overgrowth by increasing intestinal permeability and thereby increasing the passage of endotoxins from the intestinal lumen to the portal blood, leading to an increase in the production of reactive oxygen species, which stimulates hepatic inflammation [57]. Ethanol is constantly produced by the MI even in the absence of alcohol ingestion. It has been found that a diet rich in refined sugar can lead to increased levels of alcohol in the blood and that ingested ethanol is eliminated via the enzyme alcohol dehydrogenase in the liver. This enzyme converts the alcohol into acetaldehyde, which, even in small concentrations, is toxic to the body [58]. The production of ethanol by the MI in obese and eutrophic patients with NASH suggests that it may contribute to the development and progression of the disease [59].

Another product of the metabolism of nutrients by bacteria that may be toxic to the liver is trimethylamine N-oxide. The IM can promote the conversion of choline to trimethylamine, which will then reach the liver by the circulation gate and will be converted into trimethylamine N-oxide [42]. The increase in the production of this compound leads to a decrease in the exportation of hepatic very-low-density lipoprotein and the modulation of bile acid synthesis, which has detrimental effects on the liver, such as increased liver fat deposition and inflammatory and oxidative lesions and decreased glucose metabolism [60].

NAFLD patients are characterized by a significant increase in circulating levels of endotoxins, which may represent an important early marker of potential liver abnormality [61]. Furthermore, gut dysbiosis may be associated with changes in the level of serum metabolites, including branched-chain amino acids and aromatic amino acids. Moreover, BCAAs are increased in individuals with IR, which is considered the major risk factor for hepatic steatosis [62].

5. Prebiotics and probiotics and the intestinal microbiota

Current studies have shown that the use of prebiotics and probiotics can modulate the MI in several diseases, including NAFLD [63–65].

Probiotics improve the composition of the MI, which is associated with a reduction in liver inflammation, a decrease in LPS concentrations and an improvement in aminotransferase concentrations [66,67]. Probiotic supplementation can improve inflammatory factors [68,69] and improve metabolic parameters of NAFLD, such as visceral fat, total cholesterol and IR [70]. In addition, the consumption of probiotics has been associated with a decrease in low-density lipoprotein levels in animals and in humans with hypercholesterolemia [71,72]. Cano *et al.* demonstrated that the consumption of the *Bifidobacterium pseudocatenulatum* probiotic

bacteria improved glucose tolerance, inflammatory status and hepatic steatosis [73].

Prebiotics can modulate the IM by increasing beneficial bacteria in the intestine, particularly Bifidobacteria and Lactobacilli, and inhibit bacterial activities that are detrimental to host health [74]. In addition, in the large intestine, inulin-type fructan and resistant starches are completely catabolized by the microbiota and are transformed into SCFAs, bacterial biomass and organic acids such as lactic acid and gases (carbon dioxide, hydrogen and methane) [66]. Data suggest that altering the microbiota through the use of prebiotics leads to decreased hepatic lipogenesis and blood TG levels and increases the concentration of SCFAs; however, the ability to modulate the intestinal microbiota and liver function in patients with NAFLD should be investigated [75–77].

Recent studies have shown that the use of prebiotics and probiotics can be an alternative therapy in the treatment of NAFLD. In an experimental model of NAFLD, it was observed that the use of synbiotics can reduce hepatic aminotransferases and IL-6 expression. However, the histology showed that the synbiotic was not able to prevent the progression of the disease [78]. A randomized clinical trial demonstrated that the use of multistrain probiotics was able to modulate the IM, thereby significantly reducing fatty liver and aminotransferase activity and reducing the expression of TNF-α and IL6 in NAFLD patients [79]. Another randomized study showed that synbiotic supplementation improves hepatic steatosis, aspartate aminotransferase levels, lipid profile and homeostatic model assessment) in NAFLD patients [64]. A study in NASH patients also showed that the use of synbiotics (*Bifidobacterium longum* and fructooligosaccharide), in combination with lifestyle modification (diet and exercise), improved aspartate aminotransferase, low-density lipoprotein, and TNF-α, leading to decreased endotoxins, IR and steatosis [80]. In addition, a study conducted by Eslamparast *et al.* corroborated the conclusion that the use of synbiotics combined with physical exercise improves aminotransferase levels, TNF-α and NF-kβ [81].

In conclusion, some evidence has demonstrated the role of the IM in the pathogenesis of NAFLD/NASH; however, it is necessary to explore how the IM can be modulated to improve NAFLD/NASH progression. Future randomized studies involving diet and/or probiotics should be performed.

Abbreviations

GI   gastrointestinal
IM   intestinal microbiota
LPS   lipopolysaccharides
NAFLD   nonalcoholic fatty liver disease
NASH   nonalcoholic steatohepatitis
NF-kβ   nuclear factor-kappa B
SCFA   short-chain fatty acid
TNF-α   tumor necrosis factor-alpha

Conflict of interest

The authors have no conflicts of interest to declare.

References

[1] Cani PD, Delzenne NM, Amar J, Burcelin R. Role of gut microflora in the development of obesity and insulin resistance following high-fat diet feeding. Pathol Biol (Paris) 2008;56:305–9.
[2] Karagiannides I, Pothoulakis C. Obesity, innate immunity and gut inflammation. Curr Opin Gastroenterol 2007;23:661–6.
[3] Machado MV, Cortez-Pinto H. Gut microbiota and nonalcoholic fatty liver disease. Ann Hepatol 2012;11:440–9.
[4] Manoharan I, Suryawanshi A, Hong Y, Ranganathan P, Shanmugam A, Ahmad S, et al. Homeostatic PPARα signaling limits inflammatory responses to commensal microbiota in the intestine. J Immunol 2016;196:4739–49.
[5] Fasano A, Physiological. pathological, and therapeutic implications of zonulin-mediated intestinal barrier modulation: living life on the edge of the wall. Am J Pathol 2008;173:1243–52.
[6] Powrie F. Immune regulation in the intestine: a balancing act between effector and regulatory T cell responses. Ann N Y Acad Sci 2004;1029:132–41.
[7] Xu J, Gordon JI. Honor thy symbionts. Proc Natl Acad Sci U S A 2003;100:10452–9.
[8] da Silva ST, dos Santos CA, Bressan J. Intestinal microbiota; relevance to obesity and modulation by prebiotics and probiotics. Nutr Hosp 2013;28:1039–48.
[9] Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718–23.
[10] Mahowald MA, Rey FE, Seedorf H, Turnbaugh PJ, Fulton RS, Wollam A, et al. Characterizing a model human gut microbiota composed of members of its two dominant bacterial phyla. Proc Natl Acad Sci U S A 2009;106:5859–64.
[11] Ley RE. Obesity and the human microbiome. Curr Opin Gastroenterol 2010;26:5–11.
[12] Bajzer M, Seeley RJ. Physiology: obesity and gut flora. Nature 2006;444:1009–10.
[13] Santacruz A, Collado MC, García-Valdés L, Segura MT, Martín-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010;104:83–92.
[14] Jandhyala SM, Talukdar R, Subramanyam C, Vuyyuru H, Sasikala M, Nageshwar Reddy D. Role of the normal gut microbiota. World J Gastroenterol 2015;21:8787–803.
[15] Musso G, Gambino R, Cassader M. Interactions between gut microbiota and host metabolism predisposing to obesity and diabetes. Annu Rev Med 2011;62:361–80.
[16] Hildebrandt MA, Hoffmann C, Sherrill-Mix SA, Keilbaugh SA, Hamady M, Chen YY, et al. High-fat diet determines the composition of the murine gut microbiome independently of obesity. Gastroenterology 2009;137:1716–24.
[17] Tilg H, Kaser A. Gut microbiome, obesity, and metabolic dysfunction. J Clin Investig 2011;121:2126–32.
[18] de La Serre CB, Ellis CL, Lee J, Hartman AL, Rutledge JC, Raybould HE. Propensity to high-fat diet-induced obesity in rats is associated with changes in the gut microbiota and gut inflammation. Am J Physiol Gastrointest Liver Physiol 2010;299:G440–8.
[19] Park JS, Seo JH, Youn HS. Gut microbiota and clinical disease: obesity and nonalcoholic fatty liver disease. Pediatr Gastroenterol Hepatol Nutr 2013;16:22–7.
[20] Paolella G, Mandato C, Pierri L, Poeta M, Di Stasi M, Vajro P. Gut–liver axis and probiotics: their role in non-alcoholic fatty liver disease. World J Gastroenterol 2014;20:15518–31.
[21] Wang R, Li H, Yang X, Xue X, Deng L, Shen J, et al. Genetically obese human gut microbiota induces liver steatosis in germ-free mice fed on normal diet. Front Microbiol 2018;9:1602.
[22] Sharma V, Garg S, Aggarwal S. Probiotics and liver disease. Perm J 2013;17:62–7.
[23] Soares JB, Pimentel-Nunes P, Roncon-Albuquerque R, Leite-Moreira A. The role of lipopolysaccharide/toll-like receptor 4 signaling in chronic liver diseases. Hepatol Int 2010;4:659–72.
[24] Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxemia with obesity: is it real and is it relevant? Biochimie 2016;124:11–20.
[25] Scott KP, Gratz SW, Sheridan PO, Flint HJ, Duncan SH. The influence of diet on the gut microbiota. Pharmacol Res 2013;69:52–60.
[26] Spencer MD, Hamp TJ, Reid RW, Fischer LM, Zeisel SH, Fodor AA. Association between composition of the human gastrointestinal microbiome and development of fatty liver with choline deficiency. Gastroenterology 2011;140:976–86.
[27] Boursier J, Diehl AM. Implication of gut microbiota in nonalcoholic fatty liver disease. PLoS Pathog 2015;11, e1004559.
[28] Trenell MI. Sedentary behaviour, physical activity, and NAFLD: curse of the chair. J Hepatol 2015;63:1064–5.
[29] Walker AW, Ince J, Duncan SH, Webster LM, Holtrop G, Ze X, et al. Dominant and diet-responsive groups of bacteria within the human colonic microbiota. ISME J 2011;5:220–30.
[30] Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R, Gordon JI. The effect of diet on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice. Sci Transl Med 2009;1:6ra14.
[31] Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterology 2013;144:967–77.
[32] Day CP, Saksena S. Non-alcoholic steatohepatitis: definitions and pathogenesis. J Gastroenterol Hepatol 2002;17:S377–84.
[33] Farrell GC, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. Hepatology 2006;43:S99–112.
[34] Angelico F, Del Ben M, Conti R, Francioso S, Feole K, Fiorello S, et al. Insulin resistance, the metabolic syndrome, and nonalcoholic fatty liver disease. J Clin Endocrinol Metab 2005;90:1578–82.
[35] Betrapally NS, Gillevet PM, Bajaj JS. Changes in the intestinal microbiome and alcoholic and nonalcoholic liver diseases: causes or effects? Gastroenterology 2016;150:1745–55.
[36] Lakhani SV, Shah HN, Alexander K, Finelli FC, Kirkpatrick JR, Koch TR. Small intestinal bacterial overgrowth and thiamine deficiency after Roux-en-Y gastric bypass surgery in obese patients. Nutr Res 2008;28:293–8.
[37] Madrid AM, Poniachik J, Quera R, Defilippi C. Small intestinal clustered contractions and bacterial overgrowth: a frequent finding in obese patients. Dig Dis Sci 2011;56:155–60.

[38] Miele L, Valenza V, La Torre G, Montalto M, Cammarota G, Ricci R, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49:1877–87.

[39] Wigg AJ, Roberts-Thomson IC, Dymock RB, McCarthy PJ, Grose RH, Cummins AG. The role of small intestinal bacterial overgrowth, intestinal permeability, endotoxaemia, and tumour necrosis factor alpha in the pathogenesis of non-alcoholic steatohepatitis. Gut 2001;48:206–11.

[40] Brun P, Castagliuolo I, Di Leo V, Buda A, Pinzani M, Palù G, et al. Increased intestinal permeability in obese mice: new evidence in the pathogene-sis of nonalcoholic steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2007;292:G518–25.

[41] Shanab AA, Scully P, Crosbie O, Buckley M, O'Mahony L, Shanahan F, et al. Small intestinal bacterial overgrowth in nonalcoholic steatohepatitis: associa-tion with toll-like receptor 4 expression and plasma levels of interleukin 8. Dig Dis Sci 2011;56:1524–34.

[42] Machado MV, Cortez-Pinto H. Diet, microbiota, obesity, and NAFLD: a danger-ous quartet. Int J Mol Sci 2016;17:481.

[43] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, et al. Metabolic endo-toxemia initiates obesity and insulin resistance. Diabetes 2007;56:1761–72.

[44] Spruss A, Kanuri G, Wagnerberger S, Haub S, Bischoff SC, Bergheim I. Toll-like receptor 4 is involved in the development of fructose-induced hepatic steatosis in mice. Hepatology 2009;50:1094–104.

[45] Verdam FJ, Rensen SS, Driessen A, Greve JW, Buurman WA. Novel evidence for chronic exposure to endotoxin in human nonalcoholic steatohepatitis. J Clin Gastroenterol 2011;45:149–52.

[46] Da Silva HE, Teterina A, Comelli EM, Taibi A, Arendt BM, Fischer SE, et al. Non-alcoholic fatty liver disease is associated with dysbiosis independent of body mass index and insulin resistance. Sci Rep 2018;8:1466.

[47] Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Grata-doux JJ, et al. *Faecalibacterium prausnitzii* is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–6.

[48] Duarte SMB, Stefano JT, Miele L, Ponziani FR, Souza-Basqueira M, Okada LSRR, et al. Gut microbiome composition in lean patients with NASH is associated with liver damage independent of caloric intake: a prospective pilot study. Nutr Metab Cardiovasc Dis 2018;28:369–84.

[49] Scheppach W, Weiler F. The butyrate story: old wine in new bottles? Curr Opin Clin Nutr Metab Care 2004;7:563–7.

[50] Sheng L, Jena PK, Liu HX, Kalanetra KM, Gonzalez FJ, French SW, et al. Gender dif-ferences in bile acids and microbiota in relationship with gender dissimilarity in steatosis induced by diet and FXR inactivation. Sci Rep 2017;7:1748.

[51] Trottier J, Caron P, Straka RJ, Barbier O. Profile of serum bile acids in nonc-holestatic volunteers: gender-related differences in response to fenofibrate. Clin Pharmacol Ther 2011;90:279–86.

[52] Leung C, Rivera L, Furness JB, Angus PW. The role of the gut microbiota in NAFLD. Nat Rev Gastroenterol Hepatol 2016;13:412–25.

[53] Boursier J, Mueller O, Barret M, Machado M, Fizanne L, Araujo-Perez F, et al. The severity of nonalcoholic fatty liver disease is associated with gut dys-biosis and shift in the metabolic function of the gut microbiota. Hepatology 2016;63:764–75.

[54] Loomba R, Seguritan V, Li W, Long T, Klitgord N, Bhatt A, et al. Gut microbiome-based metagenomic signature for non-invasive detection of advanced fibrosis in human nonalcoholic fatty liver disease. Cell Metab 2017;25:1054–62.

[55] Schneeberger M, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, et al. *Akkermansia muciniphila* inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. Sci Rep 2015;5:16643.

[56] Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, et al. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013;57:601–9.

[57] Volynets V, Küper MA, Strahl S, Maier IB, Spruss A, Wagnerberger S, et al. Nutri-tion, intestinal permeability, and blood ethanol levels are altered in patients with nonalcoholic fatty liver disease (NAFLD). Dig Dis Sci 2012;57:1932–41.

[58] Engstler AJ, Aumiller T, Degen C, Dürr M, Weiss E, Maier IB, et al. Insulin resis-tance alters hepatic ethanol metabolism: studies in mice and children with non-alcoholic fatty liver disease. Gut 2016;65:1564–71.

[59] Zhu L, Baker RD, Zhu R, Baker SS. Gut microbiota produce alcohol and contribute to NAFLD. Gut 2016;65:1232.

[60] Dumas ME, Barton RH, Toye A, Cloarec O, Blancher C, Rothwell A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 2006;103:12511–6.

[61] Harte AL, da Silva NF, Creely SJ, McGee KC, Billyard T, Youssef-Elabd EM, et al. Elevated endotoxin levels in non-alcoholic fatty liver disease. J Inflamm (Lond) 2010;7:15.

[62] Pedersen HK, Gudmundsdottir V, Nielsen HB, Hyotylainen T, Nielsen T, Jensen BA, et al. Human gut microbes impact host serum metabolome and insulin sensitivity. Nature 2016;535:376–81.

[63] Bradford V, Dillon J, Miller M. Lifestyle interventions for the treatment of non-alcoholic fatty liver disease. Hepat Med 2014;6:1–10.

[64] Mofidi F, Poustchi H, Yari Z, Nourinayyer B, Merat S, Sharafkhah M, et al. Syn-biotic supplementation in lean patients with non-alcoholic fatty liver disease: a pilot, randomised, double-blind, placebo-controlled, clinical trial. Br J Nutr 2017;117:662–8.

[65] Cho MS, Kim SY, Suk KT, Kim BY. Modulation of gut microbiome in nonalcoholic fatty liver disease: pro-, pre-, syn-, and antibiotics. J Microbiol 2018;56:855–67.

[66] Mokhtari Z, Gibson DL, Hekmatdoost A. Nonalcoholic fatty liver disease, the gut microbiome, and diet. Adv Nutr 2017;8:240–52.

[67] Wong VW, Won GL, Chim AM, Chu WC, Yeung DK, Li KC, et al. Treatment of nonalcoholic steatohepatitis with probiotics. A proof-of-concept study. Ann Hepatol 2013;12:256–62.

[68] Ma X, Hua J, Li Z. Probiotics improve high fat diet-induced hepatic steatosis and insulin resistance by increasing hepatic NKT cells. J Hepatol 2008;49:821–30.

[69] Donato KA, Gareau MG, Wang YJ, Sherman PM. *Lactobacillus rhamnosus* GG attenuates interferon-{gamma} and tumour necrosis factor-alpha-induced barrier dysfunction and pro-inflammatory signalling. Microbiology 2010;156:3288–97.

[70] Shin HS, Park SY, Lee DK, Kim SA, An HM, Kim JR, et al. Hypocholesterolemic effect of sonication-killed *Bifidobacterium longum* isolated from healthy adult Koreans in high cholesterol fed rats. Arch Pharm Res 2010;33:1425–31.

[71] Pereira DI, Gibson GR. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. Crit Rev Biochem Mol Biol 2002;37:259–81.

[72] Usman, Hosono A. Effect of administration of *Lactobacillus gasseri* on serum lipids and fecal steroids in hypercholesterolemic rats. J Dairy Sci 2000;83:1705–11.

[73] Cano PG, Santacruz A, Trejo FM, Sanz Y. *Bifidobacterium* CECT 7765 improves metabolic and immunological alterations associated with obesity in high-fat diet-fed mice. Obesity (Silver Spring) 2013;21:2310–21.

[74] Blaut M. Relationship of prebiotics and food to intestinal microflora. Eur J Nutr 2002;41:11–6.

[75] Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. Br J Nutr 2010;104:S1–63.

[76] Bindels LB, Porporato P, Dewulf EM, Verrax J, Neyrinck AM, Martin JC, et al. Gut microbiota-derived propionate reduces cancer cell proliferation in the liver. Br J Cancer 2012;107:1337–44.

[77] Tarantino G, Finelli C. Systematic review on intervention with prebi-otics/probiotics in patients with obesity-related nonalcoholic fatty liver disease. Future Microbiol 2015;10:889–902.

[78] Tagliari E, Campos AC, Costa-Casagrande TA, Salvalaggio PR. The impact of the use of symbiotics in the progression of nonalcoholic fatty liver disease in a rat model. Arq Bras Cir Dig 2017;30:211–5.

[79] Kobyliak N, Abenavoli L, Mykhalchyshyn G, Kononenko L, Boccuto L, Kyriienko D, et al. A multi-strain probiotic reduces the fatty liver index, cytokines and aminotransferase levels in NAFLD patients: evidence from a randomized clin-ical trial. J Gastrointest Liver Dis 2018;27:41–9.

[80] Malaguarnera M, Vacante M, Antic T, Giordano M, Chisari G, Acquaviva R, et al. *Bifidobacterium longum* with fructo-oligosaccharides in patients with non alco-holic steatohepatitis. Dig Dis Sci 2012;57:545–53.

[81] Eslamparast T, Poustchi H, Zamani F, Sharafkhah M, Malekzadeh R, Hek-matdoost A. Synbiotic supplementation in nonalcoholic fatty liver disease: a randomized, double-blind, placebo-controlled pilot study. Am J Clin Nutr 2014;99:535–42.