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The Gut Microbiome in Alcohol Use Disorder: Implications for Health Outcomes and Therapeutic Strategies - A literature review

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Abstract

Alcohol use disorder (AUD) represents a major public health issue which affects millions of people globally and consist a chronic relapsing condition associated with substantial morbidity and mortality. The gut microbiome plays a crucial role in maintaining overall health and has emerged as a significant contributor to the pathophysiology of various psychiatric disorders. Recent evidence suggests that the gut microbiome is intimately linked to the development and progression of AUD, with alcohol consumption directly impacting its composition and function. This review article aims to explore the intricate relationship between the gut microbiome and AUD, focusing on the implications for mental health outcomes and potential therapeutic strategies. We discuss the bidirectional communication between the gut microbiome and the brain, highlighting the role of microbiota-derived metabolites in neuroinflammation, neurotransmission, and mood regulation. Furthermore, we examine the influence of AUD-related factors, such as alcohol-induced gut dysbiosis and increased intestinal permeability, on mental health outcomes. Finally, we explore emerging therapeutic avenues targeting the gut microbiome in the management of AUD, including prebiotics, probiotics, and fecal microbiota transplantation. Understanding the complex interplay between the gut microbiome and AUD holds promise for developing novel interventions that could improve mental health outcomes in individuals with AUD.

Keywords: alcohol use disorder; gut microbiome; dysbiosis

Core tip: The emerging field of research on the gut microbiome's role in AUD has revealed significant implications for health outcomes and potential therapeutic strategies. Alcohol consumption has profound effects on the gut microbiome, leading to dysbiosis and increased systemic inflammation but their association has been found bidirectional. The gut microbiome represents a promising therapeutic target for the treatment of AUD, with dietary interventions such as probiotics and prebiotics, as well as fecal transplantation showing potential in improving gut dysbiosis and reducing inflammation.

Introduction

Alcohol use disorder (AUD) represents a major public health issue which affects millions of people globally. AUD is characterized by excessive drinking and persistent alcohol-seeking behavior. It has been described as a single spectrum of problematic use and clinically significant impairment based on endorsement of at least two of a total of 12 criteria that assess behavioral and physical manifestations of heavy alcohol consumption according to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V). The terms alcohol abuse and alcohol dependence fall under the umbrella of the general term alcohol use disorder (AUD), and can be can be classified as mild (if patient meet 2 or 3 criteria), moderate (if patient meet 4 or 5 criteria), or severe AUD (if patient meet more than 6 criteria). [1]

The prevalence of AUD is increased in high/upper middle-income countries in both males and females. Some estimates show almost 6% of individuals meet the AUD criteria, leading to significant socioeconomic problems and public health losses. [1,2]

The worldwide prevalence of heavy episodic drinking surpassed 18% of total population in 2016. [3] Nevertheless, alcohol use and its effects present substantial variations across different countries. The European Union (EU) is the region with the highest alcohol consumption at a global scale, with 87% of its adolescents having consumed alcohol at least once during their lifetime, even higher compared to the United States' 70% of adolescents. [4]

Alcohol abuse is responsible for approximately 3 million deaths per year (5.3% of all deaths), along with more than 5% of the disease burden globally according to the WHO. [5] Alcohol use disorder is attributed as a causal factor for a plethora of diseases, which include communicable diseases, such as maternal, perinatal and nutritional conditions, and non-

communicable diseases, such as epilepsy, cancer, cardiovascular, digestive diseases and injuries.[3] While the physical health consequences of AUD have been well-described, the impact of alcohol on mental health is a matter of ongoing deliberation. The interplay between alcohol consumption, mental health, and physical health outcomes is complex and multifaceted. AUD syndrome is the result of cumulative effects caused by excessive alcohol consumption, a person's genetic susceptibility, and several environmental factors, as such, deep understanding of its pathophysiology could be vital for the development of an effective treatment. [6]

Abundant evidence have highlighted that alcohol dependence (alcoholism) is a complex genetic disease, with a heritability estimate as high as 50%, and a large number of variants across the genome influencing the onset and development of a person's addiction to alcohol , with some of these genes being involved in alcohol metabolism.[1] Acquiring a better understanding of the way that the environment influences genetic risk which contributes to the onset of alcoholism is of major importance in deciphering the underlying mechanisms of alcohol use disorders. [7]

The gut microbiome, the complex ecosystem of microorganisms residing in the gastrointestinal tract, has risen as a fascinating field of study, as it affects several physiological processes, including digestion, metabolism, and immune function. Recently, the gut microbiome is referred to hold a substantial role in the pathophysiology of mental illnesses such as schizophrenia, bipolar disorder, anxiety disorders, depression and AUD. Preclinical studies have indicated the influence of gut microbiota in the gut-brain axis and the bidirectional interactions between the central nervous system, the enteric nervous system, and the gastrointestinal tract, potentially affecting mental health outcomes. [8]

In this review, we will discuss the latest findings regarding the changes in gut microbiome associated with AUD, and how they contribute to the development and progression of the disorder. We will further discuss the potential mechanisms through which gut dysbiosis leads to implications on health outcomes. Additionally, we will delve into the potential therapeutic approaches targeting the gut microbiome for the treatment of AUD, such as probiotics, prebiotics, dietary interventions and fecal transplantation. By considering the gut microbiome when evaluating and treating individuals with AUD, clinicians may be able to improve the health outcomes of these patients and reduce the burden of disease associated with AUD.

Methods

A comprehensive literature search was conducted using the PubMed database to identify relevant articles for this review. The following search terms were used: "gut microbiome," "alcohol use disorder," "alcohol abuse," "alcohol consumption," "microbiota," and "microbiome." This review focused on articles published in the English language between 2010 and 2022, to ensure the inclusion of recent research while capturing significant developments in the field.

The initial search yielded a broad range of articles related to the gut microbiome and AUD. After careful evaluation, articles were selected based on their relevance to the topic. Studies that investigated the changes in gut microbiome composition and function in individuals with AUD, as well as those which explored the impact of alcohol on gut dysbiosis and associated health outcomes were included. Additionally, articles focusing on therapeutic strategies targeting the gut microbiome for the treatment of AUD were considered.

The selected articles were thoroughly reviewed, and the key findings, methodologies, and conclusions were extracted. Data related to the mechanisms linking alcohol consumption to gut dysbiosis, the implications of gut dysbiosis on health outcomes, and the potential therapeutic approaches targeting the gut microbiome were synthesized and organized in a coherent manner.

By analyzing the available literature and synthesizing the findings, this review intends to contribute to the growing body of knowledge on the gut microbiome's role in AUD and provide valuable insights for future research and clinical practice.

The composition of gut microbiome in healthy vs AUD individuals

[1] The human body is inhabited by a vast number of microorganisms that live in concordance with their host and are commonly referred to as human microbiota or microflora. The largest proportion of microbiota, approximately 70%, can be found in the gut. [1] Intestinal microflora is involved in host's physiology, regulating digestion, vitamin

production, metabolism of xenobiotics, immunological responses and at the same time conferring protection against pathogen perturbation. [9,10]

Although it was originally believed that gut contains about 1000 bacterial species, a large scale study has estimated that the collective human gut microbiota is composed of over 35000 bacterial species. Overall, the healthy gut microbiota is predominantly constituted by strict anaerobes that dominate over anaerobes from the phyla Firmicutes and Bacteroidetes. This is followed by the phyla Actinobacteria, and Proteobacteria, with minor proportions of species belonging to the phyla of Fusobacteria, Tenericutes, and Verrucomicrobia. Despite the constant appearance of this general profile remains constant, gut microbiota can exhibit temporal and spatial differences regarding distribution at the genus level and higher classification. [10, 11]

A microbiome that has been linked with a healthy host, requires an extent of resistance towards external (e.g. dietary, pharmaceutical) and internal (e.g. age) changes and the ability of resilience afterwards. Thus, microbial health does not reflect a static but rather a dynamic state. Any such disturbance could jeopardize the balance of the composition and/or regulation of microbial communities, a condition that is commonly described as dysbiosis. This condition is far more likely to occur due to inadequate presence of commensal microorganisms, possible alteration of regular microbial diversity, and competition between commensal and pathogenic species for a particular body region or nutrients. Additional external factors that favor the progression of a dysbiotic state involve malnutrition, as in the case of low dietary fibers or vitamins, food additives (e.g. preservatives, emulsifers), chronic alcohol consumption, drug abuse or certain medication (most often antibiotics, over-the-counter anti-inflammatories and chemotherapeutics), exposure to hazardous environmental agents (toxins, heavy metals or radiation), and increased stress levels (anxiety, depression). Current literature suggests that mental health disorders (as in the case of drug and alcohol abuse) are often correlated with dysbiosis. [12] Alcohol abuse is known to cause deleterious effects all over human body. Despite that, it remains to be proved whether alcohol drinking is the cause or the consequence of changes in the gut microbiota. As stated above, alcohol consumption can cause significant imbalances to the gut microbiome such as the promotion of potentially pathogenic bacteria in alcoholics with and without liver disease, resulting in dysbiosis. De Timary et al (2015), observed no differences in alcohol intake between the

dysbiotic and non-dysbiotic group, while the total concentration in bacteria and in most bacterial families, genera or species failed to recover after detoxification, suggesting that the difference in composition of the gut microbiota might not be the consequence of drinking, which raises the issue of whether the alteration in the gut microbiota could be a precursor to the development of alcohol-dependence in some subjects. [13] The majority of data deriving from human-based studies show a link between alcohol intake and bacterial abundance that does not imply causality. Current knowledge suggests that innate gut bacteria may bey key influencers of voluntary alcohol intake in rats that have been selectively bred for their increased ethanol intake. Administration of antibiotics that are non-absorbable prior to ethanol access has been reported to inhibit approximately 70% of volunteray ethanol intake. This effect has been fully observed during the first day of access to alcohol. The available data suggest that the firewall mechanisms that typically prevent increased alcohol intake tend to be suppressed by endogenous microbiota of a rat strain that is selected for their preference towards alcohol [14] Carbia et al demonstrated that the most typical patten of alcohol misuse in adolescence is linked with alterations of the gut microbiome, even prior to the development of addiction. [15] An animal- based study of Segovia-Rodríguez et al. shed light to whether intestinal microbiota could be the cause of increased alcohol intake in animals that received fecal transplantation from alcohol-dependent laboratory animals, leading to a higher alcohol consumption and to a reduced spontaneous locomotor activity compared to animals which received transplant from control animals or those treated with the buffer without feces. [16,17] This finding indicated that alterations that were induced by microbiota would affect the host in a more universal fashion. Authors proposed a synergistic mechanism of interaction between the new microbiota received and alcohol consumption. A plausible explanation of the bidirectional effect is that in the presence of alcohol, microbiota can promote a positive feedback mechanism that favors the abundance of bacteria that benefit from alcohol intake. [17] These alterations reflect a dysregulation in the microbiome-gut brain axis that could further trigger dysregulation and lead to an even more increased risk of psychopathology and especially when appearing during key windows across a person's lifetime.[15]

Most data about the influence of alcohol on the relative abundance of gut microbiome comes from animal-based studies; expose to alcohol does not alter the abundance of gut microbiota, but remarkably alternates its composition. In animal models, alcohol intake decreases the relative abundance of the genera Lactobacillus (or Sporolactobacillus) and promotes the relative abundance of the genera Blautia, Allobaculum,[18] Ruminococcus,

Coprococcus,[19] Adlercreutzia and Turicibacter,[20] Alistipes and Odoribacter,[21] resulting in memory loss, and neuropsychiatric behaviors, like anxiety and depression-like disorders. [18, 20, 21]

Different patterns of drinking, such as chronic drinking (chronic) or recent heavy drinking (acute) could lead to a different composition of gut microbiota.[22] Specifically, in a mouse model with acute alcohol consumption, an upregulation of the phyla Actinobacteria and Verrucomicrobia and the genera Bacteroidales and Lachnospiraceae was recorded,[23] while in chronic alcohol consumption Bacteroidetes, Bacteroides genus, and Akkermansia genus were present at a higher proportion.[22] Furthermore, acute alcohol consumption decreased the levels of Lactobacillus, Escherichia-Shigella, and Turicibacter, [23] while in chronic alcohol intake the relative abundance of Firmicutes phylum, Lactococcus, Pediococcus, Lactobacillus, and Leuconostoc genus was downregulated.[22]

Even though alcohol consumption could be a critical factor that influences gut microbiome in terms of function and composition, alcohol metabolism itself, along with its effects on the patient/consumer can be influenced by the microbiome, establishing a bidirectional relationship. However, existing data on alcohol's effect on gut microbiome in humans are scarce. The first studies dealing with changes in the gut microbiome of individuals with AUD concluded a reduction in the abundance of beneficial bacteria such as Bacteroidetes, Lactobacillus and Bifidobacterium, and an increase in potentially harmful bacteria, such as Enterobacteriaceae, Streptococcus and Proteobacteria. [24,25]

On the other side, alcohol dependents with higher intestinal permeability seems to present a more unique profile with a sharp reduction in the abundance of Ruminoccaceae family (and in particular in Rumminococcus, Faecalibacterium, Subdoligranulum. Oscillibacter and Anaerofilum), and an increase in Lachnospiraceae and the genera Blautia and Megasphaera.[25]

Dubinkina et al. (2017) were the first to describe the gut metagenome of patients with alcoholic disorder using shotgun (whole-genome) metagenomic sequencing. They found significant differences on the gut microbial community in gut dysbiosis, when comparing alcoholics with and without liver disease,[26] as opposed to Mutlu et al. (2012), who reported similarities between the two groups.[24] Dubinkina et al. reported only a slight overlap in the metagenomic signature of alcoholics with and without liver disease. Increased populations of Klebsiella and decreased Coprococcus, *Faecalibacterium prausnitzii*, and unclassified

Clostridiales were associated with alcoholics without liver cirrhosis, whereas both groups were characterized by reduced *Acidaminococcus sp*, as well as increased Lactobacilli and Bifidobacterium members, however with different species in each group. [26] Most recently, Bjørkhaug *et al.* (2019) confirmed via sequencing the higher relative abundance of Proteobacteria in alcohol overconsumers in a dose independent manner. Their results revealed reduced relative abundance of Faecalibacterium, a plethora of taxonomic groups inside the Firmicutes phylum, and specifically in the Clostridia and Actinobacteria class in the group of alcohol overconsumers, and a higher relative abundance of Sutterella, Clostridium, and Holdemania.[27]

A more recent meta-analysis highlighted the causal role of alcohol in gut dysbiosis confirming that alcohol consumption could favor the proliferation of some bacterial species in the gut, such as the already mentioned Bacteroidetes and Proteobacteria and suppress other species like the probiotic Lactobacillus and Bifidobacterium and the physcobiotics *Faecalibacterium prausnitzii* and *Akkermansia muciniphila*.[28]

However, the gut microbiome does not consist of bacteria only, but also includes viruses and fungi. Major differences have been observed regarding the fecal viromes of AUD patients, specifically in the composition of bacteriophage species. At least 18 bacteriophages were found to be more abundant in the control subjects, including eight bacteriophages that target Propionibacterium, five that target Enterobacteria, while the remaining seem to target Salmonella, Lactobacillus, Cronobacter, Escherichia, and Leuconostoc. Concerning the bacteriophage species which were more abundant in the AUD patient population, the findings include two bacteriophages that target Streptococcus and two that target Lactococcus. [29] Concerning mycobiome, there are scarce data investigating fungal abundance differentiation in AUD patients compared to non-alcoholic individuals. An increase in the abundance of Candida [30-33], Pichia[30,33] Kluyveromyces, Issatchenkia and Scopulariopsis [33], genus level has been reported, while Saccharomyces, Penicillium, [31], and Epicoccum[30], presented lower levels. Literature data are conflicting regarding Debaryomyces. [30,31,33] (Picture1)

Implications of Gut Dysbiosis in AUD

The dysbiosis observed in the gut microbiome of individuals with AUD can have significant implications for health outcomes. One of the most notable consequences is the increased risk of developing liver disease. Chronic alcohol consumption leads to imbalanced gut microbiome, which in turn causes hepatocytes damage, leading to the development of alcoholic liver disease (ALD), ranging from steatosis to cirrhosis. Gut dysbiosis has also been linked to the development of other chronic diseases, including cardiovascular disease and type 2 diabetes. Gut microbiota may influence brain function through neural, endocrine, and immune pathways [34] related to vagus nerve signaling.[35] In order to ameliorate the health outcomes of gut dysbiosis, it is essential to understand the role of the gut microbiome in health maintenance, and how an imbalance could affect the human body.

Inflammation, a hallmark of gut dysbiosis, plays a pivotal role in the development of these diseases. Research has shown that gut dysbiosis can lead to the production of pro-inflammatory cytokines, lipopolysaccharides (LPS), and bacterial endotoxins, resulting in increased inflammation, oxidative stress, insulin resistance and endothelial dysfunction, and other widespread effects on the body, further exacerbating the detrimental health outcomes associated with AUD.[36] The intestinal mucosa can be detrimentally affected by inflammation caused by microbiome metabolites or ethanol, leading to damage and increased permeability. Although the exact mechanism has not yet been characterized, alcohol may lead to gut inflammation via alterations in the gut microbiota by promoting an increase in pro-inflammatory bacteria and/or decrease in anti-inflammatory bacteria, and affecting cytokine expression. Accordingly, in an animal model treated with human gut microbiota, the relative population of a pro-inflammatory bacterium (Clostridium cluster XIVa) increased, whereas there was a decrease in anti-inflammatory bacteria (*Akkermansia muciniphila*, Atopobium, *Faecalibacterium prausnitzii*), compared to untreated.[37] Interestingly, this kind of alcohol-induced dysbiosis was found to mediate intestinal barrier dysfunction, as well as alcoholic liver disease, via activation of tumor necrosis factor receptor I, in intestinal epithelial cells. Inflammation is primarily facilitated via introduction of leukocytes, as well as the presence of inflammation mediators, such as histamine, ROS and leukotriene. Mucin can be altered by reduced Mucin 2 (MUC-2) expression, which in turn is caused by increased matrix metalloproteinase-9 (MMP-9) expression by epithelial cells. MMP-9 can also be induced by Claudin-1 up-regulation, inhibiting goblet cell

differentiation via Notch signalling, also leading to inflammation, due to the resulting reduction in MUC-2 expression.[37]

The amount of episodes that involve binge drinking are associated with increased responsiveness of the stimulated cytokines (IL-6 and IL-8). Moreover, blood cytokine response is stimulated(mostly increases via the TLR4 triggered cytokines IL-6, IL-8, Il-1b), as the frequency of the binge drinking events increases. Additionally, craving behavior is linked to increased levels of circulating markers of inflammation in AUD patients.[15]

Release of endotoxins by dysbiotic microbiota in the gut was also found to induce production of pro-inflammatory cytokines and ROS by the stimulated hepatic Kupffer cells. [38,39] Even a sole binge drinking event can elevate serum endotoxin, most possibly due to translocation of products derived from gut bacteria and disturbance of the innate immune response, which contribute to the deleterious effects of binge drinking.[15] For instance, gut microbiota possibly affect the intestinal barrier's integrity. The following release of cytokines could signal to the brain via activation of the vagus nerve or via signaling across the blood–brain barrier. In parallel, substances that are produced by gut microbiota are possibly absorbed reaching the brain through the bloodstream. Subsequently, the brain, can affect the gut microbiota through neuronal and endocrine mechanisms and by the adoption of health behaviors. Thus, it is obvious that a possible imbalance of gut microbiota may affect the brain and lead to dysfunctions in the form of psychiatric disorders including as emotional and cognitive alterations.

AUD patients have enhanced levels of LPS, as a result of the increased abundance of the gram-negative bacteria which produce it, and have been shown to trigger significant inflammation; high levels of LPS can cause sepsis, even septic shock, and health implications including neurodegenerative disorders such as Alzheimer's disease, via chronic neuroinflammation.[40] Previous studies have shown that alcohol and gut dysbiosis elevate the LPS serum levels that derive from bacteria. [22, 41]. LPS can release inflammatory factors such as TNF-α, IL-1, IL-6, IL-8, IL-10, IFN-γ, and MMP-9 and induce inflammation by activating TLR4 complexes [42] and subsequently switches on the hypothalamic-pituitary-adrenal axis (HPA) axis to influence the brain. The brain can affect the intestine via the HPA axis which releases adrenocorticotropic hormones, leading to elevated intestinal permeability. In alcoholics, the higher relative abundance of gram-negative bacteria also includes Enterobacteriaceae [43] and Proteobacteria [26]; their increased population highly

correlates with elevated LPS levels, and results in a more potent immunological response, compared to other phyla. These, combined with increased gut permeability, are significant contributors of AUD-related inflammation.[44]

Metabolites that derive from the gut microbiome can exert diverse functions in the body with the majority of them involved in pathways of the digestive system formation and function. These molecules range from short chain fatty acids and neurotransmitters to precursors of neurotransmitters, bile acids, hormones and vitamins. Each one of these metabolites has important impact on the brain function of the host. Notably, a decrease in the metabolic potential of alcohol dependent patients microbiome has been marked, characterized with an overall functional decrease in pathways related to methane metabolism, bacterial chemotaxis, and pyrimidine metabolism, and increase in metabolic pathways related to the phosphotransferase system.[26]

The intestinal microbiome produces a range of small molecules representing most major metabolite classes, allowing them to ferment complex dietary polysaccharides and carbohydrates, as well as dietary fibers, producing short chain fatty acids (SCFAs).[45] SCFAs (acetate, propionate, and butyrate) constitute a significant energy source for gut epithelial cells,[46] and have been associated with improvement in the function and maintenance of the intestinal barrier's integrity, for an overall protective effect against pathogens.[47] Their anti-inflammatory nature supports epithelia cell proliferation, as well as T cell differentiation in the colon, contributing to gut homeostasis.[48] The bacterial SCFA butyrate can enhance tight junction integrity by inducing the production of relevant proteins, including claudin, occludin and zonula occludes, which as a result inhibits bacterial translocation.[49] Since both the intestinal bacteria and the levels of produced metabolites have such interlinked functions with the human body, affecting barrier integrity, there should be substantial caution of the altered populations to avoid collateral harm.[50] SCFAs are part of a satiety-inducing mechanism, with propionate enhancing gluconeogenesis, and the aforementioned butyrate playing a role in increasing glucagon-like peptide-a (GLP-1) via gut cell stimulation.[51] Importantly, through the gut-brain axis, each metabolite present a neuromodulatory function, regulating the levels of critical neurotransmitters, including enteroendocrine serotonin, noradrenaline, and dopamine,) which affect numerous functions of the nervous system, as well as range of emotions, behaviors and other CNS functions. Even gamma aminobutyric acid (GABA), a predominant inhibitory

neurotransmitter, has been found to be secreted by bacterial strains, such a Bifidobacterium and Lactobacillus.[24] Lower levels of isovalerate in fecal SCFAs correlated with higher alcohol consumption in AUD patients, compared to controls, and were successfully reversed post-faecal microbiota transplantation (FMT) treatment. [15, 52, 53]   The therapeutic efficacy may be attributed to recovery in the levels of bacteria such as Alistipes, Faecalibacterium, Ruminococcus, and Ocillibacter, which have been previously associated with increased isovalerate.[54]

A metagenomic analysis in AUD patients by Litwinowicz et al. (2023) (concluded to a significant increase in facultative anaerobes (such as the Enterobacteriaceae family) , that may be a result of a simultaneous decrease in levels of butyrate-producing bacteria (Butyricicoccaceae, Lachnospiraceae, Ruminococcaceae, Oscillospiraceae), thus reducing beta-oxidation, which in turn ends up increasing oxygen levels.[55] Most of the produced butyrate gets used up for energy, with only approximately 5% remaining in circulation, however, this small fraction appears to be capable of inducing a potent anti-inflammatory response, through various pathways, including through the G-protein-coupled receptors 41 and 43 (GGPR4; GPR43),[56] inhibition of interferon gamma (IFN-γ) signaling, and induction of nuclear factor kappaB (NF-kB).[57] Butyrate's functions extends to strengthening the intestinal barrier via permeability reduction, therefore lower levels of bacteria producing it (Ruminococcaceae, Lachnospiraceae), could negatively affect AUD progression.[55,58]

The connection among gut dysbiosis and brain function is firm given the ability of the gut microbiome to affect the functions of the central nervous system. SCFAs and the condition of the gut have been shown to influence a plethora of psycho-neurological conditions that include depression, anxiety, stress, autism spectrum disorders, schizophrenia, and Parkinson disease.[59, 60] Apart from negatively affecting the progress of AUD itself, intestinal microbiota can have a detrimental effect in various factors associated with relapse, such as alcohol craving and negative emotional states, such as depression and anxiety.[13] Of note, the level of intestinal permeability was associated with behaviors, depression recovery, anxiety, and craving levels observed in individuals with reduced intestinal permeability, but not in individuals with increased intestinal permeability. This finding seems to suggest a role of the microbiome gut-brain axis in AUD. Both addiction and withdrawal are known to arise from modifications in the neuronal function. The gut-

brain axis (GBA) is regarded as the bidirectional communication pathway that links the brain with the gastrointestinal tract. Different pathways for communication between the gut and the brain involve neuronal signaling via the vagus nerve, endocrine actions via the hypothalamic-pituitary-adrenal axis, and stimulation of neural inflammation, or a wide range of metabolic changes. Several studies have proved that bacteria affect the gut-brain axis and interfere in conditions and phenotypes such as autism spectrum disorders, social behavior, anxiety, depression, eating habits as well as the amount of food consumed.[61]

Therapeutic Strategies Targeting the Gut Microbiome in AUD

Treatment outcomes in AUD can vary across patients and different medications. Most pharmacotherapies focus on limiting alcohol craving through neuromodulation, including opioid, glutamate, gamma-aminobutyric acid, and serotonin systems. While achieving abstinence is the desirable result, its rarely achieved, by merely 16% of AUD patients. The three approved AUD medications (disulfiram, acamprosate, and naltrexone) have shown moderate therapeutic efficacy, and with AUD being a heterogenous disorder, a single medication is unlikely to be effective for every patient.[62] As it is increasingly recognized that the gut microbiome is correlated with alcohol use disorders, it has emerged as a potential therapeutic target for the treatment of AUD, aiming to reverse the dysbiotic state and reduce inflammation. Dysbiosis of microbiota can be restored through different approaches.

Pro-/Pre-biotics

Dietary interventions have been investigated as a means to improve gut dysbiosis in individuals with various pathologies including AUD. A Mediterranean-style diet, rich in fruits, vegetables, whole grains, and healthy fats, is widely known for its beneficial effects on overall health, promotion of maintenance of a diverse and beneficial gut microbiota, increase in the concentration of SCFAs, and protection of the intestinal mucus layer.[63] Conversely, another study found that a diet with unsaturated fats affected the intestinal barrier, inducing inflammation and liver injury in mice exposed to chronic alcohol consumption.[64]

Another approach to modulate gut microbiota are the dietary supplements in the form of either probiotics or prebiotics since it has been proposed as an emerging therapeutic target for the management of cognitive and/or behavior pathology. The definition of probiotics states that they are "living microorganisms which, when administered in adequate quantities, present an overall benefit to the host's health".[65] Due to probiotics' potential benefits to the CNS and mental disorders, it has been proposed they be characterized as "psychobiotic," expecting low side effects, and anti-inflammatory, antidepressant, and anti-anxiety effects, ameliorating mental functions in Alzheimer's disease and Autism Spectrum Disorder (ASD).[66]

The administration of probiotics in health subjects has been linked with alterations in brain activity related to emotional memory and decision-making procedures as well as changes in functional connectivity …

The beneficial use of probiotics has been indicated in impaired social cognition and emotional functioning disorders, which have been linked to AUDs. [67,68] The administration of probiotics in healthy individual has been linked with alterations in brain activity related to emotional memory and decision-making procedures,[69] as well as changes in functional connectivity during the performance of different emotional tasks, [70,71] while stress conditions leading to rapid reaction times in an emotional recognition task and reduction of proinflammatory cytokines.[72] In autism-animal model, a probiotic administration could reverse social behavior deficits.[73] A very early study (1995) showed that consuming 100 mL of a Bacillus natto-fermented product, could reduce breath alcohol (44% reduction) and aldehyde (45% reduction) concentrations in a group of participants 1 h after drinking whisky, compared to a control group of rats.[74]

Most data about the benefits of probiotics use in alcohol disorders arise from studies of alcohol liver disease with a focus on the improvement of the liver tissue. Probiotic supplementation restores the number of fecal Bifidobacteria, Lactobacilli, and Enterococci in alcoholic patients. The proposed mechanism of action is via modulation of dysbiosis and balance restoration, which in turn promotes an anti-inflammatory microenvironment allowing the reduction of the intestinal permeability and the translocation of bacterial components (LPS) to the systemic circulation. Furthermore, endotoxemia is found to be reduced while at the same time, probiotics can prevent bacterial metabolites from reaching

the liver and triggering inflammatory responses.[75] By reducing systemic proinflammatory status and neuroinflammation, probiotics also offer an excellent alternative to relieve CNS damage reinforcing beneficial effects on addiction and, consequently, alcohol consumption. [76]

Lim et al. (2021) studied the effects of 19 probiotic species on alcohol and acetaldehyde metabolism identifying four probiotic species, which present a relatively higher tolerance to alcohol, and a more effective alcohol and acetaldehyde metabolism: *Lactobacillus gasseri* CBT LGA1, *Lactobacillus casei* CBT LC5, *Bifidobacterium lactis* CBT BL3, and *Bifidobacterium breve* CBT BR3. These species also showed high mRNA expression levels of alcohol and acetaldehyde dehydrogenases. A mixture of these four probiotics species and excipients, the ProAP4, was then administered to rats for 2 weeks in advance of acute alcohol administration. The serum alcohol and acetaldehyde concentrations were significantly decreased in the treated group than in the control. Thus, the administration of these four probiotic species, rapidly reduced blood alcohol and acetaldehyde levels in an alcohol and acetaldehyde dehydrogenase-dependent fashion. [77] Subsequently, another randomized placebo-cotroled crossover study examined the effect of Duolac ProAP4 supplementation on alcohol detoxification in humans, which lead to a reduction in both blood alcohol and acetaldehyde levels in *ALDH2*2* hetorozygotes. [78]

A number of prebiotics and probiotics alone or their combination could hold the potential of regulating brain's neurotransmition and in turn could attenuate alcohol-related addictive processes, and the related affective and cognitive-behavioral modifications. Accordingly, synbiotic supplementation was reported by Pizarro et al. to induce alterations in the relative population of gut's bacteria, tryptophan derivatives, g-aminobutyric acid and norepinephrine levels in the hippocampus and prefrontal cortex of mice, post alcohol withdrawal. Interestingly, although alcohol appeared to induce a detrimental effect in long-term memory and mobility in female mice (which was reduced by synbiotics), male mice showed no significant changes, indicating a higher alcohol tolerance in males.[79] A double-blind, placebo-controlled trial randomized participants in four groups supplemented with placebo, prebiotics, probiotics and synbiotics, respectively. This study highlighted the increase of the number of supplement-specific bacteria after probiotic administration in healthy individuals, but no improvement in the metabolism of an acute dose of alcohol was reported.[80] Furthermore, the combination of drugs that inhibit the hyper-glutamatergic

state (N-acetylcysteine and acetylsalicylic acid- NAC+ASA) with probiotics (*Lactobacillus rhamnosus* -LGG) markedly inhibit relapse ethanol intake. Two mechanisms were induced by these treatments; NAC+ASA reduced the glutamatergic tone and antibiotic+LGG reduced the dopaminergic tone, that reduced the alcohol binge–drinking relapse effect independently and complementary.[81]

Most recently, a recombinant probiotic *Lactococcus lactis* expressing human ADH1B (hADH1B) was constructed, to enhance alcohol degradation in the intestinal tract after oral administration. Alcohol's metabolism includes its decomposition from the enzyme alcohol dehydrogenase (ADH) the action of which is followed by the liver enzyme acetaldehyde dehydrogenase (ALDH); thus, as expected, the administration of hADH1B-expressing probiotic reduced alcohol absorption, and extended alcohol tolerance time and a reduction of recovery time and protected the intestine and liver from damage after acute alcohol consumption in mice.[82]

Fecal Transplantation

A number of recent studies (both preclinical and clinical) have shown that transplantation of fecal microbiota from AUD patient is capable to alter the intestinal barrier and modify brain function. In total, important alterations in the gut microbiome in time occur as a response to chronic alcohol exposure and correspond to severe intestinal barrier dysfunction and ALD development. Moreover, the altered bacterial communities of the gut may serve as a significant therapeutic target for the prevention/treatment of chronic alcohol intake induced intestinal barrier dysfunction and liver disease. [83] An animal model study showed that when sensitive to alcohol mice were fed with intestinal microbiota from resistant mice, the development of alcohol-induced liver lesions was prevented and a better gut homeostasis was observed. In total FMT treated mice indicated a similar intestinal microbiota profile to alcohol-resistant mice. [84]

A phase 1 randomised clinical trial of fecal microbiota transplant (FMT) for AUD demonstrated short-term and long-term changes in AUD patients with cirrhosis using microbial manipulation. The FMT group presented a higher number of Alistipes and Roseburia, and increased production of SCFA. In contrast, there was a reduction in stool isovalerate and 2- methylbutyrate in placebo. Alcohol craving was negatively associated with Ruminococcaceae genera after FMT, and the reverse pattern was seen with Proteobacteria genera, such as Pseudomonas and with other potential pathobionts, such as

Enterococcus. Ethanoligenens, which is associated with endogenous alcohol production, was also negatively linked with FMT. Potentially beneficial genera and those higher in post-FMT, such as Bilophila and Ruminococcus, were associated with lower alcohol-craving score after FMT. FMT subjects also showed reduced intestinal permeability, short-term decreases in inflammation, and decreased lipopolysaccharide binding protein (LBP), all of which supports FMT as a beneficial treatment for AUD patients, that often suffer from impaired intestinal barrier function.[53]

A later study in germ-free mice, colonized with microbiota from post-FMT humans, showed a striking decrease in both initial ethanol acceptance as well as ethanol preference, compared to control group. The beneficial impact was attributed to changes in the microbial taxa (increased Lachnospiraceae and Ruminococcaceae, decreased Enterobacteriaceae), which appeared comparable to those in post-FMT humans, and was complemented with improvements in intestinal barrier function, increase in SCFAs, and lower butyrate. Notably, changes in gene expression were limited in the intestine, but not the liver or prefrontal cortex, and were associated primarily with inflammation and immune response, proliferation of epithelial cells, as well as response to oxidative stress. These results promote gut microbiota and the intestinal interface as therapeutic targets to lower alcohol intake in AUD patients. [85]

Conclusion

The emerging field of research on the gut microbiome's role in AUD has revealed significant implications for health outcomes and potential therapeutic strategies. Alcohol consumption has profound effects on the gut microbiome, leading to dysbiosis and increased systemic inflammation. These alterations have significant implications for health outcomes, including the development of liver disease, cardiovascular disease, and type 2 diabetes. The gut microbiome represents a promising therapeutic target for the treatment of AUD, with interventions such as probiotics, prebiotics, and dietary modifications showing potential in improving gut dysbiosis and reducing inflammation. However, further research is needed to fully understand the intricate interactions between alcohol consumption and the gut microbiome, and to develop effective interventions that can mitigate the detrimental effects of AUD on gut health.
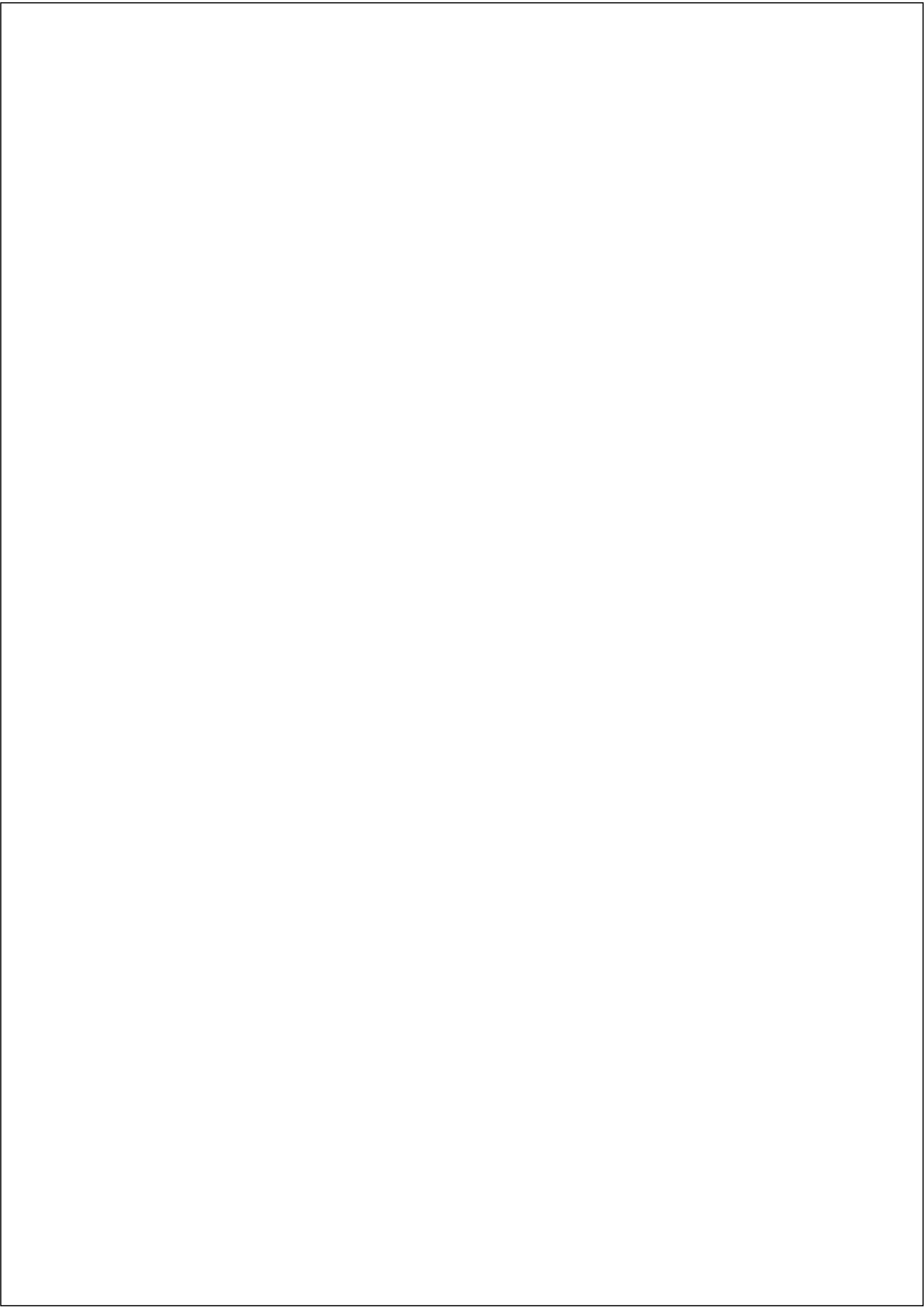
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