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Review

Role of gut microbiota: Obesity and NAFLD

LIVER

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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,

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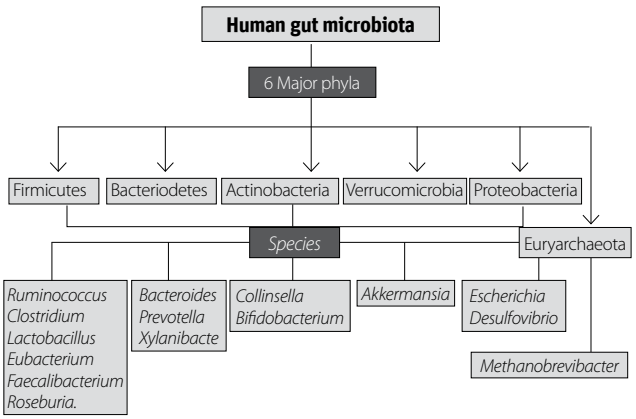

Figure 1. Human gut microbial phyla and their species. Firmicutes: *Ruminococcus, Clostridium, Lactobacillus, Eubacterium, Faecalibacterium* and *Roseburia species.* **Bacteroidetes:** *Bacteroides, Prevotella* and *Xylanibacte species.* **Actinobacteria:** *Collinsella* and *Bifidobacterium species.* **Proteobacteria:** *Escherichia, Desulfovibrio species.* **Verrucomicrobia:** *Akkermansia species.* **Euryarchaeota:** *Methanobrevibacter species.*

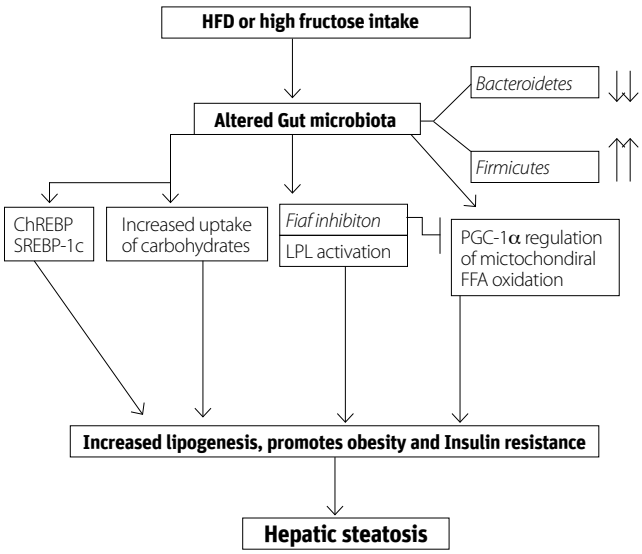

Figure 2. HFD or excess dietary fructose intake associated with altered GM composition. Disproportion of GM promotes lipogenetic pathways in the host via carbohydrate responsive-binding protein (ChREBP) and sterol responsive-binding protein (SREBP)-1c. On the other hand, Fiaf inhibition displays activation of LPL activity and inhibition of peroxisome proliferator-activated receptor co-activator-1α (PGC-1α) activity and increased lipogenetic pathways, thereby promoting obesity, insulin resistance, and hepatic triglyceride fat accumulation.

when GF mice are conventionalized (transfer of GM from CONV mice), they develop more body fat. GF mice express fasting-induced adipose factor (Fiaf), which inhibits lipoprotein lipase (LPL) activity. In CONV mice, GM inhibits Fiaf activity (9). Further studies of the same research group demonstrated that increased activation of AMP-activated protein kinase (AMPK) in the skeletal muscle and liver increases fatty acid oxidation in GF mice and protects against diet-induced obesity (24).

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

Table 1. Summary of the experimental and clinical Studies on GM changes and obesity

Model	Outcome	Reference no
ob/ob mice and lean ob/+ littermates	Obesity affects the gut microbial ecology (increasing abundance of Firmicutes and decreasing Bacteroidetes species)	25
ob/ob mice and lean ob/+ littermates along with GF mice	Obese microbiome has an increased capacity harvesting energy from diet and this trait is transmissible.	12
HFD fed C57BL/6J strain and GF mice	GF mice protected from diet induced obesity through increasing fatty acid metabolism (higher PGC-1α and AMPK activity)	24
GF mice and conventional mice	Absence of gut microbiota does not provide a general protection from diet-induced obesity.	34
ob/ ob mice fed with low fat diet and wild type lean mice fed with HFD.	Diet play an important role in modulation of gut microbiota and it depends on age.	29
Gut microbial profile in ob/ob and db/db mice	Higher abundance of Firmicutes and lower abundance of Bacteroidetes was observed in ob/ob and db/db mice.	26
Adult ob/ob mice twin pairs and germ free mice	Diet microbial changes are rapid and transmissable	35
Ob(P) rats and Ob(R) rats	Obesogenic feeding changed the composition of GM there by predisposing the host to obese phenotype and promotes central inflammation.	36
Study patients	**Outcome**	
12 Obese people and diet therapy with FAT-R or CARB-R	Obese people had fewer Bacteroidetes and more Firmicutes. Diet therapy increased Bacteroidetes species.	30
25 obese and overweight children and 24 normal weight children	Firmicutes comprising *Staphylococcus aureus* species were more prevalent in obese children.	34
Adult obese people	Obese people gut microbiota had higher abunadance of Prevotellacee (Bacteroidetes family) which are H_2 producers.	37
20 obese subjects, 9 patients with anorexia nervosa, and 20 normal-weight healthy controls	Redcution in Bacteroietes community and higher Lactobacillus species proportion was obeserved in obese patients.	31
Obese subjects and Roux-en-Y gastric bypass procedure	RYGB procedure restores the abundance of Bacteroidetes and Prevotella population.	32
33 obese, 35 over weight and 30 lean humans	The ratio of Bacteroidetes abundance was more rather than Firmicutes in overweight and obese subjects.	33
68 obese and 47 controls	Higher abundance of *Methanobrevibacter smithii, B. animalis, L. paracasei, L. Plantarum and L. Reuteri* was observed in obese individuals.	38
Obese subjects and small intestinal infusions to obese individuals from lean donors	Improves insulin sensitivity and developed butyrate producing bacteria	39
Analysis of gut microbiome of obese and lean individuals	Obese people had lower bacterial richness, overall adiposity, insulin resistance, dyslipidaemia and a more pronounced inflammatory phenotype	40
Obese and lean children cross sectional study	Higher proportion of Firmicutes-to-Bacteroidetes ratio and low relative proportions of *B. vulgatus* and higher abundance of Lactobacillus spp. Observed in Obese children.	41

ob/ ob mice: leptin deficient mice; db/db mice: leptin receptor deficient mice; GF mice: germe free mice; HFD: high fat diet; ob(P): obesity prone; ob(R): obesity resistance; FAT-R: fat restricted restricted; CARB (R): carbohydrate restricted diet

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

Patients and methodology	Outcome	Reference no
22 NASH patients and 23 healthy controls.CDXL breathe test	Prevalence of SIBO and higher TNF-α levels in NASH patients	14
10 NAFLD and 10 healthy controls. Lactulose breathe test	SIBO and endotoxins play important role in the pathogenesis of NASH	49
10 NAFLD and 10 healthy controls. Lactulose breathe test. Cisapride administration 4 weeks.	Prevalence of SIBO and higher endotoxin levels in NAFLD patients and ameliorated after 4 weeks administration of Cisapride.	50
Morbidly obese patients and hydrogen breath test	SIBO and the presence of a metabolic syndrome were independent factors of severe hepatic steatosis.	51
10 NASH, 6 steatosis patients and 12 healthy subjects. Lactulose/Mannitol test with aspirin challenge	Susceptibility to gut leakiness site is colon rather than small bowel caused endotoxemia in NASH patients	52
35 NAFLD, 27 with untreated celiac disease and 24 healthy subjects. Glucose breathe test and immunohistochemistry of small bowel TJ proteins.	Increased intestinal permeability appears to be caused by disruption of intercellular tight junctions in the intestine, and it may play an important role in the pathogenesis of hepatic fat deposition.	16
10 NASH and 16 healthy volunteers. H_2 BT	SIBO may have an important role in NASH through interactions with TLR-4 and induction of the pro-inflammatory cytokine, IL-8.	53
NASH 22, obese 25 and healthy 16 children. Faecal microbiome analysed by16S rRNA pyro-sequencing and blood ethanol levels	*Escherichia* is abundant in NASH patients and its abundance may be risk factor in driving disease from obesity to NASH.	46
22 NASH, 11 simple steatosis and 17 healthy controls. Faecal microbiome analysed by qRT-PCR technique.	Lower abundance of Bacteroidetes population in NASH patients. BMI independent association between Bacteroidetes and liver disease state was observed.	47
30 NAFLD and 30 healthy controls. Faecal microbiota and VOC profile were analysed by multitag pyrosequencing and GCMS respectively.	No different in microbial profile in two groups. Increase of ester compounds in NAFLD patients was observed.	48

SIBO: small intestinal bacterial over growth; 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,

Table 3. Animal studies on endotoxin (LPS) mediated progression of NAFLD

Model	Outcome	Reference no
fa/fa rats and ob/ob mice	Hepatic macrophage dysfunction occurs in obesity and this might promote steatohepatitis by sensitizing hepatocytes to endotoxin.	66
C57Bl/6 hyperlipidaemic model mice (LDLR (-/-) mice and ApoE (-/-) mice)	HFD induced mice showed steatohepatitis with higher expression of macrophage scavenger receptor (MARCO) mRNA in the liver.	67
ob/ob and db/db mice	Genetically obese mice display enhanced intestinal permeability, portal endotoxemia and high circulating levels of inflammatory cytokines that can contribute to liver inflammatory damage.	45
HFD fed and LPS infused C57BL/6 mice	The effects of LPS infusion in mice and HFD fed mice showed weight gain, steatosis and higher inflammatory cytokines levels.	11
HFD fed, ob/ob mice and ob/ob CD14-/- mutant mice	High-fat feeding strongly increased intestinal permeability and increased metabolic endotoxemia. Antibiotic treatment reverse the condition as well as $CD^{-/-}$ mice mimicked with antibiotic effects.	60
C57BL/6 mice fed with MCDD	LPS plays an important role in TNF-α induced hepatocyte apoptosis in a NASH model.	68
TLR4 and MD-2 knockout mice fed with a MCDD	Lower TNF-α serum levels were observed in TLR4 and MD-2 knockout mice	64
HFD fed C57BL6 mice	NLRX1 and NLRP3 inflammasome regulation play important role in NAFLD progression	76
NLRP3 and NLRP6 deficient mice fed with MCDD	Inflammasome-mediated dysbiosis is implicated in NASH progression	77

ob/ob mice: leptin deficient mice; db/db mice: leptin receptor deficient mice; fa/fa: zucker fatty rats (leptin receptor deficient); MCDD: methionine/choline deficient diet; MARCO: macrophage scavenger receptor; ApoE(-/-) mice: apolipoprotein E deficient mice; LDLR (-/-) mice: low density lipoprotein receptor deficient mice; LPS: lipopolysaccharide

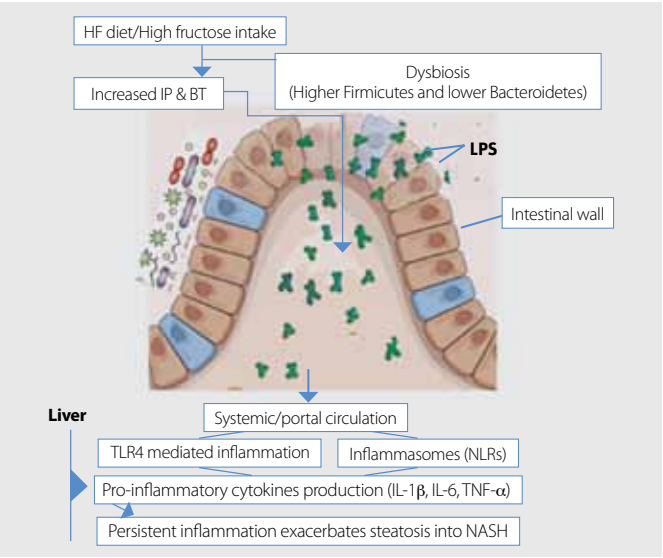

Figure 3. Gut microbiota and NAFLD progression. High fat diet/high fructose intake has shown impact on gut microbial proportion. Disproportion contributes increased intestinal permeability (IP) and bacterial transolocation (BT) in the form of lipopolysaccharides (LPS) into systemic or portal circulation. LPS induces inflammation via TLR4 and NLR mediated pathways. Persistent inflammation promotes hepatic triglyceride accumulation and NASH.

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,

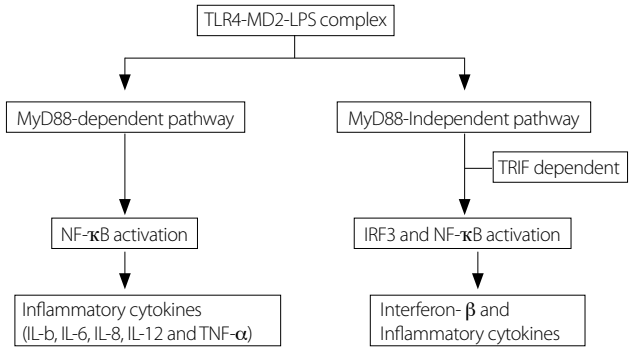

Figure 4. TLR4 mediated MyD88 dependent and MyD88 independent pathways. MyD88: myeloid differentiation factor 88; TRIF: TIR-domain-containing adapter-inducing interferon-β; IRF-3: interferon regulatory factor-3.

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 MCD diet 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-

Table 4. Human studies on endotoxin (LPS) mediated progression of NAFLD

Subjects	Outcome	Reference no
40 morbidly obese adults with NAFLD	In NASH patients, the rise in plasma levels of LBP and correlated with the increase in TNF-α gene expression in the liver.	69
40 obese children with biopsy proven NAFLD	Endotoxin levels strongly associates with an increased NAFLD activity score in children.	70
12 NAFLD patients and 6 healthy volunteers	High intake of dietary fructose may increase intestinal translocation of bacterial endotoxin, PAI-1 and contribute to the development of NAFLD in humans.	15
NAFLD-7, NASH-21 and 52 healthy controls	A mutation in the promoter for CD14, which leads to increased transcriptional activity, is associated with increased susceptibility for NASH	45
155 NAFLD patients and 23 healthy control	In human studies, serum sCD14 levels in patients with NASH increased with increasing fibrosis stage	71
NAFLD and NASH-67 and healthy control-42	Circulating microparticles from CD14 positive cells were correlated with severity of liver inflammation in patients with NAFLD	72
113 biopsy confirmed NAFLD patients.	Serum sCD14 levels are strongly correlated with the grade of liver inflammation but not the stage of liver fibrosis.	73

LBP: lipopolysaccharide binding protein; sCD14: soluble form of CD14; TNF-α: tumour necrosis factor-alpha; PAI-1: plasminogen activator inhibitor-1

toxins in the progression of NAFLD (66-73). Studies related to endotoxemia and NAFLD progression are summarized in Tables 3 and 4.

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 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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