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The Gut Microbiota in Hepatic Encephalopathy: From Recognition to Treatment

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Abstract: The role of the gut microbiota in the pathogenesis and treatment of hepatic encephalopathy (HE) has garnered increasing attention due to significant advancements in understanding the gut microbiota over recent years. A growing body of evidence from laboratory and clinical studies highlights a substantial relationship between gut microbiota and HE. Identifying the role of gut microbiota in maintaining normal cognitive function, including its influence on the gut barrier and immune cells, is essential to elucidate the mechanisms underlying the development of HE. This understanding offers novel perspectives for its prevention and treatment. This paper provides a comprehensive review of the research progress concerning the gut microbiota, HE, and their interrelationship, along with current treatment methods for HE. Furthermore, it outlines the limitations and challenges associated with microbiota-based therapeutic research.

Keywords: Gut microbiota; Hepatic encephalopathy; Gut barrier; Treatment

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1. Gut microbiota

1.1. Awareness of gut microbes

The human gut hosts thousands of microorganisms distributed across various anatomical sites, maintaining a stable, symbiotic, and mutually beneficial relationship with their hosts. Macrogenomic studies of the gut microbiota in healthy individuals have revealed considerable differences in its composition across individuals [1-3]. Each person possesses a unique gut microbiota shaped by their genetic background, physiological status, microbial interactions, environmental factors, and diet [4-6].

The relationship between the microbiome and its influences is intricate and bidirectional. External factors induce compositional changes that stabilize into an adapted microbiome state, while the microbiome also provides feedback to the host through mechanisms such as the production of specific metabolites. Over 500 microbial species inhabit the human gut, with microbial diversity typically increasing from infancy to around three years of age, at which point it reaches levels comparable to those of adults [7].

Advances in science and technology have significantly enhanced the understanding of the types of microbes present in the gut, their functions, and their roles in human health and disease. The gut microbiota is now recognized as an anaerobic bioreactor capable of synthesizing molecules that directly influence the mammalian immune system, modify the human epigenome, and regulate host metabolism [8-10].

1.2. Stages of research on the gut microbiota

In recent years, extensive research has been conducted to evaluate the correlation between gut microbiota, disease, and external environmental factors. As the depth and scope of studies continue to expand, metagenome-wide association studies (MWAS) have emerged as a focus for scientists [11]. The relationship between gut microbiota and disease remains complex. For example, in some patients with colorectal cancer [12] or arthritis [13], specific marker taxa are associated with the disease but exert a minimal effect on the overall microbial composition, such as the reduced abundance of particular bacterial species. Conversely, certain disease states are significantly linked to broader compositional changes in the microbiota. For instance, reduced species diversity or richness has been observed in patients with obesity [14] or inflammatory bowel disease [15].

It remains unclear in most cases whether microbiota dysbiosis triggers the onset of the disease or whether the disease itself induces changes in the microbiota. Furthermore, recent studies have demonstrated limited ability to explain microbial variations [16], potentially due to the low accuracy of current taxonomic classification systems [17].

1.3. Methods for studying the gut microbiota

The development of new experimental techniques and methods is crucial for advancing the study of gut microbiota. However, these methods face inherent limitations, such as amplification bias [18], primer bias [19], and restricted functional insights [20]. Whole-genome analysis offers advantages, including the ability to provide information on the relative abundance of functional genes, high-resolution identification, and population-averaged genomes through gene assembly [21,22]. However, it also presents challenges, such as bias introduced by host DNA or organelle contamination, library construction, and the assembly and annotation of reference databases. Additionally, this method struggles to distinguish between samples.

While transcriptome sequencing analysis captures intra-individual microbial dynamics [23] and directly assesses microbial activity (e.g., interference or exposure) [24], it is one of the most expensive, labor-intensive, and complex techniques [25]. It also requires the exclusion of host mRNA and is prone to contamination by rRNA [26].

To address these challenges, multi-omics analysis, absolute quantitative microbial analysis (QMP), and other methods have been developed. Multi-omics approaches complement macro-genomic studies by integrating macro-transcriptomic, macro-proteomic, and macro-metabolomic analyses [27,28]. In recent years, metabolomic studies have been employed to assess associations between gut flora, metabolites, and diseases, such as the relationships between serum metabolites and type II diabetes [29]. Additionally, macro-transcriptomic studies can directly reveal microbial gene expression and provide insights into potential microbial functions [30,31]. In contrast, macro-proteomic analyses remain limited, with only a few pilot-scale studies conducted to date [32,33]. The characteristics, advantages, and limitations of these various methods are summarized in **Table 1**.

Despite their potential, multi-omics studies face several challenges. The integration of heterogeneous data types and compositions creates a complex chain of evidence that must be analyzed holistically [27,33]. This complexity also affects the understanding of key microbiome concepts, such as the significance of functional plasticity [34]. Absolute quantitative microbiological analyses offer improvements in sensitivity and accuracy for microbiome association studies. These advances are primarily achieved through the use of internal markers [35], the

introduction of exogenous bacteria to quantify absolute bacterial abundance [36], and flow cytometry [37].

2. The gut microbiota and hepatic encephalopathy

2.1. Hepatic encephalopathy

Hepatic encephalopathy (HE) refers to brain dysfunction caused by hepatic insufficiency and/or portal system shunting. It encompasses a continuum of symptoms ranging from cognitive impairment to coma, with key clinical manifestations including altered consciousness, behavioral disturbances, and coma [38]. The association between liver disease, particularly jaundice, and emotional or behavioral disturbances dates back to Hippocrates, the father of Western medicine (460–371 B.C.) [39]. However, experimental studies in the late 19th and 20th centuries began elucidating the pathophysiological mechanisms underlying this relationship, identifying behavioral changes as consequences of chronic liver insufficiency and liver disease.

HE is classified into covert hepatic encephalopathy (CHE) and overt hepatic encephalopathy (OHE) based on the severity of its clinical manifestations [40]. It is well-documented that HE is a primary cause of hospitalization in patients with cirrhosis. Evidence suggests that OHE occurs in 30–40% of patients with cirrhosis during their clinical course [41].

2.2. Occurrence and progression

HE represents a typical model of gut-liver-brain axis disease, although its pathogenesis remains unclear. There is growing consensus that alterations in gut microbial composition and its metabolic by-products, local and systemic inflammation, and a compromised intestinal barrier (leaky gut) collectively contribute to the development of HE [42]. Among the microbial by-products, indole and ammonia are particularly neurotoxic. Indole interacts with voltage-gated sodium channels in the brain, acting as a sedative that induces coma in both human and animal models [43,44]. Ammonia disrupts pH levels, membrane potential, cellular metabolism, and neurotransmission, leading to astrocyte swelling and brain edema [45].

The composition of the sigmoid colon microbiota in patients with HE differs significantly from that of healthy individuals [46]. In HE patients, the abundance of *Roseburia* is reduced, while *Enterococcus*, *Veillonella*, *Megasphaera*, and *Burkholderia* are elevated. Cognitive performance and lower inflammation markers have been associated with *Blautia*, *Faecalibacterium*, *Roseburia*, and *Dorea*, whereas cognitive deficits correlate with *Enterococcus*, *Streptococcus*, *Burkholderiaceae*, *Veillonellaceae*, *Megasphaera*, *Rikenellaceae*, *Alistipes*, *Streptococcaceae*, *Alcaligenaceae*, *Sutterella*, *Porphyromonadaceae*, and *Parabacteroides*. Notably, *Alcaligenaceae* produce ammonia via urea degradation, potentially linking them to cognitive impairments. Bajaj *et al.* also reported that *Enterobacteriaceae*, *Fusobacteriaceae*, and *Veillonellaceae* are positively correlated with inflammation, while *Ruminococcaceae* are negatively correlated [47].

The association between altered gut microbiota and neurological deficits in patients with cirrhosis (with or without HE) has been further clarified through nuclear magnetic resonance spectroscopy and magnetic resonance diffusion tensor imaging. Patients with HE exhibit an increased abundance of *Staphylococcaceae*, *Enterococcaceae*, and *Porphyromonadaceae* compared to those without HE [48]. Animal studies have shown that *Porphyromonadaceae* is linked to cognitive dysfunction and the development of fatty liver disease [46,49,50]. Brain MRI spectra have revealed positive correlations with *Streptococcaceae*, *Enterobacteriaceae*, and *Lactobacillus*, as well as negative correlations with *Spirulinaceae*, Ruminococcaceae, and *Clostridium perfringens*. It has been established that *Spirulinaceae*, *Clostridium tumefaciens*, and *Clostridium tetradecium* dominate healthy gut

microbiota, contributing to short-chain fatty acid (SCFA) production and bile acid 7-alpha dehydroxylation [51,52]. As cirrhosis progresses, the abundance of *Lactobacillaceae* and *Streptococcaceae* decreases, while potentially harmful bacteria, such as *Streptococcaceae* and *Enterobacteriaceae*, increase [53]. Interestingly, Ahluwalia *et al.* reported an increase in *Lactobacillaceae* in the fecal samples of HE patients and cirrhosis mouse models [48,54].

Patients with cirrhosis are particularly susceptible to dysbiosis due to the diverse pathological interactions between the liver and gastrointestinal tract, which have significant clinical implications. Altered intestinal dynamics, elevated gastric pH, and reduced colonic bile acid concentrations in cirrhotic patients can lead to uncontrolled bacterial overgrowth. Furthermore, cirrhosis impairs the liver's ability to regulate systemic immune responses. Compared to controls, cirrhotic patients exhibit increased monocyte proliferation and chemotaxis but significantly reduced neutrophil activity [55]. This disruption compromises the intestinal barrier, promotes bacterial translocation, and heightens the risk of intestinal bacterial infections and liver failure [56,57].

3. Treatment of hepatic encephalopathy

Current clinical treatments for HE primarily focus on regulating gut microbiota to reduce pathogenic bacteria, bacterial urease activity, and intestinal pH. This, in turn, decreases ammonia production and absorption [51,52,58]. Common treatment methods include dietary interventions, lactulose, the antibiotic rifaximin, probiotics, and fecal microbiota transplantation (**Figure 1**). Although these approaches have demonstrated therapeutic efficacy, concerns persist among some experts regarding their long-term effectiveness and potential side effects in clinical practice. This underscores the need to develop novel treatment strategies informed by a comprehensive understanding of the underlying mechanisms of gut microbiota.

3.1. Dietary interventions

As gut microbiota are closely linked to dietary habits and play a pivotal role in HE pathogenesis, dietary interventions have been proposed as a potential treatment for alleviating HE symptoms [59]. Traditionally, it was believed that protein catabolism increased ammonia levels, leading to recommendations for protein restriction in patients with HE. However, recent studies have shown that normal protein intake is both well-tolerated and beneficial in HE, ensuring sufficient substrates for energy synthesis and liver function [60,61]. Consequently, experts now strongly recommend avoiding protein restriction in patients with HE.

3.2. Lactulose

Lactulose, a synthetic disaccharide composed of lactose and galactose, is classified as a prebiotic. A distinctive feature of prebiotics is their resistance to absorption in the gastrointestinal tract. Lactulose, along with other non-absorbable disaccharides such as inulin, fructooligosaccharides (FOS), and galactooligosaccharides (GOS), stimulates the growth and activity of beneficial gut bacteria, such as bifidobacteria [62].

Lactulose reduces ammonia production and absorption through several mechanisms:

(1) Osmotic effect: It increases osmotic pressure and lowers pH in the intestinal lumen [63].

(2) Ammonia utilization: It promotes bacterial uptake of ammonia for protein synthesis [64].

(3) Inhibition of glutaminase activity: It reduces intestinal glutamine absorption and its subsequent conversion to ammonia [65].

Numerous studies have focused on the role of lactulose in improving quality of life and cognitive function

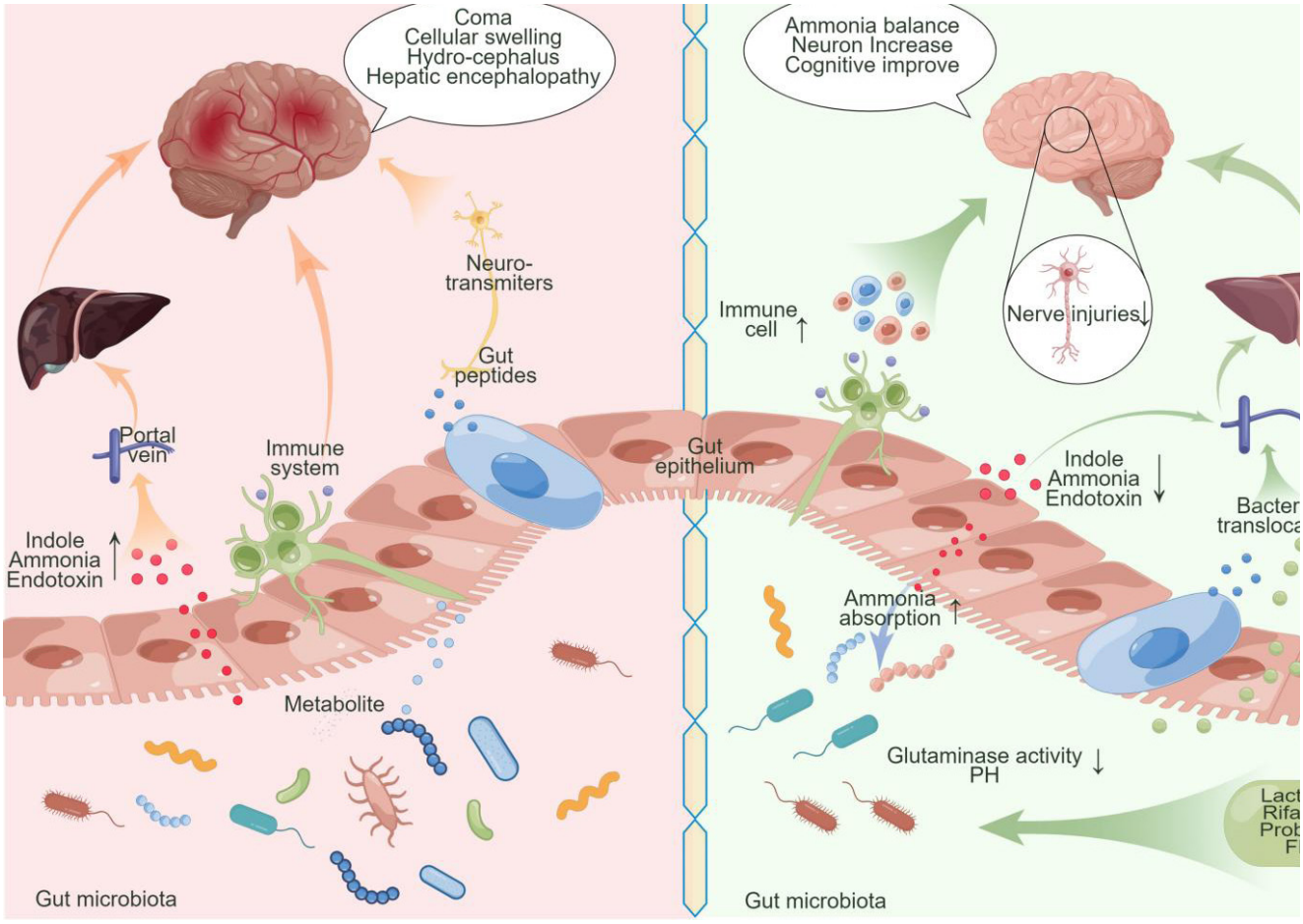

Figure 1. Schematic representation of microbiota in the progression of hepatic encephalopathy. In HE, gut dysbiosis increases metabolites like indole, ammonia, and endotoxins, which affect the brain via the portal venous, immune, and nervous systems, leading to coma, cell swelling, hydrocephalus, and HE. Treatments such as lactulose, rifaximin, antibiotics, and FMT reduce pathogenic bacteria, bacterial urease activity, glutaminase activity, and pH, while decreasing bacterial translocation. These interventions lower the production or absorption of ammonia, indole, and endotoxins, enhance immune function, reduce brain damage, restore ammonia balance, increase neurons, and improve cognition.

in HE patients. In 2014, the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) jointly recommended lactulose as a treatment for overt HE [38]. Compared to placebo or no intervention, lactulose significantly reduces the risk of overt HE, lowers blood ammonia levels, and enhances health-related quality of life [66].

In patients with mild HE, lactulose decreases arterial ammonia levels, inflammatory markers (e.g., TNF-α, IL-6, IL-18), and serum endotoxins [67]. Animal studies have demonstrated that lactulose increases neuroplasticity by promoting neurite growth and enhancing the formation of new neurons in the hippocampus. Lactulose also exerts neuroprotective effects by increasing glial fibrillary acidic protein (GFAP)-immunoreactive cells [68].

Furthermore, lactulose has been shown to reduce bacterial DNA translocation in mild HE patients, resulting in lower serum ammonia levels and improved neurocognitive performance [69]. Approximately one-third of mild HE patients experience inflammatory bacterial antigen translocation, which lactulose reduces to 16%. This effect has also been observed in a rat model of acute liver failure, suggesting that lactulose inhibits bacterial translocation

and alleviates HE symptoms by improving intestinal permeability, accelerating intestinal transit, and reducing small intestinal bacterial overgrowth [69,70].

3.3. Rifaximin

Rifaximin, a derivative of rifamycin, inhibits bacterial RNA and protein synthesis by irreversibly binding to the β-subunit of bacterial DNA-dependent RNA polymerase [71]. It targets a broad spectrum of intestinal aerobic and anaerobic bacteria [72]. In cirrhotic patients with HE-related symptoms, rifaximin has been shown to lower serum ammonia levels, significantly improve neurological signs and symptoms of overt HE, prevent HE episodes, and reduce hospitalization rates [73,74].

Rifaximin has also demonstrated efficacy in treating acute HE [75]. In two long-term randomized, non-blinded studies, rifaximin improved neurological and neuromotor abnormalities associated with cirrhosis and reduced the recurrence of HE episodes [76,77]. Bajaj *et al.* further highlighted rifaximin's effectiveness in preventing HE relapse [78].

Short-term rifaximin administration reduces blood ammonia levels, improves psychometric test scores, and decreases small intestinal bacterial overgrowth [79]. Moreover, rifaximin has a direct impact on intestinal barrier function and the metabolome [80,81]. A study investigating metabolic and microbial changes following rifaximin treatment found increased levels of eubacteria and beneficial bacterial species, reduced oxidative stress, and decreased production of aromatic amino acids and nitrogen. A reduction in *Verrucomicrobiaceae* levels was also observed in fecal samples. The development of HE, particularly mild HE, has been linked to increased Eubacterium vulgaris in the feces and colonic mucosa of cirrhotic patients [82].

Overall, research indicates that rifaximin improves HE by modulating bacterial metabolic function rather than altering overall bacterial abundance.

3.4. Combined treatment

Sharma *et al.* conducted a prospective randomized study involving 120 patients with cirrhosis to evaluate the synergistic effects of rifaximin and lactulose in the treatment of overt hepatic encephalopathy (OHE). The combination therapy of rifaximin and lactulose was found to be significantly more effective in achieving complete regression of HE compared to lactulose alone (76% vs. 44%, respectively) [83]. Additionally, the combined treatment reduced mortality rates in OHE patients relative to lactulose monotherapy.

The impact of combined rifaximin and lactulose therapy on the composition of mucosal flora was also investigated. This combination significantly reduced the abundance of *Rothschildia spp*., *Lauterichia spp*., and *Veronococcaceae*, while increasing the abundance of *Propionibacterium spp*. compared to lactulose alone [47]. Another study demonstrated that combined treatment with lactulose and rifaximin was more effective than monotherapy in improving cognitive function and reducing ammonia levels. Collectively, these findings suggest that combination therapies can enhance treatment efficacy by targeting multiple physiological levels [84].

3.5. Probiotics

In addition to sugars and antibiotics, probiotics play a crucial role in the treatment of HE. Probiotics have been shown to be effective in managing irritable bowel syndrome [85], ulcerative colitis [86], and non-alcoholic fatty liver disease [87], with an even more pronounced impact on HE. Studies indicate that probiotics increase the abundance of beneficial flora, reduce pathogenic bacteria, lower physical and psychosocial disease impact scores, and significantly reverse minimal hepatic encephalopathy (MHE), thereby reducing the occurrence of OHE [88,89].

The therapeutic rationale for probiotics is based on the hypothesis that the pathogenesis of HE is linked to harmful microbial by-products, such as ammonia and indoles. The increased concentration of these toxic metabolites, combined with the impaired clearance function of the diseased liver, results in significant pathophysiological effects. Probiotic supplementation helps reduce ammonia levels by promoting the growth of beneficial bacteria, such as *Bifidobacteria* and *Lactobacilli*, thereby restoring balance to the intestinal microbiota [90].

A recent study further revealed that probiotics significantly reduced levels of C-reactive protein, tumor necrosis factor (TNF), FABP-6, and claudin-3, while markedly increasing neutrophil oxidation in HE patients receiving probiotic intervention [91]. These effects contributed to maintaining intestinal flora homeostasis by enhancing immune adaptability.

3.6. Fecal microbiota transplantation

Although lactulose and rifaximin are standard treatments for HE, recurrent HE is associated with high rates of disability and mortality. Additionally, both treatments are associated with issues such as microbial resistance and adverse side effects. Probiotics, while beneficial, have not been shown to be superior to lactulose or antibiotics in achieving remission in HE patients [92], highlighting the need for novel therapeutic approaches.

Growing research into advanced liver cirrhosis and HE has recognized the potential of fecal microbiota transplantation (FMT) as a treatment for recurrent HE. In one case study, a male patient with HE (MELD score 10) received FMT over five consecutive weeks. Improvements in concentration, serum ammonia levels, and quality of life were observed during the study period, with no hospitalizations reported. However, the beneficial effects of FMT did not persist after discontinuation, suggesting that heterologous microbiota did not colonize the new host, and repeated treatments may be necessary to maintain the therapeutic effect [93].

Larger sample sizes are needed to support and validate these findings. A recent non-blinded randomized controlled trial (RCT) evaluating the safety of FMT for recurrent HE reported a reduced incidence of serious adverse events in the FMT group (20%) compared to the control group (80%). FMT also increased the relative abundance of commensal bacterial groups, such as *Lactobacillaceae*, *Bifidobacteriaceae*, and *Ruminococcaceae* [94].

Furthermore, antibiotic pretreatment combined with FMT has been shown to improve intestinal dysbiosis and reduce hospitalizations. Bajaj et al. demonstrated that FMT restores the diversity of intestinal microbiota diminished by antibiotic use, while also addressing changes in short-chain fatty acids and bile acids [95]. Over longer periods, patients with HE who received antibiotic pretreatment combined with FMT exhibited improvements in clinical symptoms and cognitive function [96].

These findings confirm the significant therapeutic potential of FMT in treating HE. However, current studies primarily focus on the structural and functional changes in intestinal microorganisms and the safety of FMT [97]. Further research is required to identify the specific effective flora and metabolites, as well as the precise pathways and mechanisms underlying FMT's therapeutic effects.

4. Conclusion and perspective

The gut microbiota plays a pivotal role in human health and disease. Correcting microbiota dysbiosis and restoring normal gut microbiota has been reported to alleviate disease symptoms and complications, including advanced severe liver diseases such as cirrhosis and hepatic encephalopathy (HE). However, current clinical research on HE primarily focuses on cognition, metabolites, the inflammatory environment, and the composition and function of

intestinal microbiota, with several limitations.

Most studies investigating the treatment of diseases through the improvement of intestinal flora are restricted to animal models or isolated cases. While these studies have yielded promising results, differences in systems and physiology between humans and animals pose significant challenges in generalizing these findings to humans. Furthermore, in animal studies involving mice, factors such as fecal feeding behavior and cage effects can contribute to inaccuracies [98].

It is important to note that the efficacy of intestinal microbiota-based treatments depends on factors such as the donor, the composition of the microbiota, the route of transplantation, and other variables. Additionally, the lack of long-term follow-up studies and appropriate controls hinders the assessment of potential adverse reactions, thereby affecting the broader application and promotion of these treatments.

Moreover, research on the role of gut microbiota in the early development of the nervous system is limited, and it remains unclear whether gut microbiota influences the occurrence and progression of related diseases in adulthood. To confirm that adjustments to intestinal flora can improve patient symptoms and prevent the progression of HE, more randomized controlled trials are required. Such studies should focus on changes in the composition of intestinal flora, the features of its metabolic products, their impact on the host, and the specific mechanisms involved.

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Disclosure statement

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