Role of gut microbiota: Obesity and NAFLD

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ABSTRACT
Non-alcoholic fatty liver disease (NAFLD) is a leading cause of chronic liver disease in developed countries. Obesity is the most important risk factor for metabolic syndrome and NAFLD. Accumulated evidence has revealed that gut microbial compositional changes may be associated with more energy harvesting from the diet, which promotes increased fatty acid uptake from adipose tissue and shifts lipid metabolism from oxidation to de novo production. Furthermore, changes in intestinal barrier function contribute to metabolic endotoxemia in the form of low-grade microbial inflammation. Persistent inflammation exacerbates NAFLD progression. In this review, we discuss the role of gut microbiota in obesity and NAFLD.

Keywords: Non-alcoholic fatty liver disease, obesity, endotoxin, microbiota

INTRODUCTION
Non-alcoholic fatty liver disease (NAFLD) is a common chronic liver disease, and it generally develops on the background of obesity and insulin resistance (IR) (1). It is mostly in the form of simple steatosis. However, progression to non-alcoholic steatohepatitis (NASH) happens in 20% of the cases. Cirrhosis and hepatocellular carcinoma (2,3) develop in a minority. Obesity is the main factor for metabolic syndrome and NAFLD (4-6). Growing evidence suggests the involvement of intestinal microbiota (IM) in the development of obesity and metabolic syndrome (MS), attributing a potential role in the pathogenesis of NAFLD (7).

Several animal studies have acknowledged that intestinal microbiota (IM) can exacerbate NAFLD by increasing hepatic steatosis, inflammation, and fibrosis (7-11). Additionally, IM have the ability to maximize hepatic triglyceride content through mechanisms, such as modified appetite signaling, increased energy extraction from the diet, and altered expression of genes involved in de novo lipogenesis, and by inflammation-driven steatosis (8,9,11-13). Development of NAFLD in humans is associated with changes in intestinal barrier function and higher endotoxin levels, as well (14-16). This review explores the pathogenetic association between intestinal microbiota and NAFLD in detail.

GUT MICROBIOTA
Gut microbes are useful to the host in terms of protecting it against pathogenic bacteria, digesting complex carbohydrates, allowing extraction of more energy from the diet, and regulating immune function (17-21). The IM comprise 100 trillion bacteria (1-2 kg in mass) with 2000 distinct species, with a total genome of 150 times as many genes than the human genome (22). Fecal microbiota profiling by 16S ribosomal sequencing revealed that Firmicutes and Bacteroidetes are the more predominant phyla (90% of the GM). Actinobacteria, Proteobacteria, and Verrucomicrobia are other prevalent bacterial phyla residing in the gut, and less prevalent bacterial groups are Cyanobacteria, Fusobacteria, Lentisphaerae, Spirochaetes, and TM7 (23). Gut microbial phyla and their species are illustrated in Figure 1.

GUT MICROBIOTA AND ENERGY HARVESTING CAPACITY OF THE HOST
Germ-free (GF) mice display reduced body fat compared with conventionalization (CONV) mice. Notably,
**CHANGES IN GERM-FREE COMPOSITION ASSOCIATED WITH OBESITY**

Compositional changes of GM have been seen in obese individuals. For instance, the cecal microbial profile of ob/ob mice, db/db mice was characterized, with a higher abundance of Firmicutes and lower abundance of Bacteroidetes species compared to lean mice (25). A shifted ratio in favor of Firmicutes in ob/ob mice produced more fermentation end products in the cecum (eg, butyrate and acetate) than their lean littermates. Fermented end products, called short-chain fatty acids (SCFAs), play an important role in appetite regulation. However, excess produced SCFAs are converted into triglycerides in the liver (12,26). In addition, GF mice colonized (gavage) with microbiota collected from the cecum of an ob/ob donor led to increased body fat with a higher relative abundance of Firmicutes (12). Prolonged HFD feeding (15 weeks) decreases the concentrations of fecal acetate in ob/ob mice, and it remained stable in wild-type mice (27).

Several reports demonstrated the relation between the proportion of GM and body fat in humans. However, discrepancies have been noticed in the composition of the human GM in different studies. Obese people had fewer Bacteroidetes and more Firmicutes (28), Lactobacillus species (29), and Prevotella (30) than lean controls. When obese people were allotted to either diet therapy (28) or Roux-en-Y gastric bypass (RYGB) procedure (30), the abundance of Bacteroidetes was restored. However, a contradictory outcome was observed in another study; the ratio of Firmicutes to Bacteroidetes changed in favor of Bacteroidetes rather than Firmicutes in overweight and obese subjects (31). Kalliomaki et al. reported that during infancy, overweight children showed a higher abundance of Firmicutes, comprising *Staphylococcus aureus* species; in comparison, normal-weight gut colonizers were Bifidobacterium as well as Actinobacteria (32).

A recent review by Carcilli and Saad (26) reported the metabolomics of GM. The Bacteroidetes genes are rich in the phosphotransferase system; Firmicutes genes are responsible for the transport system. In addition, most of the obesity accomplished genes belong to Actinobacteria (75%) and Firmicutes (25%), while most of the lean-enriched genes belong to Bacteroidetes (42%). These findings support the view that in humans at functional level act as the core microbiome, and alterations in the core microbiome confer the host towards an obese phenotype instead of changes in the just one bacterial phylum (12,33). A summary of studies related to GM and obesity is presented in Table 1 (34-41). The role of gut microbiota in obesity is illustrated in Figure 2.

**PATHOGENESIS OF NAFLD THROUGH GUT-LIVER AXIS**

Through the gut-liver axis, the liver receives blood from the portal vein, so it constitutes an innate immune response against gut-originated bacterial antigens (42). Structurally, two kinds of bacteria are residing in the gut: (i) gram-positive and (ii) gram-negative bacteria. The latter group contains lipopolysaccharide (LPS, also called endotoxin) in their cell wall, which can induce strong immune responses, thereby building up...
inflammation (43). Metabolic endotoxemia was observed in different kinds of chronic liver diseases (44). Obesity, metabolic syndrome, and NAFLD are now regarded as low-grade inflammatory diseases. Persistent high circulating levels of inflammatory cytokines have been shown impact intestinal barrier function by disrupting tight junction (TJ) protein complexes (45).

Small intestinal bacterial overgrowth (SIBO) and elevated endotoxin levels are involved in the pathogenesis of NAFLD. Wigg et al., in a case-control study, reported that a higher prevalence of small intestinal bacterial overgrowth (SIBO) and higher circulating TNF-α levels were observed in NASH patients in comparison to controls (14). Generally, hydrogen breath test, lactulose breath test, fecal microbial profile, and composition of the intestinal tight junction (TJ) protein complex (claudin and occludins) are analyzed to assess SIBO. Several reports have shown a tendency towards gut leakiness in NAFLD patients (14,16,46-53). Different studies related to SIBO and NAFLD are summarized in Table 2.
Table 2. Studies on SIBO associated NAFLD progression in humans

<table>
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<th>Patients and methodology</th>
<th>Outcome</th>
<th>Reference no</th>
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<td>22 NASH patients and 23 healthy controls. CDXL breathe test</td>
<td>Prevalence of SIBO and higher TNF-α levels in NASH patients</td>
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SIBO: small intestinal bacterial overgrowth; GCMS: gas chromatography and mass spectroscopy; CDXL: CD-Xylulose lactulose breathe test; H₂ BT: hydrogen breathe test; VOC: volatile organic compounds; TLR-4: toll like receptor-4; NASH: non alcoholic steatohepatitis

16S rRNA pyro-sequencing of the fecal microbial profile of NASH, obese, and healthy children revealed that abundance of ethanol-producing *Escherichia* bacteria was a risk factor in the development of disease from obesity to NASH (46). In another study, it was observed that there was a lower abundance of Bacteroidetes in NASH patients compared to simple steatosis and healthy control subjects. In addition, a BMI-independent association between Bacteroidetes and liver disease state was observed (47). Raman et al. demonstrated higher concentrations of ester compounds (VOC) in the fecal samples of NAFLD patients (48).

Germ-free mice are resistant to HFD-induced IR and steatosis. Moreover, low levels of LPS prevent GF mice from LPS-accelerated inflammation (54). Recently, it has been reported that fecal transplantation from healthy donors to obesity with MS displays improvement in insulin sensitivity (39). In another recent case report, investigators found that *Enterobacter cloacae* B29 is responsible for weight gain and obesity. Eradication therapy of this species has shown a reduction in body weight. Notably, when the same strain was introduced into GF mice, recurrence of obesity, inflammation, and serum endotoxemia was observed (55).

**ROLE OF LPS-TLR4 SIGNALING IN NAFLD PROGRESSION**

Innate immune responses are the first line of defense against invading microbes. It includes pattern recognition receptors (PRRs), which contain toll-like receptors (TLRs) and NOD-like receptors (NLRs), that recognize a variety of pathogen-associated molecular patterns (PAMPs) and endogenously recognize damage-associated molecular patterns (DAMPs) (56,57). Toll-like receptor-4 (TLR4) recognizes gut-derived LPS, activates cell signaling cascades, and induces production of pro-inflammatory cytokines (Figure 3) (58).

HFD-fed mice exhibit increased body weight associated with development of inflammation, increased fasting glucose, liver triglyceride accumulation, and steatosis. Besides, this effect is similar to those of LPS-infused mice (11). This was proven by another study; portal LPS levels were significantly elevated in prolonged HFD-induced NAFLD in rats (59). Modulation of gut microbiota through antibiotic treatment of ob/ob mice or HFD-fed mice showed reduction in metabolic endotoxemia, reduced glucose intolerance, body weight gain, fat mass development, inflammation, oxidative stress, and macrophage infiltration marker mRNA expression in visceral adipose tissue (60).

Activation of TLR4 by LPS requires the co-receptors CD14 and MD-2. This complex further activates myeloid differentiation factor (MyD88)-dependent and TIR domain-containing adapter-inducing interferon-β (TRIF)-dependent (MyD88-independent) signaling pathways. The MyD88-dependent pathway induces inflammatory cytokines through activation of NF-κB,
whereas the TRIF-dependent pathway activates interferon regulatory factor 3 (IRF-3) and NF-κB via induction of interferons and inflammatory cytokines, respectively (56) (Figure 4).

Absence of CD14 in ob/ob CD14(-/-) mice protects from HFD-induced NAFLD (60). In humans, a mutation in the promoter region for CD14, which leads to increased transcriptional activity, correlates with increased susceptibility for NASH (45). Higher expressions of MyD88 mRNA in the liver have been observed in either MCDD-fed mice (61) or high fructose-fed mice (62). MyD88 deficiency prevents steatohepatitis in mice fed a choline-deficient (CD) diet, attributing a novel role to the MyD88 signaling pathway (63). TLR4 and MD-2 knockout mice fed with a MCDD exhibit lower serum TNF-α levels than wild-type mice, explaining the pivotal role of the LPS recognition complex in NAFLD inflammation (64). Recently, Kanuri et al. reported that the higher expression of TLR 1-5 mRNA in the livers of NAFLD patients was associated with an induction of higher expression of their intracellular adapter molecule, MyD88, but not IRF3 (65).

In addition to reports mentioned above, several experimental and clinical studies have displayed the involvement of endo-
NOD-LIKE RECEPTORS

Nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) are another class of PRRs present in the cytoplasm and recognize a variety of PAMPs and DAMPs, mediating the immune response to defend against pathogen infection and endogenous damage (56). Several NLR members, including NLRP1, NLRP3, NLRP6, and NLRC4, group into large multiprotein complexes called inflammasomes to control caspase-1 activity. Inflammasome-dependent caspase-1 activation leads to the maturation and secretion of the proinflammatory cytokines IL-1β and IL-18 (74). Most DAMPs produce the generation of reactive oxygen species (ROS), which are known to activate the NLRP3 inflammasome (75). Wang et al. demonstrated the role of NLRX1 and NLRP3 inflammasomes in the development of NAFLD. HFD-fed mice exhibited higher expression of NLRP3 mRNA and lower expression of NLRX1. LPS aggravates the expression of NLRP3 inflammasomes and exacerbates NASH progression. Besides, lower expression of NLRX1 increases the expression of TNF receptor associated factor (TRAF)-6. The investigators concluded that regulation of both the NLRX1 and NLRP3 inflammasomes is a novel target for treatment of NAFLD (76). In another study, inflammasome (NLRP3+ and NLRP6+)-deficient mice fed with MCDD showed dysbiosis associated with aggravated hepatic steatosis and higher TNF-α expression. Notably, these mice, co-housed with wt (wt mice are either ASC+ or IL18+ mice) animals, displayed significant enhancement of NASH compared to age- and gender-matched singly housed wt controls. Investigators concluded that transmissible colitogenic bacteria were present in the inflammasome-deficient mice and that they were the major contributor in the aggravation of NASH (77).

CONCLUSION

Obesity is a well-documented risk factor for metabolic syndrome and NAFLD. Gut microbiota play an important role in host immune protection, energy-harvesting capacity, and micronutrient absorption. Metagenomic studies revealed that Bacteroidetes and Firmicutes are the predominant (90%) phyla constituting the GM. Gut microbial alterations contribute to the development of obesity in both animals and humans. Increased abundance of Firmicutes-to-Bacteroidetes ratio leads to greater energy-harvesting capacity from undigested carbohydrates, producing more fermentable end products (eg, butyrate). Intestinal bacterial overgrowth has an impact on intestinal barrier function through changing the composition of intestinal TJ protein complexes. A change in intestinal barrier function promotes translocation of LPS from the gut into systemic/portal circulation, leading to LPS-TLR4-mediated inflammation and the progression of NAFLD. In addition to TLRs, inflammasome-dependent (eg, NLR) production of pro-inflammatory cytokines exacerbates NAFLD progression. Currently, no specific treatment has been established to treat NAFLD. Most of the studies displayed the involvement of GM in the pathogenesis and progression of this disease. Future studies targeting GM are new avenues to treat NAFLD. Several experimental and few clinical studies have addressed the efficacy of probiotics, prebiotics, and antibiotics in NAFLD. More clinical studies are required to confirm the effectiveness of these drugs.

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