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Gut microbiota, biological and psychological alterations in alcohol use disorder

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Summary

Alcohol-use-disorder (AUD) is a major public health problem affecting 5 to 10% of the population in developed countries. Beside the severe effect on the brain, AUD induces malnutrition and affects peripheral organs such as the liver or the gut. A dysbiosis, meaning alterations in the gut microbiota, has been associated with an increased intestinal permeability in AUD patients. These symptoms are correlated with behavioural alterations, suggesting the involvement of the gut-brain axis in the development of AUD. This project aims at understanding the role of the gut microbiota in biological and behavioural alterations associated with AUD and to test an innovative therapeutic approach targeting the gut microbiota in AUD patients, in order to help them to recover upon alcohol withdrawal.

We carried out an interventional, double-blind, placebo-controlled study to supplement AUD patients with inulin, a dietary fiber with prebiotic properties, in order to modulate their gut microbiota composition. Fifty AUD patients hospitalized for a three-weeks detoxification program have been included in the study. Patients received daily supplementation with an increasing dose (4g to 16g) of inulin or maltodextrin (placebo) during 17 days.

The first part of this work is based on the data obtained at baseline. It aims at evaluating the nutritional habits with a focus on dietary fiber (DF) intake and to investigate its link with psychological symptoms in AUD patients. We found that energy intake (excluding alcoholic beverage), total fat, monounsaturated and polyunsaturated fatty acids, protein and DF intakes were lower in AUD subjects compared to healthy subjects. Ninety percent of patients had a DF intake below the recommendation. We also discovered that DF intake was negatively associated with anxiety whereas it was positively associated with sociability score adjusted for main confounders.

We then wanted to explore the relationship between gut dysbiosis and social functioning in AUD patients at baseline. We found that dysbiotic patients were younger, thinner and had a higher craving score compared to non-dysbiotic patients. Interestingly, we discovered that dysbiotic patients had a lower sociability score and a smaller and less connected social network. They displayed also a higher level of IL-8. Neither nutritional intake nor medication was different between groups.

Finally we studied the effect of inulin supplementation on gastrointestinal totelerance, gut microbiota composition, biological and behavioural alterations. Inulin was well tolerated by AUD patients and induced a decrease in α-diversity and changes in gut

microbiota composition including an increase in *Bifidobacterium*. The supplementation had no effect on depression, anxiety or craving score. However only patients treated with inulin significantly improved the sociability score. Regarding biological outcomes, inulin increased serum BDNF level and patients supplemented with inulin had higher level of aminotransferases and IL-18 at T2.

In conclusion, with this work, we demonstrated that actively drinking AUD patients had a lower intake of DF including fructans and this was associated with behavioural alterations. We demonstrated that 17 days of inulin supplementation during withdrawal had minor effect on behavioural and biological alterations. Our work supports an association between social behaviour and gut microbiota in AUD patients in both cross-sectional and intervention studies.

Further studies involving longer treatment and a larger sample size are needed to determine whether targeting the gut microbiota might be an appropriate approach to improving psychological symptoms and biological outcomes in patients with AUD.

List of abbreviations and acronyms

ACTH	Adrenocorticotropic hormone
ADH	Alcohol dehydrogenase
Ahr	Aryl hydrocarbon receptor
ALD	Alcoholic liver disease
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
AUD	Alcohol use disorder
AUDIT	Alcohol use disorder test
BA	Bile acid
BBB	Blood-brain barrier
BDI	Beck Depression Inventory
BMI	Body mass index
BSFS	Bristol stool form scale
cAMP	Cyclic adenosine monophosphate
CAP	Control attenuation parameter
cGMP	Cyclic guanosine monophosphate
CK18	Cytokeratin 18
CNS	Central nervous system
CRF	Corticotropin-releasing factor
DF	Dietary fiber
DHA	Docosahexaenoic acid
DSM	Diagnostic and Statistical Manual of Mental Disorders
ED	Eating disorder
EI	Energy intake
ELISA	Enzyme-linked immunosorbent assay
EPA	Eicosapentaenoic acid
FDA	United states Food and Drug Administration
FGF-21	Fibroblast growth factor 21

FMT	Fecal microbiota transplant
FOS	Fructo-oligosaccharides
GABA	Gamma-aminobutyric acid
GF	Germ free
GI	Gastrointestinal
GLP	Glucagon-like peptide
GOS	Galacto-oligosaccharides
HDL	High-density lipoprotein
HPA	Hypothalamic-pituitary-adrenal
HS	Healthy subject
5 HT	5-hydroxytryptamine
IBD	Inflammatory Bowel Disease
IBS	Irritable bowel syndrome
ICD	International Classification of Disease
IL	Interleukin
Iba1	Ionized calcium-binding adapter molecule 1
ISAPP	International Scientific Association for Probiotics and Prebiotics
ITF	Inulin-type fructans
JNK	c-Jun N-terminal kinase
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
MCP-1	Monocyte chemoattractant protein 1
MDD	Major depressive disorder
MEOS	Microsomal ethanol oxidising system
MFI	Multidimensional Fatigue Inventory
MMSE	Mini Mental State Examination
mRNA	messenger RNA
Muc-2	Mucin 2
MUFA	Monounsaturated fatty acids

NEFA	Non-esterified fatty acid
NMDA	N-methyl-D-aspartate
OCDS	Obsessive-Compulsive Drinking Scale
PBMC	Peripheral blood mononuclear cells
PDE	Phosphodiesterase
PF	Processed food
PGN	peptidoglycans
PLS	Partial least squares analysis
PPARγ	Peroxisome proliferator-activated receptor-γ
PSD-95	Postsynaptic density protein 95
PTSD	Post-traumatic stress disorder
PUFA	Polyunsaturated fatty acids
PVN	Paraventricular nucleus
PYY	Peptide YY
qPCR	quantitative polymerase chain reaction
rRNA	ribosomal
sCD14	Soluble Cluster of differentiation 14
SCFA	Short chain fatty acids
SD	Standard deviation
SEM	Standard error of the mean
SFA	Saturated fatty acids
STAI	State-Trait Anxiety Inventory
TDF	Total dietary fiber
TEIque	Trait Emotional Intelligence Questionnaire
TG	Triglycerides
TLRs	Toll-like receptors
TNFa	Tumor necrosis factor alpha
UPF	Ultra-processed food

VLDL	Very low density lipoproteins
VPT	Visual perspective task
VTA	Ventral tegmental area
WHO	Wordl health organization

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INTRODUCTION

1 Alcohol use disorder

Alcohol, like illicit drugs, is a psychotropic substance with a high addictive potential. For millenials, alcoholic beverages have been used by human beings and are an integral part of our culture and traditions. Indeed, alcohol is consumed for recreational purposes, accompanying almost all festive events in family and social life.

Yet, according to the World Health Organisation (WHO), alcohol consumption is responsible for 3 million deaths worldwide each year (5.3% of all deaths) and is one of the leading factors for premature death [1]. In the population from 20 till 39 years old, approximately 13.5% of all deaths are attributable to alcohol abuse. Alcohol consumption increases the risk of developing health problems such as mental disorders, including alcohol use disorders and somatic diseases such as cirrhosis, certain cancers and cardiovascular diseases, as well as injuries resulting from violence or traffic accidents [1]. If we take the case of Belgium, the average consumption of pure alcohol was 12.1 litres per capita per year in 2016, which is higher than the European average consumption of 9.8. This makes Belgium one of the countries with the highest alcohol-related disease burden. For example, alcohol was responsible for 5.4 % of all deaths and 7.7% of cancers in men and 4.2% in women in 2016.

In addition to the health implications, alcohol consumption also has important economic and social consequences making it a real public health priority [1].

1.1 Definition of AUD and epidemiology

1.1.1 Definition of AUD

Alcohol consumption is considered problematic when it reaches a certain threshold. In Belgium, one glass or standard unit corresponds to 10 g of ethanol. There is a wide disparity in consumption recommendations because alcohol units vary between countries. However, the WHO has established recommendations for safe drinking [1]:

- A woman should not exceed 2 units per day, with at least one day of abstinence per week
- A man should not exceed 3 units per day, with at least one day per week of abstinence

In Belgium, these recommendations were recently revised (without distinction according to gender) by the Superior Council of Health and are now more restrictive: 2 drinks maximum per day, a maximum of 10 drinks per week with several days of abstinence [2].

Also, on a special occasion, consumption should not exceed 4 units. Alcohol misuse includes any drinking that endangers the health, safety or leads to other alcohol-related problems. It also includes excessive drinking in a short period of time, known as binge drinking, where a man has five or more drinks within two hours and a woman has four or more drinks within two hours [1].

A number of clinical features should be considered in order to identify the presence of an alcohol misuse. The diagnosis is based on the diagnostic criteria described in the international classifications of mental disorders. Currently, there are two classifications: the Diagnostic and Statistical Manual of Mental Disorders (DSM) and the International Classification of Disease (ICD) [3]. The latest version of the ICD (ICD-11) was adopted in 2019 and the last version of the DSM (DSM-5 fifth edition) was adopted in 2015 (French version). In both classifications the diagnosis is based on a number of criteria, including the maintenance of alcohol consumption despite negative psychological, biological, behavioural and social consequences over a 12-month period. Although the DSM-5 and ICD-11 show a high level of concordance, there are discrepancies, particularly in the diagnosis of mild and moderate disorders [4]. In the present work, patients were selected upon criteria of the DSM-5.

The categorical approach of the DSM-IV, which distinguished alcohol abuse and alcohol dependence, has been replaced by a dimensional approach that groups the two disorders into one disorder called "alcohol use disorder" (AUD). Indeed, some studies have challenged the hierarchical distinction between abuse and addiction [5–7]. The DSM-5 takes into account the severity of the disorder and allows to distinguish mild, moderate and severe AUD. Moreover, it adds alcohol craving as a criterion for AUD diagnosis, which was not included in the prior edition and removes the legal issues. The different criteria are described in Table 1. The severity of AUD is assessed based on the number of criteria presented by the patient over a period of at least 12 months. An AUD will be defined as "mild" if 2 or 3 criteria are present, "moderate" if 4 or 5 criteria are present and "severe" if 6 or more criteria are present (**Table 1**).

Table 1: A comparison between DSM-IV and DSM-5, National Institute on Alcohol Abuse and Alcoholism [8]

DSM–IV			DSM–5	
In the past year, have you:			In the past year, have you:	
Any 1 = ALCOHOL ABUSE	Found that drinking—or being sick from drinking—often interfered with taking care of your home or family? Or caused job troubles? Or school problems?	1	Had times when you ended up drinking more, or longer, than you intended?	The presence of at least 2 of these symptoms indicates an **Alcohol Use Disorder (AUD).** The severity of the AUD is defined as: **Mild:** The presence of 2 to 3 symptoms **Moderate:** The presence of 4 to 5 symptoms **Severe:** The presence of 6 or more symptoms
	More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?	2	More than once wanted to cut down or stop drinking, or tried to, but couldn't?	
	More than once gotten arrested, been held at a police station, or had other legal problems because of your drinking? ****This is not included in DSM–5****	3	Spent a lot of time drinking? Or being sick or getting over other aftereffects?	
	Continued to drink even though it was causing trouble with your family or friends?	4	Wanted a drink so badly you couldn't think of anything else? ****This is new to DSM–5****	
Any 3 = ALCOHOL DEPENDENCE	Had to drink much more than you once did to get the effect you want? Or found that your usual number of drinks had much less effect than before?	5	Found that drinking—or being sick from drinking—often interfered with taking care of your home or family? Or caused job troubles? Or school problems?	
	Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, restlessness, nausea, sweating, a racing heart, or a seizure? Or sensed things that were not there?	6	Continued to drink even though it was causing trouble with your family or friends?	
	Had times when you ended up drinking more, or longer, than you intended?	7	Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?	
	More than once wanted to cut down or stop drinking, or tried to, but couldn't?	8	More than once gotten into situations while or after drinking that increased your chances of getting hurt (such as driving, swimming, using machinery, walking in a dangerous area, or having unsafe sex)?	
	Spent a lot of time drinking? Or being sick or getting over other aftereffects?	9	Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?	
	Given up or cut back on activities that were important or interesting to you, or gave you pleasure, in order to drink?	10	Had to drink much more than you once did to get the effect you want? Or found that your usual number of drinks had much less effect than before?	
	Continued to drink even though it was making you feel depressed or anxious or adding to another health problem? Or after having had a memory blackout?	11	Found that when the effects of alcohol were wearing off, you had withdrawal symptoms, such as trouble sleeping, shakiness, restlessness, nausea, sweating, a racing heart, or a seizure? Or sensed things that were not there?	

1.1.2 Epidemiology

AUD is one of the most common mental disorders affecting 5 times more men (8.6%) than women (1.7%) in 2016 [1]. However, studies show that this gap tends to narrow over the years [9,10]. A Norwegian study has shown that the drinking habits of adults have changed significantly over the past 20 years, with abstinence becoming rarer for both men and women [9]. Women's drinking was similar to men's in terms of abstinence, recent drinking (1 drink in the last 14 days) and volume of alcohol consumed (liter per year) [9].

The highest prevalence of AUD is observed in Europe (14.6% of men and 3.5 % of women) and in America (11.5% of men and 5.1% of women; **Figure 1**). In Belgium, 8.1% of the population suffer from AUD in 2016 (12.1% of men and 4.3% of women)[1].

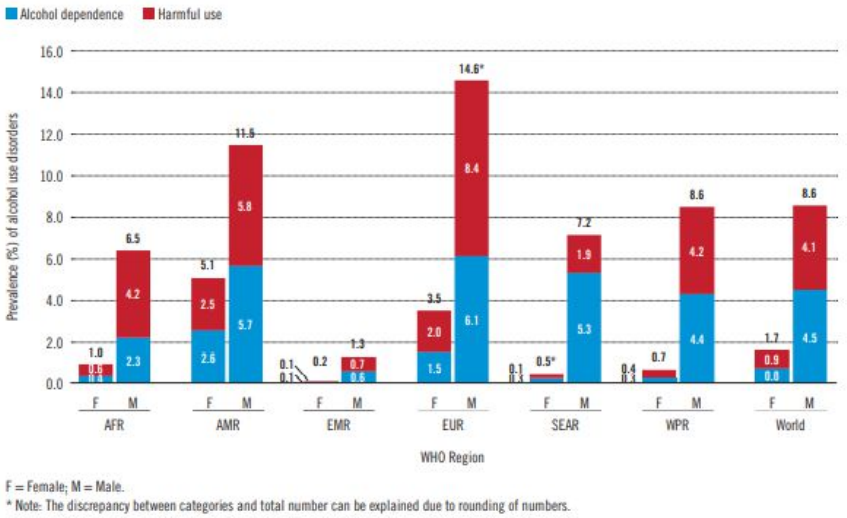


Figure 1: Prevalence (in %) of alcohol use disorder, by gender and by WHO region in the world, WHO 2016 [1].
AFR: African region; AMR: Region of the Americas; EMR: Eastern Mediterranean Region; EUR: European Region; SEAR: South-East Asia Region; WPR: Western Pacific Region

Differences in drinking habits are also observed according to socio-economic status and education level. The higher the level of education, the higher the number of drinkers and the higher the quantity of alcohol consumed. This could be explained by the greater purchasing power of higher socio-economic levels [11].

1.2 Risk factors of AUD

AUD results of complex interaction between factors directly related to the individual (biological, psychological) but also to his environment (cultural, social, family) [12].

The environment of an individual has a strong impact on the development of addictive disorders. For example it has been shown that the prevalence of AUD is higher in cultural groups that adopt a more permissive attitude toward heavy drinking or alcohol intoxication [3,13,14].

Regarding individual risk factors, subjects suffering from psychiatric illnesses such as anxiety, depression, schizophrenia, social anxiety disorders or bipolar disorder are at higher risk of addiction [15,16]. Moreover, post-traumatic stress disorder (PTSD) is strongly associated with AUD [17] and drug misuse [18,19]. More recently, it has also been shown that patients undergoing bariatric surgery were 7 times more likely to develop AUD than subjects who had not had bariatric surgery (hazard ratio=7.29, (CI): 5.06-9.48) [20].

Twin studies have highlighted the role of genetic in the development of AUD. It has been shown that when a person suffers from AUD in the family it increases the frequency of the disease in relatives, especially if the degree of family proximity is important. These studies have shown a heritability estimates from 40 to 70% [3].

1.3 Neurobiology of AUD

Ethanol is able to cross the blood-brain barrier (BBB) and acts directly on the brain. By acting on different brain neurotransmission systems, ethanol can strongly influence behaviour and interfere with certain neurobiological mechanisms involved in decision-making, motivation and emotion.

1.3.1 Implication of reward circuits in the development of AUD

Alcohol acts on many neurotransmitter systems: excitatory, inhibitory or neuromodulatory involving glutamate, gamma-aminobutyric acid (GABA), dopamine, opioids and serotonin. These neurotransmitters are present or interfere with different structures such as the mesolimbic system (ventral tegmental area (VTA), nucleus accumbens and prefrontal cortex), the amydala and the hippocampus [21], where they have been described to play a role in the development of the addiction.

a. Acute effect of alcohol

Alcohol, contrary to other drugs, does not have specific receptors but it has been shown that this molecule has pockets of binding at the level of glutamate N-methyl-D-aspartate (NMDA) receptors and $GABA_A$ receptors [22]. During an acute consumption, alcohol acts on the GABA receptors. This induces an increase in the synthesis and release of GABA neurotransmitters and in the activity of these receptors at the postsynaptic level [23–25]. Acute alcohol consumption also induces a decrease in the release of glutamate, an excitatory neurotransmitter [23,24]. As GABA is an inhibitory neurotransmitter, neuronal activity will decrease in several brain regions, including the frontal cortex, which explains the appeasement and the decrease in behavioural inhibition [25].

Alcohol generates rewarding effects by stimulating the mesolimbic dopaminergic system, which begins in the VTA and projects to the nucleus accumbens and other limbic regions of the brain [26,27]. Alcohol induces the release of dopamine in the nucleus accumbens directly by increasing the activity of the dopaminergic neurons of the VTA [28]. Indirectly, alcohol, by promoting $GABA_A$ receptor function, may inhibit GABAergic terminals in the VTA and disinhibit VTA dopamine neurons (**Figure 2**). This activation of the reward system, inducing a release of dopamine, is responsible for the euphoric and pleasurable effects of alcohol and plays a major role in positive reinforcement and thus increases the probability of using alcohol [29]. This explain the initiation and persistence of drinking and is pivotal in the development of AUD [21].

Endogenous opioids are small molecules naturally produced in the body. There are three classes of endogenous opioids: endorphins, enkephalins, and dynorphins. These molecules interact with three subtypes of opioid receptors—μ, δ, and κ [29,30]. The opioidergic system also plays a determining role in positive reinforcement by promoting the release of dopamine during the anticipation of alcohol consumption or during ingestion and thus contributes to the motivational effect [30]. Indeed, acute alcohol ingestion can induce β-endorphin release, resulting in activation of μ receptors on the GABAergic neurons in the VTA. This, in combination with alcohol's inhibition of glutamate effects on GABA neurons, could lead to decreased GABAergic activity in the VTA, and subsequently increased firing of the dopaminergic neurons, resulting in increased dopamine release in the nucleus accumbens (**Figure 2**) [30,31].

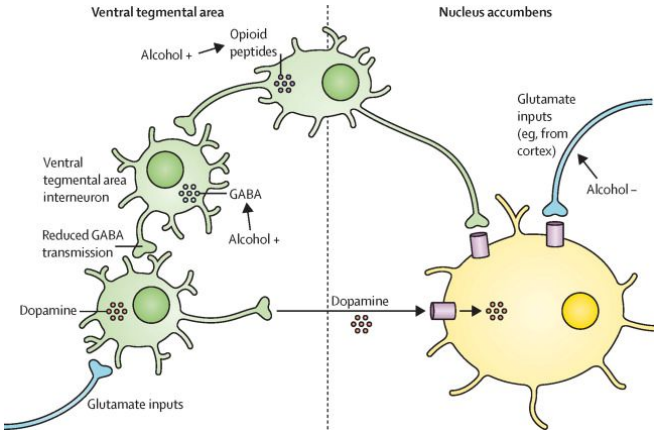


Figure 2: Effects of acute alcohol consumption on neurotransmitter systems [21]

b. Chronic effects of alcohol consumption

Reinforcement is a complex psychological process by which a stimulus or event will act to reinforce a behaviour (activating, directing and maintaining goal-directed behaviour). Positive reinforcement refers to a situation in which a rewarding stimulus, such as euphoria or pleasure induced by alcohol consumption, encourages the repetition of the drinking behaviour. This factor is common in the early stages of alcohol use and abuse, even before the addiction develops. The negative reinforcement process of the addiction involves that drinking is related to the elimination or attenuation of a negative stimulus such as withdrawal symptoms (anxiety, irritability, tremors, etc.) or the negative affects related to drinking and other. It generally develops at a later stage, when the addiction has developed. Negative reinforcement is recognized as a major factor involved in the persistence of drinking habits in AUD subjects [29].

Repeated alcohol consumption increases the reinforcing value of alcohol. The intake of alcohol becomes more and more attractive and the desire to consume evolves towards craving. Indeed, if the reward system is continuously activated, it will adapt to restore a homeostasis of neurotransmitters and their receptors [29]. The dopaminergic receptors will be less sensitive, which will require increased stimuli to obtain the same effect of the alcohol, inciting the individual to drink again or increase the doses. A phenomenon of tolerance will then set in. Regarding the GABA/glutamate balance, chronic consumption will induce an increase in the number of NMDA receptors and a decrease in the number of

GABA receptors [29]. When alcohol is cleared from the body, there is no longer any potentiation of GABA. The balance will then shift towards glutamate, inducing neuronal hyperexcitability responsible for withdrawal symptoms such as tremors, tachycardia, sweating and psychic symptoms that may even express as *delirium tremens* [32]. The individual will then consume to escape these negative stimuli (negative reinforcement).

Serotonin (or 5-hydroxytryptamine, 5-HT) may also be involved in the neurobiological processes responsible for the development and persistence of AUD [33]. Numerous studies have shown that serotonin is involved in the regulation of emotional states, particularly impulsivity and compulsivity. An alteration of the serotoninergic system could therefore contribute to behavioural disinhibition and thus facilitate the behaviour of seeking and using the substance [33]. It has been shown that acute alcohol consumption increases the production of extracellular serotonin, while chronic consumption leads to a decrease in serotonin neurotransmission in alcoholic patients [34,35]. Furthermore, alterations in serotoninergic neurotransmission have been shown to be associated with increased alcohol consumption and higher vulnerability to AUD [36].

This neurotransmitter is also involved in the mood disorders frequently observed in AUD patients, which may be involved in the repeated need to consume to avoid negative symptoms [33].

1.3.2 Implication of stress circuit

The development and persistence of AUD also involves the circuits related to the stress response. In particular, exposure to stress has been shown to increase the risk of relapse or the amount of alcohol consumed [37].

Hypothalamic-pituitary-adrenal (HPA) axis is controlled by neurons located in the paraventricular nucleus of the hypothalamus (PVN). In response to stressful situations, the PVN neurons secrete corticotropin-releasing factor (CRF) which stimulates the production of adrenocorticotropic hormone (ACTH) by the pituitary gland. The latter is released into the bloodstream and stimulates the release of cortisol by the adrenal glands [38]. Cortisol promotes adaptive responses to environmental stressors, including changes in energy metabolism, physiological, and behavioural responses [38]. CRF acts through his receptor CRF1 in the pituitary gland and through CRF1 and CRF2 receptors in other brain areas such as the amygdala [38].

Alcohol can be considered a stressor since it has been shown, in preclinical studies, to activate the HPA axis [39,40]. Chronic alcohol consumption induces a neuroendocrine

tolerance and a dysregulation of the HPA axis responsible for elevated ACTH, corticosterone, and amygdala CRF during acute withdrawal [41]. These dysregulations are associated with anxiety disorders and the acute withdrawal symptoms involved in relapse [38].

1.3.3 Neuroinflammation

A large literature arising from both animal and human studies have suggested a role for neuroinflammation in the pathophysiology of AUD as well as in other psychiatric disorders like major depressive disorder (MDD) [42–44]. The neuroinflammation is generated both from a direct effect of ethanol on the brain and from the induction of a systemic inflammation [45]. Activation of the immune system, resulting in the production of pro-inflammatory cytokines that can reach the brain, induces sickness behaviour. The later is characterized by fatigue, lassitude, inability to concentrate, irritability, loss of appetite and withdrawal from social interactions [46,47]. These symptoms are particularly close to the psychiatric symptoms observed in AUD. Knowing this, chronic neuroimmune imbalance could have physiological consequences that lead to behavioural alterations and symptoms related to AUD or mood disturbances [48].

Gene expression profiles of human alcoholic frontal cortex analysed *postmortem* show changes in immune-related genes [49]. Monocyte chemoattractant protein 1 (MCP-1) levels were increased in the VTA, *substantia nigra*, hippocampus and the amygdala of AUD patients [43]. Pro-inflammatory cytokines levels in serum and striatum have been shown to be increased after chronic voluntary ethanol consumption in mice [50].

Microglia, the innate immune cells of brain, is a pivotal actor in the neuroinflammatory processes [51]. In case of neuroinflammation its density increase leading to gliosis and microglia acquire an “activated” phenotype [52]. Microglia activation is observed in several neurological and psychiatric disorders including in AUD [53–55]. Indeed, ionized calcium-binding adapter molecule 1 (Iba1), a microglial density marker, was increased in AUD patients compared to control subjects [43]. Several preclinical studies showed that acute and chronic alcohol exposures as well as binge drinking lead to a switch in microglia phenotype which in turn leads to neurodegeneration [56–59].

Numerous studies suggest that the neuroinflammation influence drinking behaviour. For example, blocking the IL-1 receptor, which induces attenuation of ethanol-induced immune activation and neuroinflammation [60], has been shown to reduce ethanol-induced acute sedation and binge drinking [61,62]. Infusing MCP-1 into cerebral ventricles

led to increased operant ethanol self-administration in rats [63]. Finally, it has been shown that anti-inflammatory drugs, such as phosphodiesterase (PDE) inhibitors, may be an interesting target in the management of AUD. PDEs are enzymes involved in the degradation of cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP). cAMP is a critical regulator of microglial homeostasis and thus PDE is an attractive target to modulate neuroinflammatory processes [64]. In rodents a PDE inhibitor induced a reduction in alcohol consumption [65,66] but also anxiolytic and antidepressant effects [67]. In AUD subjects, it has recently been shown that a PDE inhibitor decrease craving and improve reactivity to stress and alcohol cue exposure and also the probability of heavy drinking compared to placebo [68,69].

1.4 Behavioural, nutritional and metabolic consequences of AUD

As seen previously, AUD has important neurobiological consequences. These alterations can be responsible for the occurrence of important cognitive and behavioural disorders. Acute and chronic alcohol consumption will also have repercussions on the metabolism and nutritional intake of individuals.

1.4.1 Behaviour

Psychiatric co-morbidities are common in AUD patients, affecting approximately 50 to 70% of patients [70]. Psychiatric disorders can be present before and induce AUD but alcohol can also be responsible for psychotic decompensation, anxiety and depressive disorders [71].

Chronic alcohol consumption is also associated with structural and functional brain abnormalities leading to cognitive impairment such as attention deficit, alterations in response inhibition, memory, visuospatial and motor skills [72].

More recently, emotional dysregulation has been described in AUD patients. This includes impairment in social cognition which is defined as the body of knowledge and processes that enables individuals to understand and interact with the social world [73]. Social cognition includes two main components, namely emotional processing and theory of mind. Several deficits related to social cognition have been identified in AUD subjects, such as alexithymia (difficulties in identifying and describing its own emotions), impairment in emotional decoding, lack of empathy, as well as difficulties to take the perspective of others [73,74]. AUD patients also display alterations in emotional reactivity (intensity of emotions) and regulation of emotions meaning the ability to initiate, inhibit,

maintain or modulate its own affects [73,75]. Impulsive drinking associated with AUD is a common example of impairment in emotion regulation [76].
It has been found that the social network of AUD patients was smaller compared to controls [77]. The complexity of the social network structure could be associated with social competence. Furthermore, the size and composition of the social network is a good indicator of social support, an important element in the management of AUD [78–81].

Besides the loss of control in alcohol consumption AUD patients have been shown to be more prone to develop eating disorder (ED) [82,83]. Eating disorders are associated with severe disturbances in eating behaviour and body weight. The three best characterized eating disorders are bulimia, anorexia nervosa and binge eating disorders [84]. The comorbidity of ED and substance use disorders was associated with greater symptom severity, poorer treatment outcome, and increased mortality [85]. In a recent study, the prevalence of AUD was estimated at 20.6% in patients with ED [86]. Despite the small number of studies and the large variability reported, it appears that ED and AUD are most likely to co-exist with bulimia (from 2.9 to 48.6%) and binge eating (19.9%) [82,87]. Even if co-occurence between both pathologies is not understood, they present common comorbidities such as anxiety, depression, trauma, family history as well as common personality traits such as compulsive behaviour, impulsivity, low selfesteem and emotional dysregulation which could play a role in the relationship between the two psychiatric disorders [88–90]. AUD and ED also share neurobiological mechanisms involving common disruptions of neurotransmitter function in the dopamine, serotonin, GABA, and endogenous opioid systems [89,91,92]. Increased medial prefrontal cortex activity in response to food stimuli has also been observed in bulimic subjects. This response is similar to the medial prefrontal cortex activity associated with cravings and compulsive drug use behaviours found in substance abuse [93].

All these deficits are interconnected and can potentially contribute to the development and maintenance of AUD and interfere with recovery. In particular, negative affects, such as depression, or social pressure have been shown to increase the risk of relapses after detoxification [74,94].

Tools to study mood, alcohol craving and social cognition

In this PhD work we were particularly interested to study anxiety, depression and craving for alcohol. These symptoms are correlated with each other and are a measure of negative reinforcement.
We used different validated and standardized tools to asses these symptoms:

- **The Beck Depression Inventory** (BDI) is a 21-item self-report inventory designed to measure the severity of depressive symptoms. Each item consists of 4 sentences corresponding to 4 degrees of increasing intensity on a scale of 0 to 3. The maximum score is 63. The higher the score, the greater the depressive symptoms [95].

- **The State-Trait Anxiety Inventory** (STAI Form YA) is a self-report inventory for measuring the state of anxiety. This test includes 20 questions to which the patient has to answer using a 4-point Likert scale. The scores range from 20 to 80 where higher scores indicate greater anxiety [96].

- **The Obsessive-Compulsive Drinking Scale** (OCDS) is a questionnaire that measures obsessionality and compulsivity related to craving and drinking behaviour. This questionnaire is composed of 14 questions and provides a global craving score, an obsessive score and a compulsive score [97].

Affective deficits and difficulties in interpersonal relationships also characterize AUD patients. Therefore, we used different complementatry tools to assess the social cognition:

- **The Trait Emotional Intelligence Questionnaire** (TEIque): Emotional intelligence is the set of abilities that allow us to recognize our emotions and those of others, to understand and think about them and to regulate them in order to act appropriately. This self-reported questionnaire consisting of 75 items and encompasses four factors: well-being, self control, emotionality and sociability [98].

- **The social situation test** is a self report questionnaire assessing preferences for social vs non social situations. The questionnaire included 28 situations (e.g., doing laundry alone;

going to a barbecue with friends). Patients were asked to indicate using a 7-point Likert-type scale (1 - very little or not at all to 7 - extremely or very much) how much they wanted to do the activity "now." Composite mean scores for three levels of enjoyment (high [5 items], medium [4 items], and low [5 items]) were calculated for social and nonsocial situations [99].

- **The visual perspective task** (VPT) aims to measure the ability to take the perspective of others; it therefore reflects the degree of attention to others. This computer-based task asks participants to consider either their own perspective or the perspective of an avatar in the middle of a room. Red disks will appear on the walls to the right or left. In the consistent perspective condition, both the participant and the avatar see the same amount of disks. In the inconsistent perspective condition, the participant and the avatar each see a different amount of disks. Participants must explicitly judge how many disks could be seen, either from their own perspective or from the avatar's perspective [100].

- **The Sociogram** is a complete and precise tool developed by sociologists from the UCLouvain to build a social support network. Starting with a very simple question: "Who are the people who support you in your daily life?", the respondent has to list the supporting people and then define the type of support obtained in four specific domains: finances, housing, activities and health. Supporters are then placed on Hogan's bullseye map, and the participant is then asked to link those whom he or she believed exchanged information ("who shares information about you?") [101].

1.4.2 Nutritional status

Alcohol, regardless the type, contains a large amount of calories. Alcoholic beverages contain water, pure ethanol and a variable amount of sugars [102]. In contrast, they contain limited quantity of protein and micronutrients (vitamins and minerals). Ethanol has a caloric intake of 7.1 kilocalories per gram (kcal/g) which is far from negligible when we know that carbohydrates provide 4 Kcal/g [102].

Alcohol dependence is one of the major causes of malnutrition in Western countries. Malnutrition is influenced by different factors such as the quantity of alcohol consumed and socio-economic status [103–105]. Excessive alcohol consumption is associated with increased energy intake, decreased diet quality and disorganized eating [103,105–107]. Alcohol can represent half of the calories ingested in AUD subjects, and this energy is devoid of micro and macronutrients leading to various deficiencies like water-soluble vitamins such as B vitamins, but also fat-soluble vitamins (A, D, E, K) [106,108–110]. Blood levels of minerals such as iron, magnesium, calcium, zinc and selenium can also be altered by chronic alcohol consumption [111]. Micronutrient deficiencies can be the result of a deficit in adequate food intake but also of a decrease in gastrointestinal (GI) absorption or an alteration of the hepatic metabolism caused by alcohol. Indeed, the liver contributes to the storage and/or metabolism of many micronutrients [102].

The dietary intake of AUD patients is therefore altered and can lead to several macro and micronutrient deficiencies. Taking into account the impact of nutrition is therefore essential in the context of AUD. In particular when investigating its impact on the gut microbiota, the liver and the brain, organs that are particularly sensitive to nutritional intake.

1.4.3 Gastrointestinal and liver function

Alcohol consumption has long been recognized as a major cause of liver damage and liver disease worldwide [112]. However it can also affect the GI tract leading to GI symptoms and increased risk of cancers [113].

a. Gastrointestinal function

After its ingestion ethanol is rapidly absorbed by passive diffusion through the mucosa: 20% of ethanol is absorbed in the stomach and about 70% in the small intestine. It then enters the bloodstream [114]. The majority of ethanol is metabolized in the liver

(90-98%). However, a small part can also be metabolized in the GI tract not only in the mucosal cell via the alcohol dehydrogenases (ADH) and microsomal ethanol oxidising system (MEOS), but also in a great variety of bacteria [113,114].

By being in contact with the different parts of the GI tract, ethanol induces structural and functional modifications leading to GI symptoms. In particular, it has been shown that alcohol consumption promotes gastroesophageal reflux disease, gastritis and gastroduodenal ulcer by stimulating acid secretion [115]. One of the most common GI disorders observed in acute and chronic alcohol consumption is diarrhea. This is promoted by the alteration of food absorption, the modification of intestinal motility and the increased gut permeability induced by alcohol [115].

The GI barrier is composed of a monolayer of intestinal epithelial cells sealed by tight junctions and covered by a protective mucus layer (**Figure 3**). The tight junctions regulate the paracellular permeability of the intestinal barrier and prevent the uncontrolled passage of large molecules through the epithelium, but allow the passage of ions, water and compounds of interest [116].

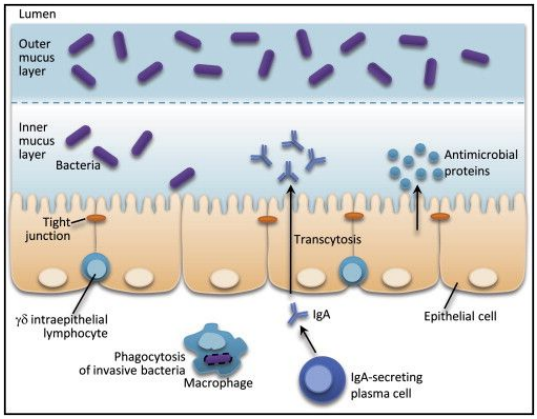


Figure 3: Key layers of the gut barrier [117]

The presence of ethanol or its derivative (acetaldehyde) in the intestine induce gut-barrier dysfunction [114,118]. Indeed, preclinical and clinical studies have found increased gut permeability in mice expose to ethanol or in actively drinking AUD patients due to disruption of the tight junctions and adherents junctions [119,120]. Ethanol detrimental effect on gut barrier function is alleviated by ablation of the mucin 2 (*muc2*) gene (coding for a protein that composes the mucus) [121]. It corroborate findings showing that

ethanol consumption leads to a larger mucus layer, which is a defensive reaction also seen in enteric infections [121–123].

The increased gut permeability enhances the translocation of luminal antigens (e.g., bacteria and endotoxins) into the portal circulation [121]. This can activate Kupffer's cells, the liver macrophages, subsequently leading to cytokines release, which can result in hepatocellular injury [124].

b. Liver function

If chronic and excessive alcohol consumption damages almost all organs, the liver as the primary site of ethanol metabolism suffers early tissue damage [125].

The spectrum of liver disease includes different stages (**Figure 4**). In early stages, repeated alcohol consumption leads to steatosis. It is characterized by the accumulation of micro lipid droplets in the hepatocytes. A number of hepatic and extrahepatic mechanisms have been associated with steatosis after alcohol ingestion such as increased lipogenesis, altered VLDL secretion and decreased fatty acid oxidation. About 90% of AUD patients develop steatosis [126]. If consumption is stopped, steatosis is reversible. However, in case of continuous consumption, the presence of fat in the liver will promote lipid peroxidation and oxidative stress which in turn damages hepatocytes causing its death. It generates apoptotic bodies that promote inflammation. Increased production of tumor necrosis factor-alpha (TNF-α) will occur in Kupffer cells. The inflammatory reaction will lead to the formation of scar tissue, characteristic of fibrosis [126]. Alcoholic steatohepatitis occurs in approximately 35% of AUD patients with a 1-year mortality rate of 20% for the severe form [127,128].

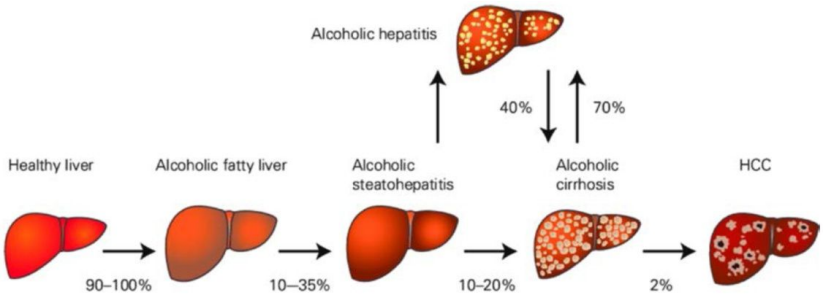


Figure 4: The natural course of alcohol-related liver disease. HCC, hepatocellular carcinoma **[129]**.

Hepatic fibrosis may still be partially reversible if alcohol consumption ceases. However, if alcohol consumption persists, chronic inflammation and fibrogenesis progress [130]. Fibrosis prevents the reconstitution of normal liver lobules and the regeneration of hepatocytes occurs in an uncontrolled manner, resulting in the formation of "regeneration nodules" characteristic of cirrhosis. This can lead to portal hypertension and/or liver failure. Alcoholic hepatitis is associated with fast progression of fibrosis and leads to cirrhosis in 40% of cases [131]. About 2% of cirrhotic patients develop primary hepatocellular carcinoma [129].

As mentioned above, not all AUD patients develop advanced liver disease. The cause is not yet well known but factors such as age, gender, drinking pattern and type of alcohol consumed, genetic factors, obesity, viral diseases (hepatitis C or B) or the composition of the gut microbiota have been identified as influencing the progression of liver disease [127,130,132].

1.5 Acute and long-term management of AUD

1.5.1 Alcohol withdrawal syndrome

Alcohol withdrawal can be particularly violent and remains fatal if left untreated. The discomfort of withdrawal (dysphoria, negative emotional state) is particularly important and contributes to maintaining consumption.

a. Clinical symptoms

Alcohol withdrawal syndrome consists in the physiological and psychological events occurring when a chronic drinker suddenly abstains from alcohol consumption. Up to 50% of AUD patients experience clinical signs of withdrawal [133,134]. These events occur 6 to 24 hours after complete cessation or reduction of alcohol use [133]. They vary considerably from one patient to another and can be manifested by: hand tremors, agitation, increased heart rate, increased blood pressure, headaches, sweating, nausea, vomiting, loss of appetite, craving for alcohol, anxiety, irritability, insomnia. More serious manifestations such as hallucinations, epileptic seizures, delirium tremens and coma can also occur [133–135]. The mild or moderate forms of alcohol withdrawal syndrome are often managed by the patients themselves without treatment and disappears within 2 to 7 days after the last consumption, while the more severe manifestations require medical treatment [133,134].

b. Neurochemical mechanisms

As seen previously (part 1.3), chronic alcohol consumption induces a neuroadaptation to counteract the neurochemical imbalance and return to a state of equilibrium. This neuroadaptation consists in reducing the number of $GABA_A$ receptors and producing more NMDA receptors. Reducing or stopping alcohol consumption suddenly unbalances the brain's neurochemistry in favor of the excitatory neurotransmitter, glutamate. This neuronal hyperexcitability is responsible for many of the signs and symptoms of the withdrawal syndrome [135].

Animal studies also highlighted that alcohol withdrawal is related to reduced release of dopamine in the striatum [136,137]. This could be responsible for the negative mood observed during alcohol withdrawal.

1.5.2 Treatments and management of AUD

AUD is a multifactorial disease with a high risk of relapse. Indeed, without treatment, the relapse rate at one year reaches 70%. It has been shown that combining psychological and pharmaceutical approaches before, during and after withdrawal reduces the risk of relapse [138].

a. Treatment of withdrawal symptoms

The pharmacological molecules that have proven to be the safest and most effective in the treatment of withdrawal symptoms are the benzodiazepines [139]. They mimic the initial effects of alcohol by stimulating the $GABA_A$ receptors. By their action they will restore the neurochemical balance in the brain and prevent neuronal hyperexcitability. They are associated with a great reduction in the risk of seizures, delirium tremens and mortality [135,140,141].

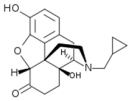
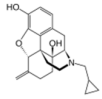
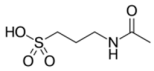
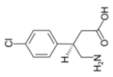
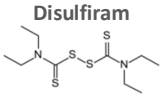
Thiamine (vitamin B1) supplementation is also part of the management of alcohol withdrawal. Indeed, thiamine deficiency is very common in AUD patients due to insufficient nutritional intake and reduced intestinal absorption and can have serious consequences during withdrawal [142]. The brain and heart cells are very sensitive to thiamine deficiency. It is an essential cofactor for the functioning of several enzymes involved in carbohydrate metabolism that generate molecules essential for brain function, including antioxidants and adenosine triphosphate (ATP), which provides energy to cells [143]. During withdrawal, neurons are hyper-excited and require more energy. In case of thiamine deficiency, the lack of ATP will induce brain lesions due to oxidative stress, cell

damage and cell death. These brain lesions in alcoholics are associated with a neurological disorder known as Wernicke-Korsakoff syndrome characterized by delusion, walking difficulties with gait enlargement and ophthalmoplegia [143,144].

b. Pharmacological treatment of AUD

Pharmacological molecules, targeting certain brain neurotransmitters or ethanol metabolism, have been developed to reduce the frequency of excessive alcohol consumption, the state of withdrawal and to improve the rate of abstinence and quality of life. Today, among these molecules, three have been approved by the Food and Drug Administration (FDA): disulfiram (Antabuse), naltrexone and acamprosate. The different medications for the treatment of AUD are presented in the **Table 2**.

Table 2: Medication used for treating alcohol use disorder

Medication	Target	Clinical use	References
Naltrexone 	μ-opioids receptor antagonist	Decreases alcohol consumption	[145,146]
Nalmefen 	μ and δ antagonist partial κ agonist	Decreases alcohol consumption	[146,147]
Acamprosate 	NMDA Glutamate receptor antagonist	Increases abstinence rates	[145]
Baclofen 	$GABA_B$ receptor agonist	Increases abstinence rates	[148–151]
Disulfiram 	Aldehyde deshydrogenase inhibitor	Decrease alcohol intake	[152]

However, clinical studies have shown mixed results or have reported low to moderate efficacy of these drugs [153]. It is therefore necessary to find new therapeutic avenues and among these, the gut microbiota could represent an interesting target.

2 The gut microbiota

2.1 Definition and current knowledge

2.1.1 Gut microbiota composition

From birth, the human body is colonized and lives in symbiosis with a large number of micro-organisms including protozoa, archaea, eukaryotes, viruses and bacteria. This combination of microorganisms creates a real ecosystem known as the "human microbiota" [154]. Among these micro-organisms, bacteria represent the most well studied group and will be the main focus of this work. Bacteria are colonizing many parts of the human body including the oral cavity, genital tract, respiratory tract, skin and GI tract [154]. It was estimated that we had 10 times more microbial cells than human cells (10:1). However this number has recently been updated estimating the ratio at 1:1 [155]. The human microbiota contains approximately 10^{13}-10^{14} microbial cells and would represent 2kg alone [155]. The microbiome, the set of gut microbial genes in an individual, represents a genetic repertoire 150 times larger than the human genome [156].

The ecosystem faces variable conditions (anatomical and chemical) from the oral cavity to the rectum. Therefore, the density and composition of bacteria differ significantly between the different parts of the GI tract (**Figure 5**). Indeed, due to the low pH, oxygen level and short transit time, the upper part of the digestive tract (stomach and small intestine) contains a lower bacterial density estimated at 10^{3}-10^{4} bacteria per ml of intestinal content [157,158]. The part with the highest number of bacteria is the colon (3.8×10^{13} bacteria) in which the transit time is longer [155](**Figure 5**).

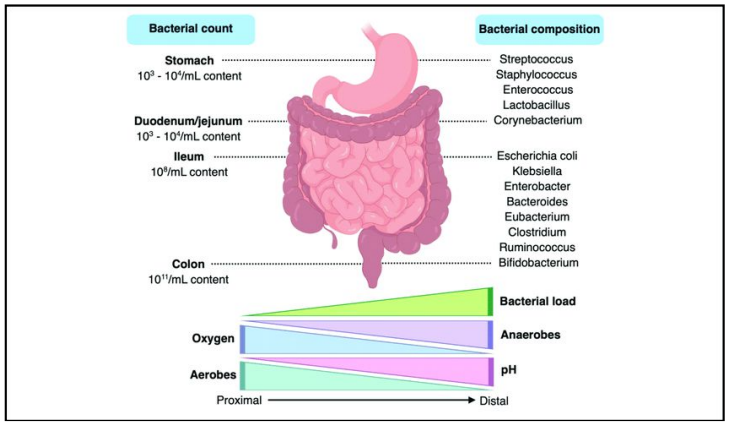


Figure 5: Composition and density of the gut microbiota according to the different parts of the gastrointestinal tract [159]

The composition of the gut microbiota is determined from birth. It has been shown that a vaginal birth predicts a gut microbiota close to the vaginal and faecal microbiota of the mother, with colonisation by *Lactobacillus*, *Prevotella* and *Bifidobacterium* [160–162]. On the contrary, a c-section birth predicts a microbial profile close to the microbiota of the mother's skin, the medical staff and the hospital environment [160–162]. Under the influence of nutrition (breast-feeding or infant milk and then dietary diversification), genetics, medical treatments received, environment and geographical location, the composition of the gut microbiota will evolve qualitatively and quantitatively during the first years of life [163]. The neonatal gut microbiota largely differs between individuals (elevated β-diversity) after birth and progressively reduced at the age of one to gradually reach that of adults. On the contrary, α-diversity, i.e. bacterial richness and evenness, increases with time [163]. These indicate that the microbiota becomes more complex and homogeneous among individuals along time. It has been shown that soon after birth, the gut microbiota is composed mainly of 2 phyla: Actinobacteria and Proteobacteria.

The adult microbiota consists of hundreds of species. It is dominated by members of the Firmicutes and Bacteroidetes phyla which constitute respectively 60-80% and 15-30% of the total bacteria [164]. The remaining bacteria, accounting for less than 10% of the total population, belong to the Proteobacteria, Fusobacteria, Actinobacteria, Verrucomicrobia, and Spirochaetes [164] (**Figure 6**).

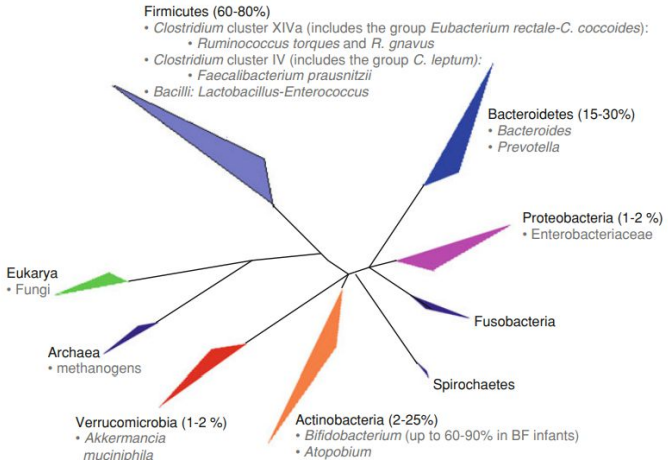


Figure 6: Phylogenetic tree of the human gut microbiota. In parentheses, the relative abundance in healthy adults. The main taxa, as well as some species known to have an impact on human health, are indicated **[164]**

As already mentioned, in adulthood the gut microbiota is more stable however some factors may still influence its composition. Indeed, it has been shown that aging, medication, environment and lifestyle including physical activity and diet can alter the microbial composition [165]. If the use of antibiotics is the most direct and effective way to target gut microbes [166,167] other drugs have important effects on microbial diversity and composition. This is the case of statins for example [168]. A recent study showed that obese participants taking statins had a significantly lower prevalence of the dysbiotic Bacteroides 2 enterotype, a gut bacterial signature, than did their obese counterparts not taking statins [168]. Antidepressants have also been shown to have an effect on bacterial composition and richness and to stop the growth of certain bacterial species such as *Escherichia coli* [169]. Finally, proton pump inhibitors use is associated with decreased gut bacterial richness and higher abundance of oral and upper GI tract bacteria especially *Streptococcaceae* [170,171]

Regarding diet, it has been shown that it is one of the main factors influencing the composition of the gut microbiota [172]. For example, a Western diet, consisting of saturated fat, sugar and meat products, decreases the total number of bacteria and the abundance of *Bifidobacterium* and *Eubacterium* species considered as beneficial bacteria. This diet has often been associated with alterations of the intestinal barrier and metabolic disorders [173–175]. In contrast, a Mediterranean diet, rich in unsaturated fatty acids, polyphenols and dietary fibers but lower in saturated fat, meats, and dairy products, is known to increase microbial diversity and *Lactobacillus*, *Bifidobacterium*, and *Prevotella*, and decreases *Clostridium* [175,176]. This diet is associated with a decreased risk of cardiovascular disease, certain cancers and neurodegenerative diseases [177–179]. It has also been shown that an acute change in diet, such as a strictly animal or plant-based diet, changes the microbial composition within 24 hours of initiation of the diet, with a return to baseline values within 48 hours of diet cessation [172].

Recently, another factor has been revealed as influencing microbial composition, the social interactions. In wild baboons, it has been shown that individuals belonging to the same group have similarities in terms of microbial composition [180]. It has also been shown that spouses/partners have a more similar microbiota and more bacterial taxa in common than siblings [181]. In addition, married people harbour more diverse and richer microbial communities than people living alone [181].

Tools to assess the gut microbiota composition:

The determination of the composition of the gut microbiota is based on two main techniques: 16S rRNA gene sequencing and whole genome shotgun sequencing. These analyses provide the relative abundance of bacterial taxa, as well as comparisons of α-diversity (diversity within the sample; one value per sample) and β-diversity (diversity between samples; pairwise values for all sample combinations).

16S rRNA gene sequencing: This technique is based on the sequencing of the 16S ribosomal DNA which codes for the small 16S ribosomal subunit. Ribosomes are ribonucleoprotein complexes that are essential for messenger RNA (mRNA) decoding and protein synthesis (translation). It is composed of a small subunit and a large subunit. Each subunit is composed of one or more ribosomal RNA (rRNA) molecules and proteins. In prokaryotes, which have 70S ribosomes, there is a small subunit (30S) and a large subunit (50S). The 30S subunit contains one molecule of 16S RNA and the 50S subunit contains two molecules of RNA.

The 16S rRNA gene is composed of regions of conserved sequence (almost identical for most bacteria) and hypervariable regions or sequences, which are phylogenetically distinct for a particular genus and species. It is the sequencing of the latter region that will allow us to recognize the bacterial taxon.

Quickly, the DNA is extracted from the stool and then, 16S rRNA genes are amplified via polymerase chain reaction (PCR). These amplified sequences, called amplicons, will then be grouped according to their genetic relationship and counted to give an estimate of their relative abundance in a sample [165,182].

This technique has been used in this thesis for the characterization of the gut microbiota of AUD patients.

Advantages: 1) it is cost effective, 2) data analysis can be performed by established pipelines, and 3) there is a large body of archived data for reference.

Whole genome Shotgun sequencing:

This approach bypasses PCR and allows the sequencing of all (fragmented) DNA extracted from the sample under analysis, including that of unclassified bacteria and viruses using next-generation sequencing technology.

After filtering out unwanted DNA (e.g., human DNA from a human stool sample), the remaining sequences can be used to build *de novo* genomes or align sequences to a reference database [182].

Advantages: More informative especially at lower taxonomic levels

2.1.2 The key functions of the gut microbiota

The gut microbiota, as a symbiont, has essential functions for the human body. It is often considered an "organ" because it plays an important role in shaping host immunity, vitamin synthesis, digesting food, regulating intestinal endocrine function and neurological signalling, modifying drug action and metabolism, and producing many molecules that influence the host.

a. Nutrient metabolism

The intestinal bacteria feed on food components ingested by the host such as carbohydrates, proteins and lipids but also on host-derived components such as epithelial mucus. The gut microbiota uses these substrates to produce energy for cellular processes and growth. Via the process of fermentation, the microbiota produces several gazes and numerous metabolites that influence human health and metabolism. Fermentation depends on the composition of the microbiota but also the transit time and the availability of the substrates to be fermented. The intestinal microbiota also has a key role in the synthesis of certain vitamins [183].

- Carbohydrate fermentation

The most active location for carbohydrate fermentation is the proximal colon. It is in this part of the digestive tract that the abundance of available carbohydrates is high and the pH is low (5.5-6.0) [184]. The majority of intestinal bacteria possess the enzymes necessary for the saccharolytic fermentation of carbohydrates that have not been digested in the upper intestine. These carbohydrates will first be converted into pyruvate and acetyl CoA (**Figure 7**). Pyruvate will then be converted into lactate, succinate and propionate while acetyl-CoA will be transformed into acetate, butyrate and ethanol (**Figure 7**). Acetate (2 carbons), propionate (3 carbons) and butyrate (4 carbons) are the most abundant short chain fatty acids (SCFA) in the human body. They are produced in the colon with a ratio of 60:20:20 respectively [183,185]. This fermentation also induces the production of gases (CO_2, CH_4 and H_2). SCFAs can influence their own production as they promote the decrease of pH in the colon, which will influence the microbial composition which in turn will affect the amount of SCFA produced [186]. The balance between the different SCFA depends on the phenomenon of cooperation between bacteria called cross feeding. This mechanism consists in using either products from the degradation of carbohydrates or the final products of metabolism produced by another microorganism [187]. For example, a cross feeding has been demonstrated between members of the genus *Bifidobacterium* and butyrate producing bacteria such as *Eubacterium*, *Anaerostipes*, *Roseburia* and more recently *Faecalibacterium prausnitzii*. Indeed *Bifidobacterium* will produce acetate during the fermentation of carbohydrates which will then serve as an energy source for the butyrate producing bacteria [187–190].

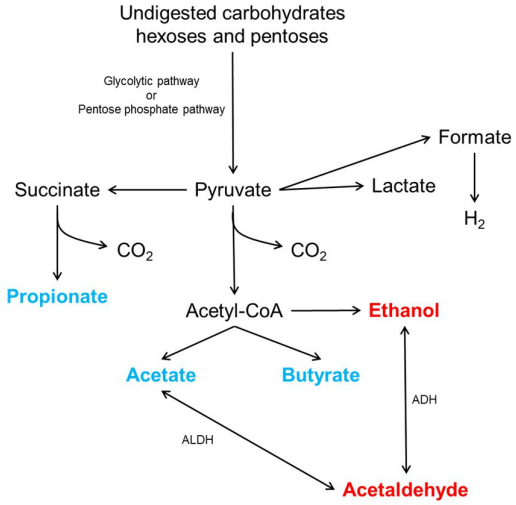


Figure 7: Simplified representation of carbohydrate fermentation in the human gut , adapted from Macfarlane S. and Macfarlane GT, 2003 [191] The short chain fatty acids predominantly found in colon appear in blue.

After their production, SCFAs will be rapidly absorbed by colonocytes to generate ATP. Those that have not been metabolized are transported to the liver via the portal vein where they will serve as a substrate for the hepatocytes. Acetate will allow the synthesis of fatty acids and cholesterol. Finally, a small proportion of SCFA will reach the bloodstream, namely 36% for acetate, 9% for propionate and 2% for butyrate [192]. Studies in animals using PET imaging has shown that very low amount of acetate or butyrate reach the brain (approximately 2% and 0.006% respectively) [193,194]. In human, PET studies did not allow to detect acetate in the brain [195,196].

After carbohydrate fermentation, some bacteria, called ethanol producers, which possess the enzymes aldehyde dehydrogenase (ALDH) and alcohol ADH can catalyse the reduction of acetate to acetaldehyde and finally ethanol [197].

- Protein fermentation

The gut microbiota also has proteolytic power and allows the transformation of proteins into short peptides, amino acids (AA) and derivatives, branched-chain fatty acids (BCFA), and gases [198]. These proteins can have an exogenous (via the diet) or endogenous (pancreatic enzymes, host tissues, and mucus protein) origin. While saccharolytic fermentation occurs mainly in the proximal colon, protein fermentation occurs in the distal colon [198,199]. Despite the efficiency of digestion and absorption in the small intestine and depending on the amount of protein ingested, short peptides and AAs are available for fermentation by the gut microbiota [199].

As with carbohydrate fermentation, bacterial protein metabolism also produces SCFA, but in smaller amount [199,200]. The fermentation of branched-chain amino acids (BCAA - valine, isoleucine and leucine) induce the production of BCFAs (isobutyrate, valerate, isovalerate) which are considered as reliable markers of proteolytic fermentation because they are produced exclusively by the fermentation of BCAAs [199] (**Figure 8**). Within 24 hours of a high-protein diet, it has been shown that BCFA production increases, which demonstrate that gut microbiome can rapidly respond to changes in diet [172].

The decarboxylation of AAs leads to the production of polyamines (**Figure 8**). Numerous amine-producing species belonging to genera such as *Bifidobacterium*, *Clostridium*, *Lactobacillus*, *Escherichia* and *Klebsiella* have been identified in the gut microbiota [201]. Gut microbes can synthesize cadaverine, agmatine, putrescine, spermine and spermidine that are present at millimolar concentrations and could impact host health [202,203].

Fermentation of aromatic AAs generates many bioactive end products such as phenol and p-cresol from tyrosine, or indole from tryptophan (**Figure 8**) [204,205].

Finally, the sulfur amino acids (methionine, cysteine, taurine) are fermented by sulphate reducing bacteria which results in the production of H2S (**Figure 8**) [199].

Bacteroides has been identified as the predominant proteolytic taxon in human fecal samples [206]. *In vitro* work has shown that a protein-rich fermentation increases the abundance and richness of *Bacteroides* [200]. Finally, a positive correlation has also been shown between *Bacteroides* abundance and habitual meat consumption [207,208].

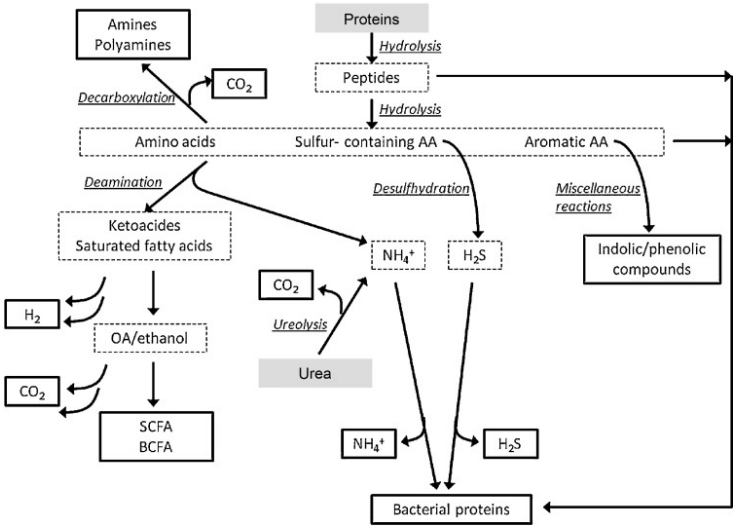


Figure 8: Pathways of protein metabolism by gut microbiota [209]

- Vitamin synthesis

Gut bacteria have been shown, in preclinical and clinical studies, to be both suppliers and consumers of B and K vitamins. Although dietary B vitamins are generally absorbed through the small intestine, B vitamins produced by bacteria are absorbed primarily through the colon [210]. A cross-feeding has been demonstrated between vitamin-producing and non-producing bacteria. Indeed, it seems that a large part of the vitamins produced by the microbes are used by other bacteria as an energy source. The bioavailability for the host is thought to be relatively low [211,212].

Regarding vitamin K, it has been shown that germ-free rats raised without dietary vitamin K supplementation have low prothrombin levels and develop haemorrhage, whereas their conventional counterparts have normal prothrombin levels and normal coagulation activity [213].

All these works suggest that the composition and function of the gut microbiota affect the host's use of vitamins and, by extension, its health.

b. Bile acid metabolism

Bile acids (BA) are metabolites that are synthesized from cholesterol to form the two primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA). BA, cholesterol, phospholipids and bilirubin are the major components of bile. The synthesis of BA includes

many steps and involves different enzymes located in the endoplasmic reticulum, mitochondria, cytosol, and peroxisomes of cells. Before their secretion into the bile, primary BAs are conjugated with a taurine or glycine molecule. This conjugation increases the detergent properties of the molecules. In the duodenal lumen, BAs act as emulsifiers of lipids and dietary fat-soluble vitamins, thus promoting their intestinal absorption. Conjugated BAs will then be absorbed in the terminal ileum. About 95% of the BAs secreted into the bile will be reabsorbed into the ileum and then transported by the portal vein to the liver where it will be excreted again into the bile [214].

Approximately 5% of BAs escape the enterohepatic cycle and reach the colon where they will interact with the gut microbes. These BAs will be metabolized by the gut microbiota into secondary bile acids as opposed to primary bile acids synthesized by the liver. The gut bacteria possess different enzymes that allow different transformations, including deconjugation, oxidation and epimerization. As a result, about twenty secondary BAs have been identified in human feces [212,215].

A bidirectional relationship exists between the intestinal microbiota and the BAs. Indeed, BAs have antimicrobial properties that affect the composition of the intestinal microbiota. The microbiota can also influence the metabolism of BAs since it can modify their structure and properties. The variation of BA composition can influence the physiology of the host and it has been shown that a disruption of BA-microbiota crosstalk was associated with several GI, metabolic and inflammatory disorders [214].

c. Modulation of gut peptides and hormones levels

GI peptides are important to control metabolic health of the host [216]. Their synthesis have been shown to be modulated by the composition of the gut microbiota [216,217]. Changes in the composition of the gut microbiota can directly affect the numbers of enteroendocrine cells which can modulate the production of GI peptides [216]. Other proposed mechanisms involved the SCFA produced by bacterial fermentation which are able to stimulate the release of satiety hormones such as peptide YY (PYY), leptin, ghrelin and glucagon-like peptide-1 (GLP-1) via the stimulation of G protein-coupled receptors (GPR) 41 and 43. GPR41 is predominantly expressed in the small intestine and GPR43 in the colon [218,219]. It has also been shown that SCFA and other microbial metabolites like lactate are able to modulate the ghrelinergic system, through the activation of the growth hormone secretagogue receptor (GHSR) [220,221]. A recent

study shown that the bacterial CLPB protein is able to modulate PYY secretion and food intake in C57Bl/6 and ob/ob mice [222].

d. Maintenance of structural integrity of the gut mucosal barrier and immune function

The GI tract is composed of physical and biological barriers that are essential to maintain intestinal integrity and immune homeostasis. These barriers isolate the host from the external environment, but also regulate the immune system, the absorption of nutrients and limit the access of microorganisms, whether commensal or pathogenic. Mucus, IgA, antimicrobial peptides and immune cells minimize contact between microorganisms and the surface of epithelial cells, thereby limiting tissue inflammation and microbial translocation. The gut microbiota has a key role in maintaining the integrity of this intestinal barrier. Indeed, it has been shown that SCFA upregulate the expression of anti-microbial peptides which can inhibit the growth of pathogenic bacteria by disrupting their bacterial inner membrane [223,224]. The gut microbiota also contributes to modulate the intestinal mucus layer. Studies in GF mice have shown that the mucus of GF mice is altered compared to conventional mice [116]. Because of its high polysaccharide content, the mucus layer is a source of nutrients for certain bacteria, the so-called “mucus degraders”. Conversely, some bacteria could influence the synthesis and secretion of mucins [116]. For example, *Bacteroides thetaiotaomicron* can modulate the thickness and composition of the mucus layer by increasing goblet cell differentiation and modulating the expression of mucin-related genes [225]. It has also been shown that certain SCFA such as butyrate and propionate increase the expression of *muc2* genes that encodes a prominent member of the mucin protein family that is crucial for the structure of the mucus layer [226].

The colonization of the digestive tract by the gut microbiota during early-life allows the maturation of the mucosal and systemic immune system. A dialogue is then established between the gut microbiota and the immune system, the former teaching the latter a certain immune tolerance against commensal bacteria [227].

The interaction between the gut microbiota and the host is mediated by different factors and particularly the microbial-associated molecular patterns (MAMPs). Among MAMPs we find lipopolysaccharides (LPS), peptidoglycan (PGN), lipoprotein, flagellin [227]. The host is able to distinguish commensal microbiota from pathogenic microbiota

by pattern recognition receptors (PRRs), such as cell surface Toll-like receptors (TLRs) and cytoplasmic nucleotide-binding oligomerization domain (NOD) proteins [228,229]. Despite the constant presence of MAMPs, commensal microbes do not generally induce inflammatory responses; on the contrary, they can play an important role to enhance immune function [229]. As an example *Faecalibacterium prausnitzii*, a bacteria decreased in several immune-related disorders like Crohn's disease, exhibits anti-inflammatory properties namely by favoring the production of IL-10 by peripheral blood mononuclear cells (PBMC) and decreasing TNFα in the gut [230].

Metabolites produced by gut bacteria may also have anti-inflammatory properties. This is the case of butyrate produced by *F. prausnitzii*, among others. In addition to being an important source of energy for colonocytes, butyrate can exert direct immunomodulatory effects through the inhibition of NF-κB, inhibition of IFN-γ production and signalling, and upregulation of peroxisome proliferator-activated receptor-γ (PPARγ) [231].

Tryptophan catabolites could also affect systemic inflammation. For example, it has been shown that indoleacrylic acid inhibits pro-inflammatory cytokines production in human PBMCs [232].

2.2 Link between the gut microbiota and health

2.2.1 Microbiota in host physiology and pathologies

Over the last two decades, more and more studies have focused on the role of the gut microbiota on host health. Changes in the composition of the gut microbiota have been observed in a large number of pathologies with metabolic components such as obesity, diabetes or chronic intestinal diseases in comparison with healthy subjects [233–236].

The use of GF mice, has been pivotal in better understanding host-microbe interactions. Historically, it was Louis Pasteur in 1885 who hypothesised that animals without bacteria would not be able to survive because of the close synergy between microbes and their hosts [237]. From the 1960s onwards, work on GF mice increased. One of the major discoveries made with GF mice was the importance of the gut microbiota in metabolism and obesity. Indeed it has been shown that GF mice are leaner than conventional mice and are protected from diet-induced obesity [238–240]. Researchers have also shown that GF animals exhibit abnormalities in the immune, metabolic and GI systems and more recently a plethora of neurobiological and behavioural alterations

[165,241,242]. Indeed, GF mice display reduced anxiety-like behaviour as well as deficits in cognitive functions, such as working memory [165,243,244].

The punctual suppression of the microbiota through the use of antibiotic treatments has confirmed these findings. For example, it was shown that antibiotic use during the perinatal period increases the risk of developing childhood diseases that may persist into adulthood. Namely, maternal use of antibiotics during pregnancy or breastfeeding is a risk factor for the development of allergy or asthma, inflammatory bowel disease, obesity and poorer neurocognitive outcomes later in life [245–249]. These models, based on depletion of the gut microbiota by antibiotic therapy, have confirmed that the gut microbiota can modulate host behaviour. Indeed, the causal role of the gut microbiota in neurobiological and behavioural alterations has been demonstrated by transferring the gut microbiota from depressed [250], alcoholic [251] or schizophrenic [252] subjects to microbiota-depleted rodents that mimic the behavioural and physiological characteristics of these diseases. It has also been shown that fecal microbiota transplantation (FMT) from obese subjects to mice induces cognitive alterations [253]. In addition, microbiota transfer from young mice (4 months) into aged recipient mice (20 months) can also counteract age-related cognitive and neurobiological (inflammation) alterations [254].

The intestinal microbiota seems to have an important role in a large number of psychiatric or neurodegenerative diseases and a growing number of studies are trying to elucidate the mechanisms by which the intestinal microbiota can influence brain function and behaviour [165].

2.2.2 Gut-brain axis: communication pathways

The use of GF animals, animals treated with antibiotics or probiotic agents allowed to highlight different potential communication pathways between the gut and the brain. The gut-brain communication is bidirectional and includes endocrine, neural, immune and vagal pathways (**Figure 9**).

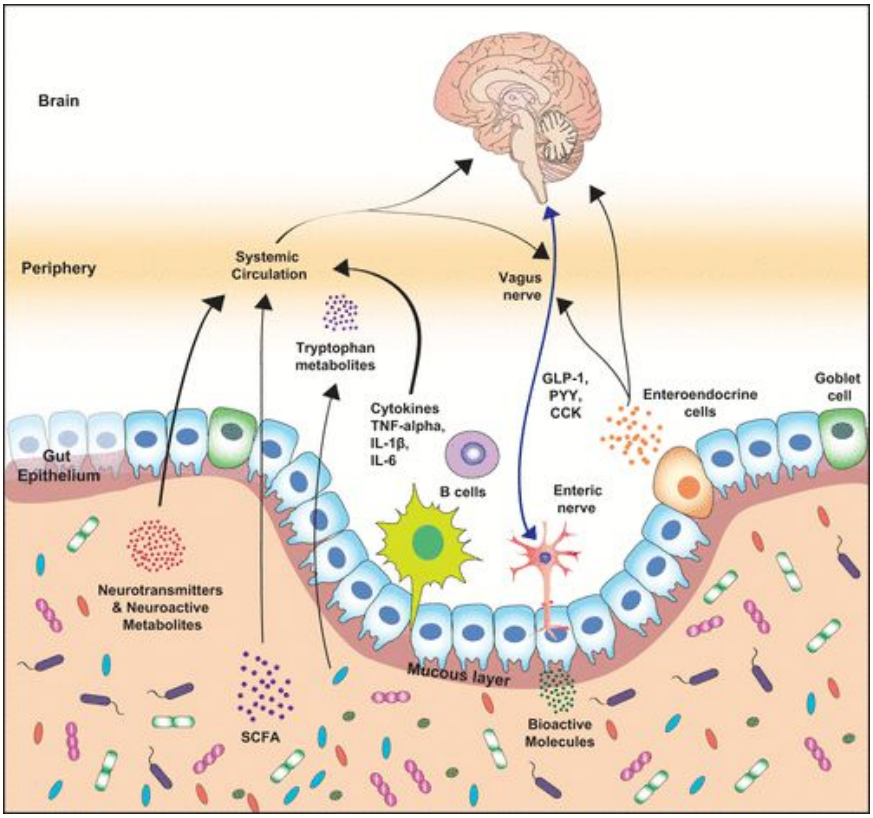


Figure 9: Different potential pathways involved in the bidirectional communication between the gut microbiota and the brain [165]

a. Hypothalamo-pituitary-adrenal axis

The HPA axis is the main efferent pathway linking the brain to the gut. It is well known that exposure to stress, inducing activation of the HPA axis, has an impact on the composition of the gut microbiota. Chronic exposure to stress in adulthood or even early life has been shown to alter the composition of the gut microbiota in mice [255,256].

Cortisol release, resulting from activation of the HPA axis, has also been shown to affect immune cell activity, both locally and systemically. This induces the release of pro-inflammatory cytokines in the GI tract that can affect intestinal permeability as well as the gut barrier function [257–259].

The use of GF mice has demonstrated that microbial composition can influence HPA axis development and stress response. GF mice subjected to contention stress exhibited hyperactivation of the HPA axis resulting in elevated circulating corticosterone levels compared to conventional mice. Interestingly, this exaggerated stress response was reversed in mice colonized with *Bifidobacterium infantis* [260]. Moreover, *Lactobacillus* and *Bifidobacterium* have been found to restore stress-induced HPA axis dysfunction in preclinical models [261–263].

b. Vagus nerve

The vagus nerve is one of the main components of the parasympathetic nervous system. Its territory of innervation is the most extensive and allows the collection of information from different visceral organs [264]. Approximately 80% of its fibers are afferent, allowing the transmission of information on the state of the GI, respiratory and cardiovascular systems to the central nervous system (CNS). The remaining fibers are efferent and provide feedback to the viscera [264].

Preclinical studies using vagotomy have demonstrated the importance of the vagus nerve in the communication between the gut and the brain. Indeed it has been shown that the vagus nerve can be a sensor of inflammation and thus modify the inflammatory response [264]. The anti-inflammatory effect of vagus nerve is mediate through the HPA axis or neurotransmitters release (norepinephrine, acetycholine) that could bind to macrophage to inhibit inflammatory cytokines release [264]. It has also been shown that the vagus nerve receives and responds to signals from bacterial metabolites, gut hormones (leptin, ghrelin) and the release of neurotransmitters such as serotonin [264].

Studies have highlighted that some bacteria could influence host behaviour via the vagus nerve. For example, ingestion of *Lactobacillus rhamnosus* JB1 in conventional mice was shown to decrease anxiety and depressive-like behaviour concomitantly with alterations in brain GABA receptor expression [265]. Similarly, in an autistic mouse model (Shank3B-/- mouse), *Lactobacillus reuteri* supplementation reversed anti-social behaviour in mice [266]. These effects were no longer present in vagotomized mice suggesting the involvement of the vagus nerve in signalling between the gut and the brain. However, despite the growing number of studies implicating the vagus nerve in gut-brain communication, the way by which gut microorganisms activate vagal afferents is not yet well understood.

c. Immune system

In the gut, under physiological conditions, the immune system is constantly stimulated by the presence of trillions of microbes that participate in the maintenance of homeostasis.

Imbalances in the composition of the gut microbiota can lead to an increased intestinal permeability that can favour the translocation of bacterial endotoxins such as lipopolysaccharides (LPS) or peptidoglycans (PGN) into the bloodstream [267,268]. LPS and PGN are recognized by TLR4 and TLR2 receptors, respectively, whose activation leads to a signal transduction cascade resulting in the activation of transcription factors and ultimately the production of proinflammatory cytokines such as TNF-α, IL-1β and IL-6 [269,270]. These cytokines can then induce neuroinflammation and influence behaviour [268].

Modulation of the composition of the gut microbiota can also indirectly influence the immune system via changes in the levels of bacterial metabolites (BAs, SCFA, AA derived metabolites, tryptophan derivatives) [271] as described in the following section (2.2.2 part d).

d. Implication of the gut microbiota-derived metabolites

The intestinal microbiota produces a whole range of neuroactive molecules which can contribute to the communication between the gut and the brain.

SCFAs, the main metabolites produced by the bacterial fermentation of dietary fibers in the GI tract, influence the communication between the gut and the brain directly or indirectly via the immune, endocrine, vagal or humoral pathways [192]. For example, SCFAs promote the integrity of the intestinal barrier, intestinal immune homeostasis and modulate cytokine production [192,272]. They may therefore indirectly affect brain function by regulating inflammation. Animal studies have shown that SCFAs are able to cross the BBB via monocarboxylate transporters and affect microglial morphology and function [192,273,274]. It has also been shown that SCFAs, particularly butyrate, can modulate the production of neurotrophic factors such as brain-derived neurotrophic factor (BDNF), signal the brain via vagal nerve and induce biosynthesis of neurotransmitters in the brain [114].

Some neurotransmitters such as catecholamine and GABA can be produced by some bacteria belonging to *Lactobacillus*, *Bifidobacterium, Bacillus* and *Escherichia* species [165]. It has been shown that GF mice that received the microbiota from specific pathogen-free mice had higher levels of GABA in colon and plasma but not in the brain compared to GF mice [276,277]. An increase in neurotransmitter production by gut bacteria therefore does not necessarily reflect an increase at the brain level and other studies are needed to better understand the contribution of gut derived neurotransmitters on brain function.

Aromatic AA metabolites such as indole derivatives or p-cresol also influence brain function and behaviour [278,279]. For example AHR ligands like indoxyl sulfate has been shown to exert neuroprotective effects in mice model of multiple sclerosis [279].

During neurodevelopment several molecules produced by the gut microbiota have been shown to be pivotal for brain development [280]. This work showed that imidazole propionate, hippurate, N,N,N-trimethyl-5-aminovalerate, trimethylamine-N-oxide among others are depleted in the brain of fetuses originating from germ-free mothers while their addition to *in vitro* culture promote axonogenesis [280].

2.2.3 Gut-liver-brain axis

We talk a lot about the gut-brain axis but the liver is an important intermediary in this axis. All metabolites produced by gut bacteria pass through the portal system and reach the liver where many of them are metabolized. These metabolites then enter the systemic circulation where they can influence other organs including the brain.

Liver dysfunction will influence 1) systemic inflammation, which is extremely harmful to the brain 2) the accumulation of metabolic products and toxins with neurotoxic effects, such as ammonia, which is responsible for hepatic encephalopathy and leads to neurological and psychiatric disorders [281].

Fibroblast growth factor 21 (FGF-21), an hepatokine able to cross the BBB, has been shown to influence brain function [282–284].

The gut liver-brain-axis is an interesting avenue to investigate in the context of AUD as it is well known that ethanol alters gut microbiota, liver and brain function.

2.3 Role of the gut microbiota in alcohol use disorder

2.3.1 Link between the gut and the liver in AUD

As mentioned in the part 1.4.3b, only 30% to 40% of AUD patients develop alcohol liver disease. The gut microbiota through its ability to modulate gut barrier integrity and immunity is thought to be an important contributor of hepatic dysfunction in AUD.

It was first shown in preclinical and clinical settings that ethanol exposure increased serum endotoxin (LPS) levels which were positively correlated with the severity of liver damage [285–287]. The administration of antibiotics or probiotics like *Lactobacillus* attenuated the hepatic alterations highlighting the involvement of the gut microbiota in the development of hepatic alterations [288–290].

Among the mechanisms that could be responsible for the increase of bacterial endotoxins in the systemic circulation, an alteration of the composition of the intestinal microbiota has been proposed [291]. Studies in AUD patients have shown a decrease in Bacteroidetes, *Ruminococcaceae*, especially *Faecalibacterium prausnitzii*, *Bifidobacterium*, *Lactobacillus* and an increase in Proteobacteria, *Lachnospiraceae* and *Enterobacteriaceae* [124,292–295]. AUD patients also display increased gut permeability compared to healthy subjects [119,287,296,297]. This is known to promote elevation of LPS and PGN in the portal bloodstream that stimulate pro-inflammatory cytokines release which can in turn induce activation of hepatocytes and Kupffer's cells and perpetuate inflammation and liver fibrosis [120]. This is corroborated by a recent study that demonstrate that

alterations in duodenal mucosa-associated microbiota, as well as elevated translocation of microbial products and/or microbes themselves, are associated with liver disease progression in AUD patients [298]. Moreover, it has been shown that leaky gut in AUD patients was associated with more pronounced alteration of the gut microbiota composition and function, the so-called gut dysbiosis [293,294]. It is reinforced by the fact that these patients have high levels of phenol, known to alter gut barrier function, and on the contrary low levels of indoles which have been shown to counteract the detrimental effects of LPS in the liver [293,299]. Indeed indoles have been shown to contribute to intestinal and systemic homeostasis by activating the AhR receptor and promoting IL-22 production, stimulating mucus production, enhancing tight junction proteins and exerting anti-inflammatory and anti-oxidant properties (**Figure 10**) [300].

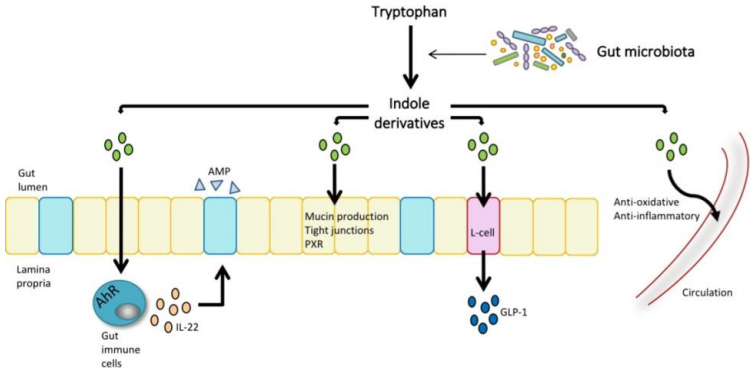


Figure 10 : Potential mechanisms of action by which indole derivatives may have a beneficial effect on the liver disease [300]

2.3.2 Link between the gut and the brain in AUD

It has been shown that the gut microbiota can exert an effect on the brain via multiple pathways. To date, the role of the gut microbiota in the development or maintenance of psychological symptoms in AUD patients is still unclear. However, it has been shown that AUD patients with the most altered gut microbiota and higher gut permeability had the most severe psychological symptoms such as anxiety, depression or alcohol craving [293]. Inflammation also seems to have an important role in the negative reinforcements of the drinking behaviour since correlations have been observed between systemic proinflammatory cytokines and alcohol craving [119,301]. Recently, the causal role of the gut microbiota in the development of psychiatric symptoms has been

demonstrated. Indeed, mice transplanted with the gut microbiota of AUD patients showed an increase in depressive-like behaviour and a decrease in social behaviour associated with neurobiological alterations (myelination, neurotransmission, inflammation) [302].

The gut microbiota influences the secretion of gut derived peptides such as GLP-1, ghrelin or PYY [303]. These peptides have been shown to be altered in AUD patients and may impact craving for alcohol [304,305]. For example, it has been shown that ghrelin modulates several processes such as reward, mood, memory and stress response [306–308]. Observational studies indicate that AUD individuals have reduced peripheral ghrelin levels during alcohol drinking and increased levels during abstinence [304,309–311]. Positive correlations were also observed between ghrelin levels, alcohol craving and brain activity in response to alcohol cues [309,312].

Finally, we have seen that gut bacteria are able to produce a large number of neuroactive molecules. A recent study showed that SCFA-producing bacteria, such as *Faecalibacterium prausnitzii*, could play a role in regulating the metabolic pathway of tryptophan, some of whose derivatives are correlated with alcohol craving [313]. Further studies investigating the relationship between bacterial metabolites and neurobiological or behavioural alterations are needed to understand their involvement in the psychological disturbances observed in AUD patients.

All these observations support the fact that the modulation of the gut microbiota represents an interesting target in the management of AUD.

It is known that AUD patients suffer from malnutrition (part 1.4.2) and it is mentioned in part 1.4.1 that eating disorders are co-occurring with substance use disorder and in particular with AUD. It is also well established that AUD patients suffer from poly-addiction [314]. For example, it has been shown that 50-75% of them are smokers [315,316]. These factors (i.e: diet, tobacco and drugs use, eating pattern disturbances) are important to consider when studying the gut microbiota as it has been suggested that they may influence each other [83,317–322]. Indeed, both AUD and ED affect nutritional intake and it is known that the diet has an important effect on the gut microbiota. It has been shown that alterations in the gut microbiota in both psychiatric disorders were correlated with anxiety and depressive behaviour, potentially indicating a common mechanism of influence exerted by the gut microbiota on these psychiatric disorders [83,293,323]. In particular, it has been suggested that gut peptides such as ghrelin may be involved in the dysregulation of the reward system observed in both disorders [83].

Finally, a recent study in mice showed that smoking induces alterations in the composition and function of the gut microbiota in the short and long term, including an increase in dimethylglycine and N-acetylglycine. These metabolites were associated with the metabolic disturbances observed during smoking cessation [319]. This suggests a cross talk between addictions, eating behabiour and the gut microbiota.

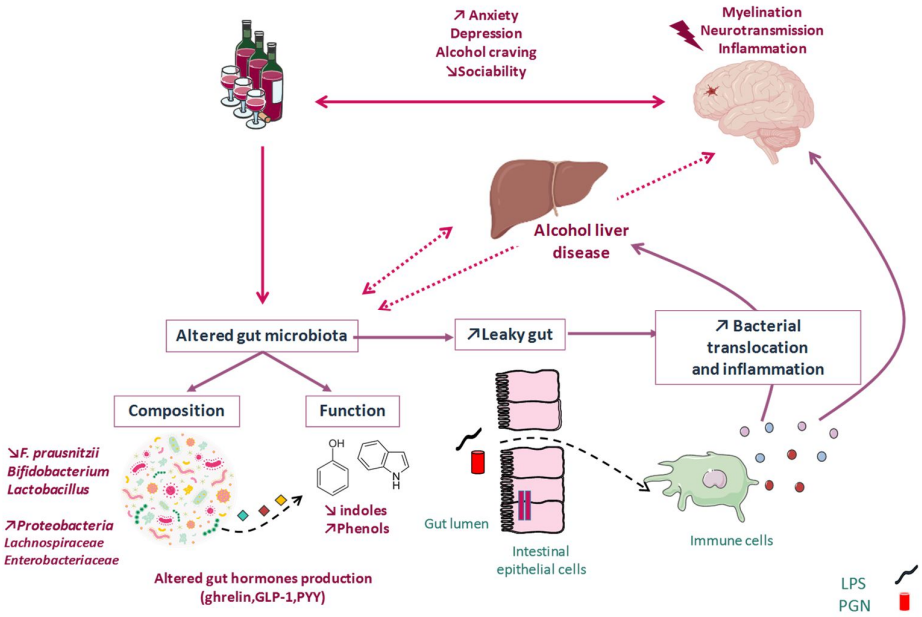


Figure 11: Summary of the main findings on the gut-brain axis in alcohol use disorder

2.4 Microbiota modulating strategies

Lifestyle has an influence on the composition and function of the gut microbiota. It has been shown that short dietary intervention as well as long-term dietary habits influence the composition of the gut microbiota [172]. Exercise also has an influence on the diversity and composition of the gut microbiota. In particular, it has been shown that elite athletes appear to have greater gut microbial diversity and an increase in SCFA-producing bacterial species [324].

The gut microbiota is a dynamic ecosystem that can be voluntarily modified using several tools: pro- or prebiotics, symbiotics (probiotic + prebiotic), postbiotics or FMT (**Figure 12**).

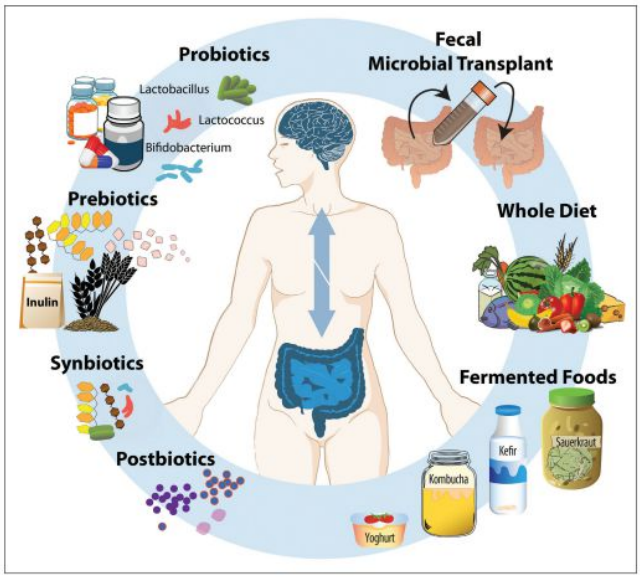


Figure 12: Different microbiota-targeted interventions [325]

Probiotics are defined as "living microorganisms that, when administered in adequate amounts, confer a health benefit on the host" [326]. The most widely studied probiotics are *Saccharomyces boulardii*, *Bifidobacterium* and *Lactobacillus*, particularly in the context of digestive disorders due to antibiotics, gastroenteritis and IBD [327]. In the last decade, *Bifidobacterium* and *Lactobacillus* have been widely studied for their psychobiotic effect (beneficial effect on mental health). The use of these probiotics or multiple probiotic strains has shown beneficial effects on stress, anxiety or depression in healthy subjects or patients with psychiatric disorders [328–331]. In recent years, probiotics or new

generation microorganisms such as *Faecalibacterium prausnitzii* or *Akkermansia muciniphila* are increasingly studied in the context of metabolic, psychiatric or neurodegenerative diseases [332–335].

Postbiotics have been defined as “preparation of inanimate microorganisms and/or their components that confers a health benefit on the host” [336]. It is thought that postbiotics can modulate gut microbiota composition, influence gut barrier, vagus nerve activity and immune function [325].

Bacterial metabolites are also of great interest since they are potential mediators of the effects of the gut microbiota on health [183]. Studies in humans are currently limited but it has been shown for example that, in overweight adults, SCFAs modified feeding behaviours (increased satiety) by stimulating the release of anorexigenic hormones, such as GLP-1 and PYY [337]. In healthy subjects, SCFAs administered directly into the colon attenuated the cortisol response to psychosocial stress [338].

FMT is part of the innovative therapeutic approaches that consists to the transfer of gut microbiota from one individual to another. This approach has been successfully applied to the treatment of *Clostridium difficile* infection. [339–341]. Since then it has been used in several pathological contexts such as obesity [342,343], autism [344] and even AUD [345]. However, FMT remains a complex approach requiring a good choice of donor, which may be complex in clinical practice. Indeed, so far we are not able to define what a "healthy" intestinal microbiome is.

In this work we used a prebiotic approach, by using inulin supplementation, which has been shown to have several health benefits.

3 Prebiotics

3.1 Definition

The concept of prebiotics was introduced over 20 years ago by Gibson and Roberfroid. It was defined as “a non-digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, and thus improves host health”[346]. The components were expected to promote the growth of health-promoting bacteria, mainly *Bifidobacterium* and *Lactobacillus* species. According to this definition, three prebiotic components were identified: fructo-oligosaccharides (FOS), inulin and galacto-oligosaccharides (GOS).

Since then, the definition of prebiotics has been widely discussed and has been expanded thanks to new discoveries and technologies developed in the field [347,348]. In particular, it has been shown that bacteria other than bifidobacteria and lactobacilli were modified by prebiotics and could have positive effects on health [348]. The definition of prebiotic has evolved to include the specificity of substrate fermentation by commensal bacteria, the entire microbiota (other body sites such as skin, vaginal mucosa...), the causal role of altering the gut microbiome in improving host health, and the inclusion of non-carbohydrate products as prebiotic ingredients [347].

In 2017, the International Scientific Association for Probiotics and Prebiotics (ISAPP) published a consensus and researchers agreed on the following definition: "a substrate that is selectively used by host microorganisms and confers a health benefit". The main changes were that the microbes responding to prebiotics had to be health-promoting bacteria, without specifying which ones. Previous definitions focused on the distal part of the GI tract, the new one does not specify a body site thus opening the way to other targets such as the skin, mouth or the urogenital tract. Finally, the definition implies that non-carbohydrates as well as categories other than food components should be included, that health benefits must be documented and that animal use is also covered [348].

Inulin, FOS, and GOS remain the most studied prebiotics so far, but new compounds may fit the definition of prebiotics such as polyphenols or polyunsaturated fatty acids. However, further studies are needed to confirm their prebiotic status [349].

In this thesis, we will focus on inulin-type fructans (ITF).

3.2 Inulin-type fructans

ITF are water-soluble fibers found in certain plants, in fruits and vegetables such as asparagus, onions, garlic, leeks, artichokes, Jeruzalem artichokes, scorzonera, chicory root and in some cereals [350]. ITF are oligomers and polymers of fructose linked by β-2,1 bonds with a terminal α-linked glucose (**Figure 13**). Depending on the length of the carbon chain, ITFs are called inulin (degree of polymerization 2-60) or FOS (degree of polymerization 2-8) [351].

The plant most used by manufacturers to extract ITF is chicory. Chicory inulin, called native inulin, is an unfractionated inulin extracted from fresh roots. FOS can be obtained either following the enzymatic hydrolysis of inulin and is then called oligofructose or synthesized enzymatically from sucrose [352].

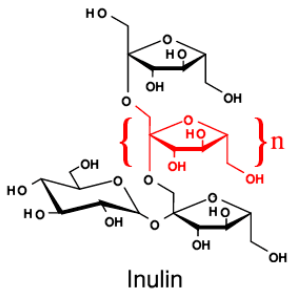


Figure 13: Chemical structure of inulin-type fructan

Due to their chemical structure, ITFs are resistant to hydrolysis by mammalian digestive enzymes and to intestinal absorption. This is why they are classified as "non-digestible" oligosaccharides. When they reach the colon, ITFs are selectively fermented by bacteria that present the enzyme β-fructosidase, such as *Bifidobacterium*. Indeed, numerous studies have demonstrated that supplementation with inulin-type fructans increases the growth of bifidobacteria in healthy individuals [353,354]. The monomers obtained by hydrolysis can then be used as growth substrate for microbial fermentation, leading to the production of SCFAs.

ITF has been studied extensively, particularly in the context of obesity, and has been shown to have positive effects on GI health and metabolism [351]. More recently, some studies have shown a role on the brain and behaviour.

3.3 Impact of inulin type fructan on health

3.3.1 Gut microbiota and gastro-intestinal tolerance

a. Impact of ITF supplementation on gut microbiota diversity and composition

The effect of ITF supplementation on microbial diversity in humans has shown contrasting results. A recent meta-analysis including 5 randomized controlled trials showed that inulin had no effect on α-diversity compared to a placebo [354]. Several studies have shown a decrease in microbial diversity with ITF supplementation. This is the case of Reimer *et al* who showed that ITF led to a decrease in the number of bacterial species compared to placebo in overweight/obese adults supplemented for 12 weeks [355]. In overweight/obese children, ITF supplementation induced a decrease in Shannon and Simpson indices [356]. Furthermore, another study conducted in healthy adults with mild constipation showed that ITF supplementation decreased the observed richness compared to placebo, while other diversity indices were not changed [357].It is therefore difficult to conclude on the effect of ITF on α-diversity, but so far, no study has demonstrated that ITF can increase it.

In their meta-analysis, Le Bastard *et al* selected 6 studies that investigated the effect of ITF on β-diversity to see if there were changes in overall microbiota architecture. Among them, 3 studies reported a significant change after supplementation [354].

Interestingly, another study found significant inter-individual variation in the response to ITF supplementation [358]. Indeed, it has been shown that the effect of ITF on the composition of the gut microbiota could depend on the amount of fiber usually consumed or on the baseline microbiota of individuals [358–360].

The impact of inulin supplementation on gut microbiota composition has been extensively studied in healthy, overweight/obese or inflammatory bowel disease (IBD) subjects. The results may vary from one study to another due to the different sequencing techniques used or the various study designs. However, in the vast majority of studies, an increase in the relative abundance of *Bifidobacterium* is observed [354,356,361–363]. This effect is called the "bifidogenic" effect. ITF thus seems to promote the growth of this bacterial genus, even at low doses and over a short time. Indeed, Bouhnik *et al*. used increasing doses of short-chain FOS over a period of 7 days (0, 2.5, 5, 10, 20 g/d) and

showed that all doses, except the lowest (2.5 g/d), significantly increased the number of bifidobacteria in healthy adults [364].

Besides the genus *Bifidobacterium*, other bacterial genera of interest have also been shown to be modified by ITF supplementation. Some studies have found consistent results showing that ITF supplementation increases the relative abundance of *Lactobacillus* and *Faecalibacterium* while decreasing *Bacteroides* [354,363] .

No study has investigated the effect of ITF in AUD patients. As mentioned previously (part 2.3), AUD patients have an altered gut microbiota, therefore it would be very interesting to study the impact of ITF supplementation on gut dysbiosis in these patients as well as the effect on host physiology.

b. Impact of ITF supplementation on gastro-intestinal tolerance

Fermentation of ITF by the gut microbiota generates SCFAs, hydrogen, carbon dioxide, methane and hydrogen sulphide [365]. Inulin improves intestinal function and contributes to softer stools and easier excretion [366]. SCFAs play an important role in the GI tract. For example, it has been shown that SCFAs, via the increase in the number of excitatory cholinergic neurons, affect GI motility by stimulating the contractile activity of the colon in rats [367]. In subjects suffering from constipation, inulin has been shown to increase stool frequency [366].

Through the gas production, ITF ingestion can also increase flatulence, bloating and abdominal pain [184,361,368–371].

The degree of ITF polymerization has been shown to affect GI tolerance [372]. FOS, which has a shorter chain, is characterized by rapid fermentation while inulin by slower fermentation [373]. According to the authors, rapid fermentation leads to a greater production of gas and an increase in water absorption in a short period of time, which increases the intensity of GI symptoms [373].

Globally, most studies report an increase in some GI symptoms after ITF ingestion and in particular an increase in flatulence. However, the symptoms are moderate and ITF is relatively well tolerated by the subjects.

3.3.2 Metabolism and liver disease

The effect of ITF on health has been most frequently studied in the context of diseases with a metabolic component such as obesity or diabetes. It has been shown in both animals and humans that ITF supplementation improves glucose homeostasis [353,374,375] and reduces plasma triglycerides (TG) and cholesterol levels [363,376–378]. Several studies have also shown that FOS modulate GLP-1 and ghrelin levels in rats resulting in increased satiety [151,152]. This last observation was also found in healthy individuals supplemented with FOS or fed a diet rich in ITF for 2 weeks [371,381].

Regarding the effects on the liver, preclinical studies have shown a reduction in intrahepatic TG concentration with FOS supplementation [382,383]. It is hypothesized that the production of SCFA via ITF fermentation and in particular propionate would have an important role in hepatic lipid metabolism [382,384]. In obese rats, FOS supplementation induced a decrease in hepatic steatosis [385].
ITF supplementation in obese, diabetic or non-alcoholic steatohepatitis subjects induced a decrease in AST levels [363,386,387].

ITF has also been shown to affect gut barrier function. In high-fat diet-fed mice, FOS supplementation restores *Bifidobacterium* levels and reduces the impact of high-fat diet-induced metabolic endotoxaemia and inflammatory disorders [353,374,388]. Indeed, ITF increases the levels of bifidobacteria known to have an essential role for intestinal health by preventing colonization by pathogens via the production of SCFA or antimicrobial components or by stimulating the immune system [389].

In the specific context of AUD, a recent study conducted in mice exposed to ethanol (ethanol-containing modified Lieber-DeCarli liquid diet) showed that inulin supplementation allowed 1°) to modulate the intestinal microbiota of the mice and 2°) to improve liver disease by decreasing the levels of pro-inflammatory cytokines and AST and ALT [390]. The authors then showed that inulin attenuated the ethanol-induced inflammatory response in the same model potentially via SCFA production [391].

In humans, to our knowledge, no study has investigated the role of ITF on metabolism or liver damage in AUD patients.

3.3.3 Cognition, neurobiological alterations and behaviour

To date, relatively few studies have investigated the role of ITF on cognition or behaviour, either in animals or in humans (see **Table 3**).

In mice, ITF supplementation improves cognitive abilities, neuronal activation and reduces the density of Aβ protein in the brain (see **Table 3**). It has also been shown that ITF supplementation decreases brain inflammation, stress reactivity, anxiety and depressive-like behaviour (see **Table 3**).

In humans, studies have shown an effect of ITF on cognitive performances, emotional competence and anxiety (see **Table 3**).

Further studies are needed to understand the mechanisms by which prebiotics may affect neurobiology and behaviour, focusing on the communication pathways between the gut and the brain.

Table 3: Summary of studies investigating the impact of inulin type fructan on neurobiology, cognition and behaviour

Species	ITF	Time of treatment	Effect	Reference
Human				
Healthy men	Inulin-propionate ester	Acute	↘Striatal anticipatory reward responses to high energy food	[392]
Healthy subjects	Oligofructose-enriched inulin	Acute	↗ free recall performance and recognition memory ↗subjective mood	[393]
Healthy subjects	Oligofructose-enriched inulin	14 days	No effect on well-being, mood or cognitive tasks	[394]
IBD	Short-chain FOS	4 weeks	↘ Anxiety scores	[395]
Obese subjects	Native inulin combined with dietary advice to consume inulin-rich vegetables	3 months	↗emotional competence ↗emotional competence (PEC Total) and mood (SPANE NE) in patients with high level of *Coprococcus* at baseline	[396]
Healthy subjects	FOS or Bimuno®-GOS (B-GOS®	3 weeks	No effects of FOS	[397]
Participants with frailty syndrome	Inulin +FOS Darmocare Pre(®)	13 weeks	Beneficial effect on 2 criteria of frailty: ↗Grip strength ↘ Exhaustion	[398]
Mouse				
C57BL/6 HF diet	Oligofructose enriched inulin	9 weeks	↗Neuronal activation in the arcuate nucleus.	[399]
d-galactose-treated Balb/c mice	FOS	7 weeks	↘brain Aβ density in the cortex and hippocampus ↗ cognitive functions	[400]
APOE4 transgenic (E4FAD) mice	Inulin	16 weeks	↘ Inflammatory gene expression in the hippocampus.	[401]

Middle-aged (10 months) or young adult mice (8 weeks) C57BL/6	Oligofructose-enriched inulin	14 weeks	↘brain Ly-6C^{hi} monocytes infiltration in middle-aged mice ↘Ly-6C^{+} microglia in middle aged mice ↗learning and reduced anxiety-like behaviour in young adult mice	[402]
C57BL/6	FOS, GOS, or both FOS and GOS	3 weeks	↘Anxiety and depressive like behaviour, stress-induced corticosterone release ↘ Crhr1 expression in the hippocampus. ↗ prosocial behaviour in the resident-intruder test ↗ BDNF hippocampal expression	[403]
Alzheimer's disease Model, APP/PS1 transgenic mice	FOS	6 weeks	↘ cognitive deficits ↗ increased expression of synapsin I, postsynaptic density protein (PSD-95) and GLP-1 ↘ phosphorylated c-Jun N-terminal kinase (JNK) level	[404]
Wistar rats	Oligofructose-enriched inulin	14 days	↗ cognitive performances (learning discrimination)	[405]

AIM AND OBJECTIVES

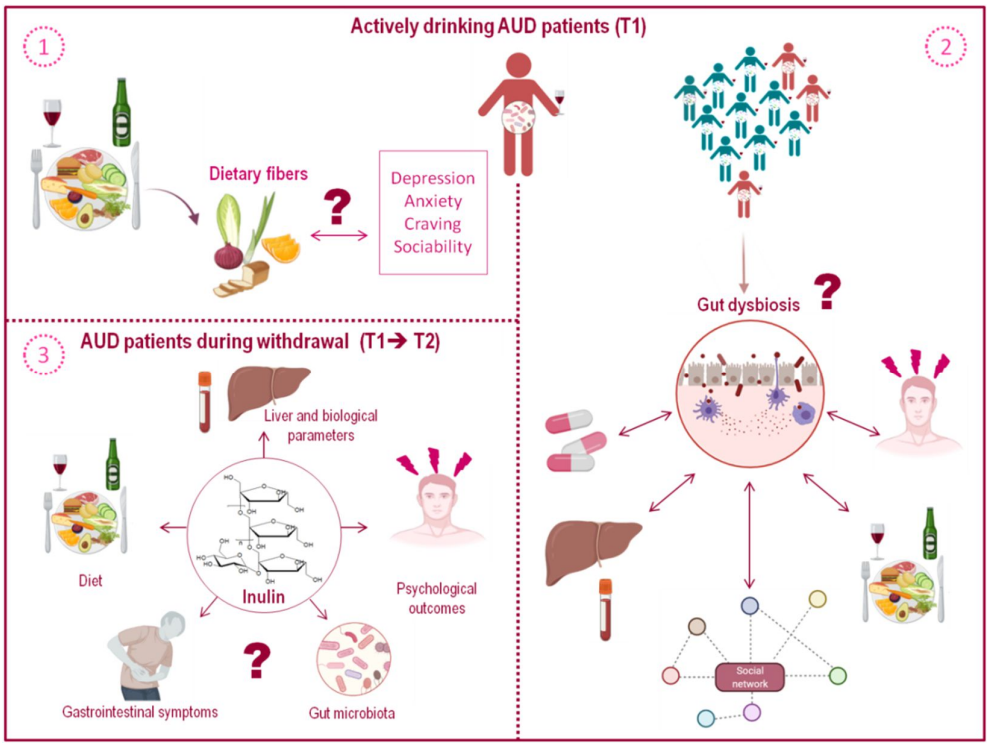


Figure 14: Schematized objectives of this thesis

Acute and chronic alcohol consumption is known to have negative impact on the gastrointestinal tract, the liver, and the brain. Numerous studies have also highlighted that AUD patients are at risk of malnutrition.

Chronic alcohol abuse induces a leaky gut and alterations of the gut microbiota composition and function in AUD patients. This dysbiosis was positively associated with the severity of psychological symptoms suggesting the involvement of the gut-brain axis in the development or maintenance of AUD.

The aim of this PhD work is to investigate the role of the gut microbiota in biological and behavioural alterations associated with AUD and to test an innovative therapeutic approach targeting the gut microbiota, in order to help AUD patients to recover upon alcohol withdrawal.

This work is divided into three chapters which aim to: (**Figure 14**):

1°) evaluate the nutritional habits and especially the dietary fiber intake of AUD patients and to investigate its link with psychological symptoms

2°) explore the relationship between gut dysbiosis and social functioning in AUD patients

3°) to determine the gastrointestinal tolerance of inulin and its impact on gut microbial disturbances, behavioural alterations and biological parameters in patients with AUD. To achieve this objective, we conducted an intervention study with inulin supplementation versus placebo in AUD patients.

In the first chapter, we describe the nutritional intake of actively drinking AUD patients compared to healthy individuals with a focus on dietary fibers which have never been studied in this pathology. Then, we have investigated the link between nutrient intake and psychological symptoms of AUD.

Previous studies have shown that only a portion of AUD patients have an altered gut microbiota. The second chapter reports data allowing to better understand the factors that could be related to gut dysbiosis with a focus on sociability. We have selected AUD patients with the larger gut microbiota composition alteration versus healthy subjects. These subjects have been compared in terms of sociodemographic characteristics,

biological factors, psychological symptoms, social functioning, nutritional intake and medication.

In the third chapter of this thesis, we evaluate the impact of inulin supplementation on gastrointestinal tolerance, biological (inflammation, metabolism, liver damage) and psychological outcomes in AUD patients. A randomized, double-blinded, placebo-controlled trial was conducted in 50 AUD individuals who received during 17 days of inulin/maltodextrin supplementation.

All the studies reported in this thesis are based on data obtained at St Luc hospital in a sample of 50 AUD patients, diagnosed according to the DSM-5 criteria, hospitalized for a detoxification program.

The detoxification program is highly standardized, with all patients being hospitalized in the same conditions. It consists in 2 weeks of alcohol withdrawal in the hospital separated by one week where the patients go back home (**Figure 15**). After verification of the inclusion criteria (described below), patients were informed about the study and signed an informed consent form if they agreed to participate on the first day at admission (always a Monday). AUD patients start the clinical study by performing the first psychological tests on Monday afternoon. On Tuesday morning, blood and fecal samples were collected. In the afternoon, a saliva sample was collected to measure the cortisol. The prebiotic/placebo treatment starts on Wednesday and continues until the end of the detoxification program. Gastrointestinal tolerance towards the treatment was monitored every two days, via self-reported questionnaire. During the week at home all the patients received oral and written advices on how to take the treatment and how to fill out the food diary and the gastrointestinal tolerance questionnaire.

The same psychological and biological tests were performed at the end of the detoxification (week 3) on Thursday and Friday.The inclusion and exclusion criteria of the study are presented in the **Table 4**.

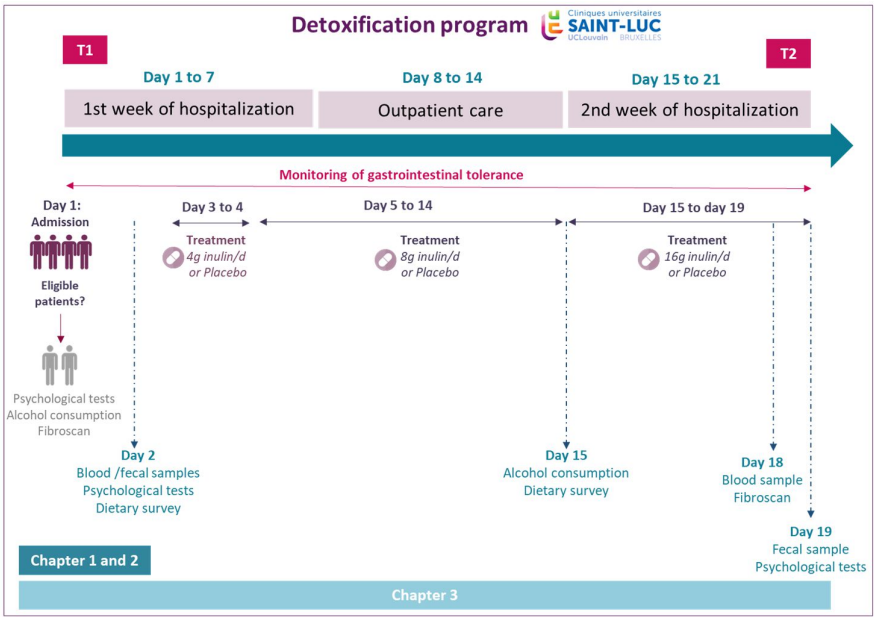


Figure 15: Design of the interventional study

Table 4: Inclusion and exclusion criteria of the Gut2Brain study

Inclusion criteria	Exclusion criteria
Male or female	Another addiction, except smoking
Aged between 18 and 65	Psychiatric comorbidity as described in the Diagnostic and Statistical Manual of Mental Disorders 5 (DSM5)
French speaking	Antibiotic, probiotic or fibers recent (<2 months) treatment (or other molecule modifying intestinal transit)
Alcohol drunk less than 48h before study enrolment	Non-steroidal anti-inflammatory drugs or glucocorticoids recently taken (<1 month)
	Obesity: Body Mass Index>30kg/m2
	Bariatric surgery
	Type 1 or 2 diabetes
	Chronic inflammatory diseases (Crohn disease, coeliac disease, rheumatoid arthritis)
	Patients with known cirrhosis or significant hepatic fibrosis (≥F2) detected by Fibroscan (> 7.6 kPa)
	Pregnancy

In the chapter 1 and 2 AUD patients at baseline were compared to 14 healthy subjects, recruited using flyers posted in Brussel's public setting, who consumed socially low amount of alcohol (Alcohol use disorders test [AUDIT] score <8 in males and <7 in females). The healthy subjects were matched for age, sex and BMI. The inclusion/exclusion criteria were the same as for AUD patients except for the alcohol related items.

RESULTS AND DISCUSSION

CHAPTER 1: Dietary fiber deficiency as a component of malnutrition associated with psychological alterations in alcohol use disorder

AUD patients have unbalanced eating habits, with alcohol accounting for up to 50% of their energy intake. Furthermore, human and animal studies have shown that chronic alcohol consumption leads to mucosal damages that will interfere with the absorption of micro- and macronutrients contributing to malnutrition.

Nutrition is now well recognized as a factor influencing psychological symptoms. In particular, it has been shown that a Mediterranean diet rich in polyphenol, fiber and polyunsaturated fatty acids is associated with a lower risk of psychiatric illness. Dietary fiber, known to influence the composition of the gut microbiota, is a healthy nutrient that has been shown to have a strong impact on health, including mental health. However, dietary fiber intake in patients diagnosed with AUD has never been evaluated.

The first objective of this chapter was **to evaluate the nutritional intake of actively drinking AUD patients and especially dietary fibers intake**. We took advantage of a tool developed in the FiberTag project to measure total, soluble and insoluble fiber intake of AUD patients compared to healthy subjects.

The second objective was **to study the link beween nutritional intake and negative affects (depression, anxiety), alcohol craving and sociability in AUD patients**.

The results of this first chapter have been published in *Clinical nutrition VOLUME 40, ISSUE 5, P2673-2682, MAY 01, 2021.*

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Original article

Dietary fiber deficiency as a component of malnutrition associated with psychological alterations in alcohol use disorder

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3.1 Abstract

Background & Aims: Chronic alcohol consumption can cause malnutrition that may contribute to alcohol-induced organ injury and psychological disorders. We evaluated the link between nutrient intake, especially dietary fibers and different parameters reflecting mental health and well being, namely anxiety, depression, alcohol craving, sociability, fatigue and intestinal comfort in AUD patients.

Methods: Cross-sectional data from 50 AUD patients, hospitalized for a 3-week detoxification program were used. Three 24-hour recalls allowed to calculate dietary habits and nutrient intakes, that was also assessed in healthy subjects (HS). Diet quality was measured using the NOVA score. Psychological factors and intestinal discomfort were evaluated using validated self-administered questionnaires.

Results: Energy intake (excluding alcoholic beverage), total fat, monounsaturated and polyunsaturated fatty acids, protein and DF intakes were lower in AUD subjects compared to HS. Ninety percent of patients had a DF intake below the recommendation. AUD patients consumed more than twice as much ultra-processed food than HS. Fructan intake was negatively associated with anxiety (p=0.04) adjusted for main confounders. Total DF, insoluble, soluble DF and galacto-oligosaccharide intakes were associated with higher sociability score. Soluble DF intake was associated with better satisfaction of bowel function (p=0.02) and a lower intestinal discomfort (p=0.04).

Conclusions: This study reveals that insufficient DF intake is part of AUD-related malnutrition syndrome, and is associated with higher anxiety, lower sociability score and intestinal discomfort. Our results suggest that an adequate intake of DF might be beneficial for recovery from AUD.

Trial registration:NCT03803709, https://clinicaltrials.gov/ct2/show/NCT03803709

Keywords: alcohol use disorders; nutrition; dietary fibers; psychological symptoms; sociability; gastrointestinal symptoms

Abbreviations

AUD: Alcohol use disorders; AUDIT: Alcohol use disorder test; BDI: Beck depression inventory; BMI: Body mass index; BSFS: Bristol stool form scale; DF: Dietary fiber; DSM-5: Diagnostic and Statistical Manual of Mental Disorders; EI: Energy intake; FA: Fatty acid; FOS: Fructo-oligosaccharides; GOS: Galacto-oligosaccharides; HS: Healthy subject; MFI: Multidimensional fatigue inventory; MMSE: Mini-mental state examination; MUFA: Monounsaturated fatty acids; OCDS: Obsessive compulsive drinking scale; PF: Processed food; PUFA: Polyunsaturated fatty acids; PSQI: Pittsburgh Sleep Quality Index; SFA: Saturated fatty acids; STAI: State-trait anxiety inventory; UPF: Ultra-processed food.

3.2 Introduction

Alcohol Use Disorders (AUD) is a chronic relapsing disease with significant physical and mental health consequences. It is one of the most common mental disorders affecting 8.6% of men and 1.7% of women worldwide [1]. AUD is associated with mood and social disorders [2, 3]. Chronic alcohol consumption leads to metabolic disturbances and is associated with several nutritional deficiencies [4–6]. Indeed, alcohol abuse where alcohol can account for more than 50% of energy intake, compromises key nutrient intake and is one of the main causes of malnutrition in Western countries [7].

Many studies demonstrated that alcohol abuse leads to gastrointestinal symptoms especially diarrhea [8]. It also decreases nutrient absorption and alters liver metabolism, thereby disrupting nutrient availability and related physiological processes [4, 6]. In addition, alcohol consumption affects eating behaviour that can contribute to malnutrition [9]. Our previous study showed that chronic alcohol abuse is associated with a decrease in energy intake from food, characterized by a reduction in fat and carbohydrate intake [10].

It is now well established that nutrition plays an important role in the development of mental illness. Notably, unbalanced diet or ultra-processed food, characterized by food rich in fat, sugar, salt and additives, is associated with mood disorders, including depression [11–14]. Numerous epidemiological studies have also reported that a diet rich in fibers is associated with a reduced risk of several chronic diseases and a lower prevalence of depressive symptoms [15–17]. Indeed, dietary fiber (DF) is considered a healthy nutrient and the European Food Safety Authority recommends an intake of 25 grams per day for adults [18, 19]. Dietary fibers with prebiotic properties, such as fructans, are known to have a significant effect on the gut microbiota, which may play a key role due to its potent effects on brain function and behaviour [20–22]. As AUD patients are characterized by alterations of gut microbiota composition [2, 23–25] and have significant cognitive, emotional, and social impairments, nutrition could be an important factor to consider in the treatment of patients as it might influence recovery from AUD [26]. However, DF intake has never been studied in the context of AUD. Our hypothesis was that the unbalanced diet of AUD patients and in particular the low fiber intake could have an impact on patients' sociability, mood and alcohol craving. We thus evaluated dietary habits with a special focus on the DF and fructans intake in a population of AUD patients and in healthy subjects (HS). The focus on fructans – including fructo-oligosaccharides (FOS) and other non-digestible oligosaccharides like galacto-oligosaccharides (GOS), is

motivated by the fact those prebiotic DF can exert beneficial effects on behaviour in other contexts [20,26].

3.3 Materials and Methods:

Subjects

A total of 50 AUD patients hospitalized for a 3 week-detoxification program in the alcohol-detoxification unit at Saint-Luc Academic Hospital, Brussels, Belgium were recruited. These patients were initially enrolled in a randomized, double-blind, placebo-controlled study assessing the impact of fiber supplementation on the gut-liver-brain axis. The severity of AUD was evaluated using the DSM-5 criteria [27]. Ninety-four percent of patients have been diagnosed as severe AUD (presence of at least 6 symptoms out of 11) and six percent with moderate AUD (presence of 5 symptoms). No other psychiatric diseases have been diagnosed in these patients. All consecutive patients, from October 2018 to December 2019, aged from 18 to 65 years old and who did not suffer from other addictions (except tobacco) were included. Patients were eligible if they had been drinking until the day of admission to the detoxification unit and if they did neither suffer from inflammatory bowel disease, other chronic inflammatory diseases (such as rheumatoid arthritis) or cancer, nor from metabolic disorders such as obesity (BMI≥ 30 kg/m2), diabetes and bariatric surgery, or severe cognitive impairment (MMSE < 24). We also excluded subjects who had taking antibiotics, probiotics, or prebiotics in the 3 months prior to enrolment and those who were taken non-steroidal anti-inflammatory drugs or glucocorticoids within 1 month before inclusion. Patients with known cirrhosis or significant liver fibrosis (≥F2) detected by Fibroscan (> 7.6 kPa) at the day of admission were also excluded from the study. Thirteen healthy controls matched for age, gender and BMI with no AUD (Alcohol use disorders test [AUDIT] score <8 in males and <7 in females) were also recruited using flyers posted in Brussel's public setting. The inclusion/exclusion criteria were the same as for AUD patients except for the alcohol related items. The protocol of this study was approved by the “Comité d’éthique Hospitalo-facultaire des cliniques universitaires” (2017/04JUL/354 and 2014/14AOU/438) and all participants provided written informed consent. The study was registered at ClinicalTrials.gov under identification number NCT03803709.

Dietary assessment

All participants were interviewed on day 2 of alcohol withdrawal by a trained dietician who administered three 24-h dietary recalls (two weekdays and one weekend day in order to limit the source of variance [407]) during a face-to-face interview. The dietician asked the patients who arrived on Monday at the hospital to report their food consumption of the previous Saturday, Friday and Thursday (day -2,-3 and -4 before the inclusion). This procedure is classically used in the hospital because of the difficulty for patients to go back further in their memory. During this interview, food quantities were determined using validated photographs, exact quantity (grams/milliliters) or household measures.

The information collected in these interviews were then entered into the dietary software (Nubel®) to analyze the nutritional values of the diet and the detailed intake of the various macro- and micronutrients. In cases where information was missing from the Nubel software, the French food composition database (CIQUAL 2017) was used to complete the data [408]. Daily mean energy and nutrient intakes were calculated from all dietary records. The results were expressed in quantities and in proportion of total energy intake (EI). The lipid intakes were also expressed in proportion of total fatty acids (FA). Micronutrient intakes were compared with the dietary guidelines for the Belgian adult population [409].

Twenty-seven categories of foods were formed by summing the amounts of food consumed expressed in grams per day [410]. Vegetables were separated into several classes to estimate the consumption of fructan-rich foods such as bulbs, rhizomes, roots and tubers [411]. The 27 categories were: roots and tubers, bulbs and rhizome, other vegetables, pasta/noodles, rice, potatoes, pulses, fruits, nuts and seeds, eggs, poultry, meat, fish and seafood, pizza/pie or sandwich, processed meat, biscuits or cakes, chocolate, soda and sweets, snack/chips, cheese, other dairy products, cereal products, bread, coffee, tea, fruit or vegetable juices, olive oil.

All foods and beverages were classified according to the NOVA food classification system consisting of four groups (unprocessed foods, culinary ingredients, processed foods and ultra-processed foods) [412]. The Nova score presented in this study excluded alcohol beverages. The proportion of processed foods (PF) and ultra-processed foods (UPF) in the diet (% grams/day of the total diet excluding alcoholic beverage) was calculated for each patient, as well as the frequency of meals (breakfast, morning snack, lunch, afternoon snack, dinner and evening snack).

During the dietary surveys, patients were also asked whether they had eaten alone or accompanied during the 3 meals of the day. From these data we constructed a quantitative score after considering the frequency of meals and the existence of company during meals (number of accompanied meals/total number of meals). The higher the score, the more the patients were used to eat with others.

Fiber intake

To properly evaluate the different types of fibers ingested, we used a database developed in the FiberTAG project [413]. This database allowed us to calculate insoluble fibers, soluble fibers including fructans (especially fructo-oligosaccharides [FOS]) and galacto-oligosaccharides (GOS). DF content in all food products and ready meals (e.g., pastries, breads) was calculated using the composition of foods from traditional recipes.

Psychological symptoms assessment

AUD patients were tested for depression, anxiety and alcohol craving with self-reported questionnaires (French versions): the Beck Depression Inventory [BDI][414], the State-Trait Anxiety Inventory (STAI form YA) [96], and the Obsessive-Compulsive Drinking Scale [OCDS][415] as described elsewhere [293]. The sociability was tested using the social situation questionnaire that assesses preferences for social (vs. nonsocial) situations. This questionnaire is composed of 28 situations characterized as social or not and pleasant or not. The patient reports how much he wants to do each activity using a Licker scale ranging from 1 to 7. This test is thus composed of 6 sub scores from 1 to 7: social high pleasant, social medium pleasant, social low pleasant and non-social high pleasant, non-social medium pleasant and non-social low pleasant [99]. Fatigue and sleep quality were also assessed using self-report questionnaires. The Multidimensional Fatigue Inventory (MFI-20) is a 20-items questionnaire that covers different dimensions of fatigue: General Fatigue, Physical Fatigue, Mental Fatigue, Reduced Motivation and Reduced Activity [416]. Sleep quality was measured using the Pittsburgh Sleep Quality Index (PSQI). The PSQI measures several aspects of sleep, including subjective sleep quality, sleep latency, sleep duration, usual sleep efficiency, sleep disorders, sleep medication use and daytime dysfunction. The overall score, which we used in this work, is calculated by adding up the seven component scores, resulting in an overall score ranging from 0 to 21. A low score indicates a healthier quality of sleep [417].

Other variables

Socio-demographic characteristics and anthropometric measures were collected at admission in a face-to-face interview. Age, age of loss of control and duration of drinking habits were reported in years. Marital status was defined as couple/married, divorced/separated or single and educational level as primary, secondary and superior. The amount of alcohol consumed the week before hospitalization was measured in gram per day using the time-line follow back approach [418]. Physical disabilities were assessed using the Health of the Nation Outcome Scales (HoNOS) completed by psychiatrists [419]. Patients with severe cognitive impairments were excluded by using the Mini Mental State Examination (MMSE) [420].

Gastrointestinal discomfort

Gastrointestinal discomfort was evaluated using a questionnaire initially used to evaluate the symptoms of irritable bowel syndrome[421] for which a French version was developed by gastroenterologists at St Luc hospital. This questionnaire allowed the patients to report the intensity of abdominal pain and bloating, their satisfaction about their intestinal transit and whether gastrointestinal symptoms affected their daily life by using visual analogic scale from 0 to 100.A total score is obtained by adding the 4 scales (abdominal pain, bloating, transit satisfaction and consequences on daily life). The maximum score was 400. A score below 60 indicates normal bowel function, a score between 60 and 139 corresponds to mild symptoms, between 140 and 239 to moderate symptoms and >240 to severe symptoms. Patients also indicated the frequency of stools and completed the Bristol Stool Form Scale (BSFS) [422]. The BSFS is a scale for identifying stool types ranging from the hardest (type 1) to the softest (type 7).

Statistical Analysis

Sociodemographic characteristics and nutritional data between groups were compared using Student's t test or Mann Whitney's test for continuous variables. Categorical variables were analyzed using Chi square or Fisher's test. The effect size and power of the statistical tests were calculated for fiber intake which is the main outcome of the study. Hedges' g were largely higher than 0.8 for total fiber, insoluble and soluble fibers (1.2, 1.6 and 1.2 respectively) and the powers were respectively 92, 95 and 93%, which indicates a high association strength.

Principal component analysis (PCA) was used to compare the dietary pattern of AUD patients and HS.

To assess which food products contributed to patients' main sources of fibers, quantities of food products were compared across tertiles of total dietary fiber intake using a Jonckheere-Terpstra test.

To address the second objective, bivariate correlation analyses (Pearson's correlation) were performed to assess the relationships between nutrients and psychological outcomes. A relevance association network from the similarity matrix derived from the partial least squares analysis (PLS) was made in order to select the most important nutrients associated with the psychological symptoms[423]. This analysis was performed with macro and micronutrients and the MFI sub-scores and the PSQI were not included in the analyze because of missing values (n=29). An edge was drawn between two variables if the estimated correlation coefficient exceeded 0.25. This was performed using the mixOMICS v5.2. Then, we used linear models to confirm the multivariate associations between the nutrients and the psychological measurements. We first adjusted for the age, gender and level of education (model 1). We subsequently added the energy intake, BMI and the smoking status in model 2. Finally, we added the quantity of alcohol consumed per day in model 3.

P values <0.05 were considered statistically significant. Statistical analyses were performed using SAS version 9.4, R studio version 3.5.1 and Graphpad Prism 8.0.

3.4 Results

Forty-nine AUD patients underwent the dietary interview. One outlier was excluded from the analysis because of excessive energy intake (more than 3 standard deviations from the mean). The final population was composed of 48 AUD patients.

We first compared the nutritional intakes of AUD patients with those of HS recruited for this purpose. Then we studied the link between nutrient intakes and psychological outcomes in the same AUD patients.

Nutritional habits of AUD patients compared to healthy subjects

Socio-demographic data are reported in Table 1. AUD patients and HS were similar in terms of age, sex, BMI and marital status. However, AUD patients were less educated, smoked more and had a lower mean MMSE score than HS. There were no differences in professional status or income sources between AUD patients and HS. In average AUD patients consumed 132 ± 73 g of ethanol/day while HS consumed 7.6 ± 10.4 g/day. Three patients had a HoNOS score of 2 or 3 for physical illnesses or disabilities and the rest had no or minor physical health problem (data not shown).

Meal frequency in AUD patients and healthy controls is shown in Table 1. The number of breakfasts, morning snacks and lunches were lower in AUD subjects compared to controls.

We calculated the NOVA score to evaluate the importance of processed (PF) and ultra-processed food (UPF) in the diet of patients. Patients tended to have a higher NOVA score than HS (2.1 ± 0.7 vs 1.7 ± 0.4, p=0.0516; Table 1). They consumed significantly more UPF (reached 28% of the total food intake) than HS (Table 1).

Table 1: Baseline characteristics of healthy subjects and AUD patients in the intervention study

	HS n=13	AUD n=48	*p* [a]
Sociodemographic characteristics			
Age (y)	46.0 ± 10.9	48.5 ± 11.4	0.52
Female, n (%)	6 (46.1)	18(35.4)	0.48
Marital status, n (%)			0.12
Couple/ married	9 (69.2)	18 (37.5)	
Single	3 (23.1)	22 (45.8)	
Separated/divorced	1 (7.7)	8 (16.7)	
Educational level, n (%)			0.001
Primary	0 (0.0)	5 (10.4)	
Secondary	0 (0.0)	17 (35.4)	
Superior	13 (100.0)	26 (54.2)	
Employment status, n(%)			0.11
Employed	11 (84.6)	27 (56.3)	
Unemployed	1 (7.7)	16 (33.3)	
Retired	1 (7.7)	5 (10.4)	
Income sources			0.10
Professional activity	11 (84.6)	27 (56.2)	
Allocations [b]	1 (7.7)	20 (41.7)	
No income or financial support from family or friends	1 (7.7)	1 (2.1)	
Clinical examination			
Weight (kg)	71.0 ± 10.9	72.6 ± 12.4	0.79
BMI (kg/m^2)	23.6 ± 3.0	24.0 ± 3.3	0.72
MMSE score	29.5 ± 0.5	28.0 ± 2.3	0.01
Smoking, n (%)	2 (15.4)	39 (81.2)	<0.001
Alcohol history			
DSM-5 AUD score	0.0 ± 0.0	8.4 ± 1.8	<0.001
Age of loss of control (y)	-	32.3 ± 11.2	-
Number of alcohol withdrawal cures	-	2.2 ± 2.2	-
Duration of drinking habit (y)	-	15.8 ± 10.7	-
Alcohol consumption (g/d)	7.6 ± 10.4	132.0 ± 73.1	<0.001
AUDIT score	3.1 ± 2.4	-	-

Meal frequency (alcohol not included), n (%)			
Breakfast	13 (100,0)	20 (41.7)	<0.001
Morning snack	7 (53.8)	8 (16.7)	0.01
Lunch	13 (100.0)	36 (75.0)	0.01
Afternoon snack	10 (76.9)	26 (54.2)	0.13
Dinner	13 (100.0)	45 (93.7)	0.22
Evening snack	1 (7.7)	12 (22.9)	0.18
Nova score[c]	1.7 ± 0.4	2.1 ± 0.7	0.05
PF intake[b] (% of total food intake)	13.3 ± 5.1	14.1 ± 11.2	0.65
UPF food intake[b](% of total food intake)	13.2 ± 12.9	27.8 ± 23.6	0.04

Values are means ± standard deviation.

[a] p values were calculated using a T-test or Mann Whitney Wilcoxon's test and Chi2 test or Fisher's test for categorical variables.

[b] Invalidity, disability, compensated unemployment, retirement, social integration income.

[c] The Nova score, the percentage of processed and ultra-processed food were calculated without taking into account alcoholic beverages.

AUD, Alcohol use disorders group; AUDIT, Alcohol Use Disorders Test; BMI, Body mass index; CT, Control group; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; HS, healthy subjects; MMSE, Mini Mental State Examination.

The principal component analysis of the food groups revealed that AUD patients ate more pizza, pie or sandwiches, processed meat and sweets and soda and consumed less vegetables, pulses, cereal products, olive oil, nuts and fruits than healthy subjects (Supplementary Figure 1).

Actively drinking AUD subjects exhibit insufficient macro and micronutrient intake

Energy intake (EI) provided by food – excluding alcoholic beverages- was significantly lower in AUD than in HS volunteers (Figure 1A). AUD patients consumed less protein and fat than HS when intakes were expressed in g/d (Figure 1B). When quantified in relative proportions of EI, protein, carbohydrate and fat intakes were all significantly decreased in AUD patients compared to HS (Figure 1C). AUD patients had lower intakes of any type of fat (saturated fatty acids [SFA], monounsaturated fatty acids [MUFA] and polyunsaturated [PUFA]) compared to HS (Figure 1E). Among PUFA, both n-6 and n-3 PUFA (EPA and DHA) intakes significantly decreased in AUD subjects (Figure 1E; 1F). In AUD patients, SFA per total fat was higher than in the HS group ($p=0.0162$; Supplementary Table 1). AUD patients consumed less cholesterol and trans fat than HS (Supplementary Table 1).

Supplementary Table 2 reports the intake of micronutrients in AUD patients and healthy subjects taking into account alcoholic beverages. The intakes of calcium, selenium, folates and of vitamins B12, C, D and E were significantly lower in AUD patients compared to HS ($p<0.001$). Nearly 80% of patients had calcium intakes below the Belgian recommendations but the proportion was not different from that of HS (Supplementary Table 2)[409]. Fifty-eight percent of patients had insufficient selenium intake *versus* 23% of HS ($p=0.0311$). Seventy-one percent and 75% of patients had too low intakes of vitamin B12 and C below the norms, respectively. Fifty-six percent of AUD patient had a folate intake below the recommended value compared to 8% of HS ($p=0.0017$). Vitamin D and Vitamin E intakes were insufficient for 92% and 85% of AUD patients versus 69% and 23% of HS respectively ($p=0.0554$ and $p<0.001$; Supplementary Table 2).

Actively drinking AUD patients have a low intake of dietary fibers

AUD patients had a lower total (13g/d), soluble and insoluble dietary fiber intake as compared to HS (Figure 1G).There were no differences between AUD patients and HS for fructans (Figure 1G), fructo-oligosaccharides (FOS) (0.9 ± 0.9 g for AUD patients vs 1.1 ± 0.9g for HS, $p=0.1234$) and galacto-oligosaccharides (GOS) intake (0.2 ± 0.4 g for AUD patients vs 0.4 ± 0.5g for HS, $p=0.1322$). Ninety percent of the AUD patients had a DF

intake below the recommended value of 25g per day compared to 50% of HS (Fisher's exact test p=0.0030; data not shown).

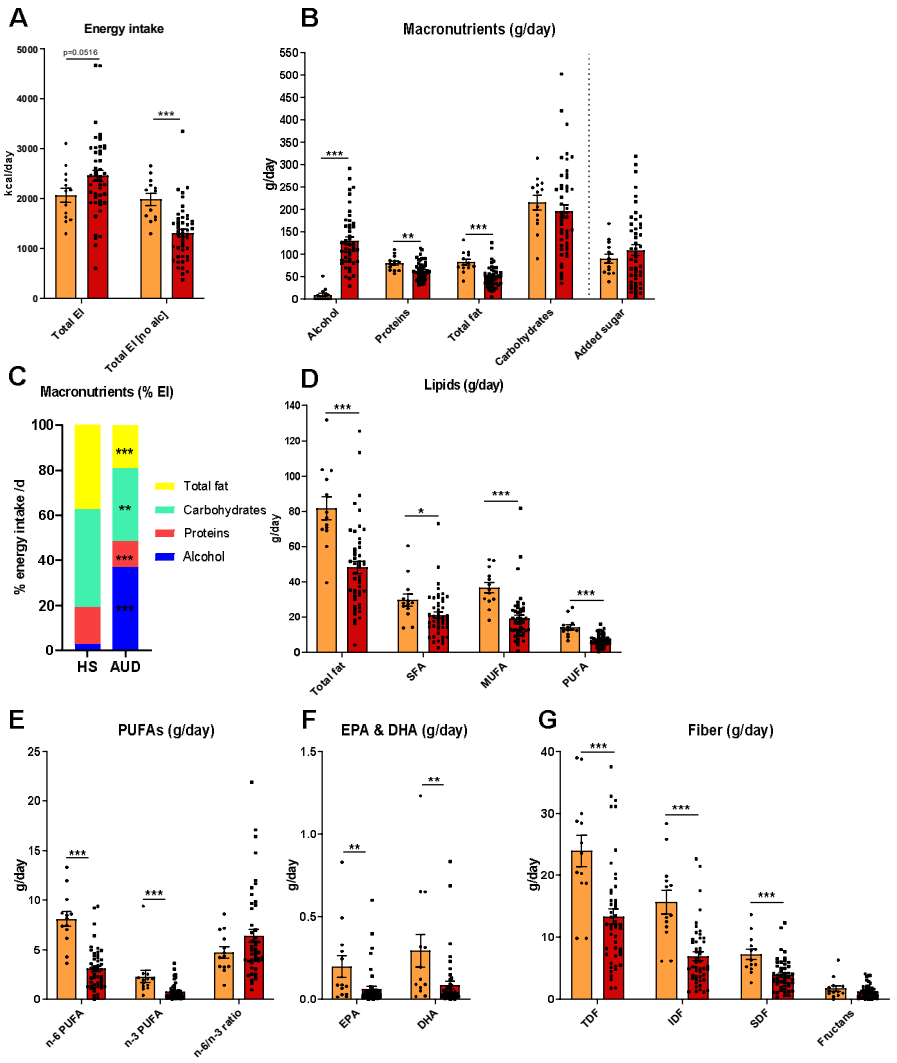

Figure 1: Macronutrient intakes in active drinking AUD patients and in healthy subjects

Values are mean±SEM. *p<0.05, **p<0.01,***p<0.001. N=13 for HS group and n=48 for AUD group. P values were calculated using Ttest or Mann Whitney Wilcoxon's test.

AUD, alcohol use disorder; DHA, Docosahexaenoic acid; EI, energy intake; EPA, Eicosapentaenoic acid; FA, Fatty acid; FOS, Fructo-oligosaccharide; GOS, Galacto-oligosaccharide; HS, healthy subjects; IDF, Insoluble dietary fibers; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SDF, Soluble dietary fibers; TDF, Total dietary fibers.

The supplementary table 3 presents the distribution of the food products according to the tertiles of fiber consumed by AUD patients. The patients in the 3rd tertile (high fiber consumers) consumed more roots and tuber, bulbs, vegetables, pulses, potatoes, fruits, bread, coffee, fruit or vegetable juice and olive oil than AUD patients in the 1st tertile (low fiber consumers). The alcohol consumption did not significantly differ between the three groups (Supplementary Table 3).

TDF intake was associated with higher protein and carbohydrate intake (r=0.46, p=0.001; r= 0.30, p= 0.04) and lower ethanol and UPF intake (r= -0.38, p=0.009; r= -0.31, p=0.03; Figure 2). TDF, SDF and fructans were positively correlated with potassium, magnesium, vitamin B1, C and folate intake (Figure 2).

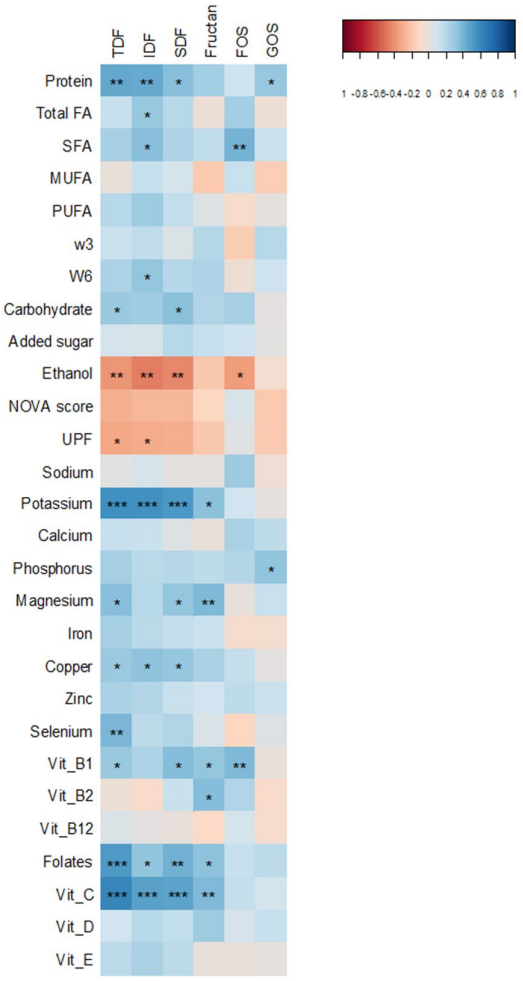

Figure 2: Correlations between dietary fiber and other nutrient intakes in AUD patients

Pearson partial correlations adjusted for total energy intake. N=48. *p<0.05, **p<0.01,***p<0.001.
AUD, Alcohol use disorder; FA, Fatty acid; FOS, Fructo-oligosaccharide; GOS, Galacto-oligosaccharide; HS, healthy subjects; IDF, Insoluble dietary fibers; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SDF, Soluble dietary fibers; TDF, Total dietary fibers; UPF, ultra-processed food.

Association between nutrient intake and psychological symptoms in AUD patients

Because inadequate nutrition could affect mood, we hypothesized that some nutrients could be involved in the negative emotional state in AUD patients. Therefore, we tested the correlations between nutrient intake, UPF and psychological symptoms (Figure 3). Our results revealed a positive correlation between the proportion of UPF in the diet and the obsessive sub-score of alcohol craving scale, the sleep quality and the mental fatigue scores meaning that patients who consumed high quantity of UPF had higher obsessive thoughts about alcohol, a higher mental fatigue and a lower sleep quality (Figure 3A). A tendency was observed between the proportion of UPF in the diet and the depression score ($r= 0.32$; $p=0.0555$). Positive correlations were observed between n-6 PUFA, n-3 PUFA and the sociability score. Total fat and MUFA intakes were associated with a better sleep quality and less physical fatigue. Negative correlations were also found between potassium intake and depression, anxiety, sleep quality and physical fatigue scores (Figure 3B). Magnesium intake was negatively correlated with the general and physical fatigue scores while vitamin B12 was positively correlated with depression and mental fatigue scores (Figure 3B). TDF, SDF, IDF and GOS intakes were associated with higher score of sociability and fructan intake with a lower anxiety (Figure 3A). Meaning that patients who consumed more fiber wanted to do more social activities and were less anxious.

In order to highlight the most relevant associations between nutrients and mood we performed a PLS analysis (Figure 3C). It confirmed that, among all macro and micronutrients, DF were the most correlated with psychological outcomes. Indeed, this analysis revealed that fructan, n-6 PUFA and potassium intakes were negatively associated with the anxiety score. TDF, SDF and IDF intakes were associated with sociability (Figure 3C).

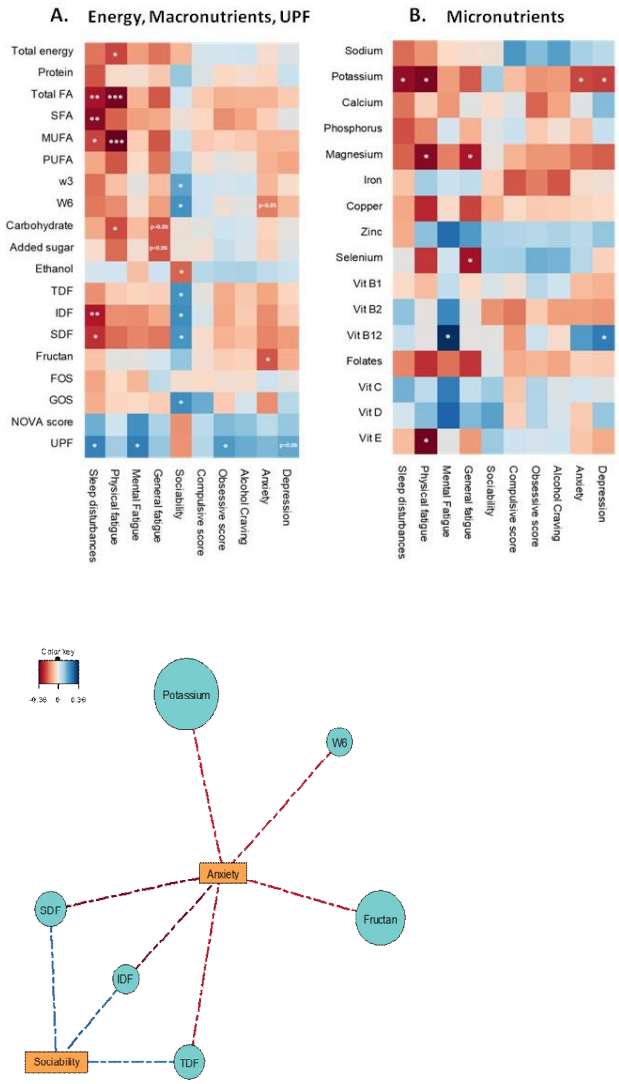

Figure 3: Correlations between nutrient intakes and psychological symptoms in AUD patients.

Pearson correlations. N=48 for all the parameters (excepted for fatigue and sleep disturbances scores n=29). *p<0.05, **p<0.01,***p<0.001.

A. Correlations between the energy, macronutrient and UPF intakes and the psychological outcomes. B. Correlations between the micronutrients and the psychological outcomes. C. Relevance associations network of partial least squares analysis with macro/micronutrients and psychological symptoms (excepted fatigue scores). A cutoff value of 0.25 was applied. AUD, Alcohol use disorder; FA, Fatty acid; FOS, Fructo-oligosaccharide; GOS, Galacto-oligosaccharide; HS, healthy subjects; IDF, Insoluble dietary fibers; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SDF, Soluble dietary fibers; TDF, Total dietary fibers; UPF, ultra-processed food.

Finally, we wanted to confirm these associations using multivariate linear models in order to take into account the different confounding factors (Table 2). When we adjusted for potential confounders, TDF, IDF, SDF and GOS were significantly associated with a higher sociability score regardless of the model suggesting a minimal confounding effect (Table 2 and Supplementary Table 4). After adjustment for sociodemographic factors, a significant inverse association was observed between fructan intake and anxiety score. This association remained significant after adjustment for energy intake, smoking habits, BMI (model 2) and alcohol consumption (model 3). Indeed, higher fructan intake was related to a decreased level of anxiety (Table 2). We did not find any significant association between the other types of fiber and the anxiety score. However, the sociability score was negatively correlated with anxiety score ($r=-0.43$, $p=0.002$; data not shown). For the n-6 PUFAs, the associations did not reach significance when the different confounding factors were taken into account (Supplementary Table 4). Potassium intake was associated with a decreased depression score but only in models 2 and 3. The association was no longer observed between potassium intake and anxiety after taking into account the different confounding factors (Supplementary Table 4).

We also wanted to investigate whether eating alone or accompanied could be linked to a poor nutritional quality and more severe psychological symptoms in AUD. To do so we constructed a correlation matrix. We found that eating accompanied was positively correlated with the sociability score ($r=0.33$, $p=0.03$). No association were found between nutrients or UPF intakes and sharing a meal (Supplementary Figure 2).

Table 2: Associations between dietary fiber intake and psychological measurements in AUD patients

	TDF		IDF		SDF		Fructans	
	β [95% CI]	p	β [95% CI]	p	β [95% CI]	p	β [95% CI]	p
Depression								
Model 1[a]	-0.04 [-0.46 ; 0.38]	0.84	-0.10 [-0.83 ; 0.62]	0.77	-0.76 [-2.12 ; 0.60]	0.26	-1.48 [-4.77 ; 1.82]	0.37
Model 2[b]	-0.07 [-0.56 ; 0.42]	0.77	-0.15 [-0.99 ; 0.68]	0.71	-1.04 [-2.63 ; 0.54]	0.19	-1.74 [-5.37 ; 1.88]	0.34
Model 3[c]	-0.07 [-0.62 ; 0.47]	0.79	-0.17 [-1.12 ; 0.78]	0.72	-1.26 [-3.06 ; 0.52]	0.16	-1.77 [-5.37 ; 1.98]	0.34
Anxiety								
Model 1[a]	-0.30 [-0.82 ; 0.22]	0.26	-0.67 [-1.56 ; 0.22]	0.14	-1.33 [-3.02 ; 0.35]	0.12	-4.44 [-8.38 ; -0.50]	**0.03**
Model 2[b]	-0.38 [-0.99 ; 0.22]	0.21	-0.85 [-1.87 ; 0.17]	0.10	-1.71 [-3.68 ; 0.25]	0.08	-5.07 [-9.38; -0.75]	**0.02**
Model 3[c]	-0.25 [-0.92 ; 0.41]	0.44	-0.65 [-1.81 ; 0.50]	0.26	-1.36 [-3.58 ; 0.86]	0.22	-4.62 [-9.03 ; -0.21]	**0.04**
Sociability								
Model 1[a]	0.05 [0.01; 0.10]	**0.02**	0.09 [0.006 ; 0.17]	**0.04**	0.16 [0.01 ; 0.32]	**0.04**	0.26 [-0.12 ; 0.64]	0.18
Model 2[b]	0.07 [0.02 ; 0.12]	**0.01**	0.11 [0.02 ; 0.20]	**0.02**	0.23 [0.05 ; 0.40]	**0.01**	0.32 [-0.09 ; 0.73]	0.13

Model 3[c]	0.07 [0.01 ; 0.13]	**0.02**	0.11 [0.01 ; 0.21]	**0.04**	0.22 [0.02 ; 0.42]	**0.03**	0.27 [-0.15 ; 0.70]	0.19
Alcohol Craving								
Model 1[a]	-0.03 [-0.22 ; 0.17]	0.78	-0.07 [-0.41 ; 0.27]	0.67	-0.18 [-0.82 ; 0.46]	0.57	-0.01 [-1.57 ; 1.54]	0.98
Model 2[b]	-0.08 [-0.30 ; 0.14]	0.47	-0.16 [-0.53 ; 0.22]	0.40	-0.41 [-1.12 ; 0.31]	0.26	-0.24 [-1.89 ; 1.41]	0.77
Model 3[c]	-0.02 [-0.26 ; 0.22]	0.85	-0.05 [-0.47 ; 0.36]	0.79	-0.23 [-1.04 ; 0.57]	0.55	-0.02 [-1.69 ; 1.65]	0.98

N=48 for all the parameters. IDF, Insoluble dietary fibers; SDF, Soluble dietary fibers; TDF, Total dietary fibers.

[a] Linear model adjusted for age, gender, educational level.

[b] Linear model adjusted for age, gender, educational level, energy intake, BMI, tobacco

[c] Linear model adjusted for age, gender, educational level, energy intake, BMI, tobacco, alcohol consumption.

Association between nutrient intake gastrointestinal discomfort in AUD patients

We compared the gastrointestinal symptoms of AUD patients with those of HS. Almost the half of the patients suffered from abdominal pain while no HS reported pain (data not shown). AUD patients were less satisfied about their bowel function (48.5 ± 29.7 vs 75.5 ± 29.6, p=0.006; Supplementary Figure 3B) and reported that their symptoms had more impact on their daily life (33.8 ± 29.8 vs 10.9 ± 19.6 p=0.008; Supplementary Figure 3C). The total score of intestinal discomfort was significantly higher in AUD patients compared to HS (129.0 ± 82.1 vs 51.2 ± 68.8, p=0.002; Supplementary Figure 3E). There was no difference concerning bloating intensity and stool frequency. The consistency of stool evaluated by the BSFS was significantly different between the two groups. AUD patients were more likely to report a score of 6 and 7 and less likely to report a score of 4 (Supplementary Figure 3F).

We then tested the link between gastrointestinal symptoms and macronutrients in AUD patients. We found a positive correlation between the satisfaction of bowel function and the intake of SDF (r=0.35, p=0.02; Supplementary Figure 4A). A negative correlation was observed between the total score of GI discomfort and the intake of SDF (-0.32, p=0.04; Supplementary Figure 4B).

3.5 Discussion

This study describes the dietary habits and nutrient intakes of actively drinking AUD patients compared to healthy individuals and evaluates the link between nutritional intakes, especially DF and psychological outcomes.

We confirmed that AUD patients displayed disorganized food habits compare to HS. As previously described by our group, AUD patients had less frequent breakfast and lunch [106]. The energy intake provided from food sources was significantly lower in AUD patients, alcohol accounting for 37% of their total energy intake. Consequently, AUD patients ate less of all macronutrients (lipids, proteins, carbohydrates). These results corroborate previous studies suggesting that energy derived from alcohol replaces energy from other macronutrients [106,424,425]. The decrease in energy and macronutrient intake could be explained by an alteration in the balance of appetite regulatory peptides [106,426,427]. Several studies have also shown an increase in leptin levels in AUD patients and a decrease in ghrelin compared to controls [106,310,428,429]. AUD is associated with negative emotional state and depressive symptoms that could also affect energy intake and food choices [430,431].

Total, MUFA, n-6 and n-3 PUFA were lower in AUD patients than in HS. This is due to a reduction in the consumption of fish, nuts, seeds, dairy products and beneficial fats like olive oil. Few studies have explored the relationship between alcohol consumption and fat intake and have shown a decrease in SFA, MUFA and PUFA in heavy drinkers and binge-drinkers [425,432].In addition, numerous studies reviewed by Borsoleno *et al* have shown that ethanol alters the absorption and metabolism of lipids leading to essential lipid deficiency [433]. Therefore, a decrease in essential fat intake, together with alterations of their absorption, could lead to several metabolic and neurobiological alterations that could favor mood and cognitive disturbances [433–436]. Furthermore, n-3 PUFA supplementation in substance abusers reduces anger and anxiety level in a 3-month trial [437]. Our results reveal positive correlations between n-3 and n-6 PUFA and sociability and negative correlations with anxiety score. However, these associations do not persist when taking into account potential confounding factors like sociodemographic characteristics, BMI, energy intake and tobacco use. The observation of a relationship between lipid metabolism dysregulation and the importance of symptoms of AUD as well as changes in myelin synthesis has recently been highlighted by a translational study from our research group [251]. Together, these data stress the importance of lipid intake and metabolism in the development of AUD.

Our data show correlations between UPF, depression, mental fatigue, alcohol craving and sleep quality in AUD patients. Two large French and Spanish cohorts have highlighted a positive association between depressive symptoms and the consumption of UPF in global population and university graduates respectively [438,439].

We also observed a lower intake of calcium, selenium, folate, vitamin B12, C, D and E. These deficiencies are common in patients suffering from chronic alcohol abuse [111,440] and may affect the cardiovascular, skeletal and nervous systems [111,441]. Potassium intake in active AUD patients was inversely associated with depression score, which is in line with previous studies in general population and elderly [442,443].

The originality of our work is to demonstrate that total dietary fibers, soluble and insoluble fiber intakes were lower in AUD patients compare to HS. To our knowledge, the fiber intake has never been studied in AUD patients. A Finnish study showed that heavy drinkers, in the general population, had lower fiber intake compared to non-drinkers [444]. A recent study reveals that higher DF consumers have a 30% decrease in all cause and cardiovascular related mortality [445]. In addition, most DF – in particular the fermented ones- interact with the gut microbiota and DF is one of the most important nutrients that modulates the gut microbiota composition [446]. We have already shown that the gut microbiota of alcoholic patients is altered [293]. It has been shown in animal studies that gut microbiota could influence anxiety and social behaviour [251,447,448]. In humans, microbial changes caused by fermented foods induce changes in mood [20, 72]. Interestingly, our work reveals that fructan intake is associated with a decreased anxiety score and TDF, SDF, IDF and GOS intakes with an increased sociability score. Several studies support the link between DF and/or prebiotic intake and depression. A study performed in 3394 older Chinese adults revealed that TDF intake is associated with a decreased prevalence of depressive symptoms [450]. GOS supplementation, associated with a gluten and casein free diet, induces a significant improvement in anti-social behaviour in autism spectrum disorders children [451]. SDF and IDF intakes are associated with better sleep quality in our work.Few studies have investigated the relationship between fiber intake and sleep quality. One study in 26 adults found that low fiber intake and high SFA and sugar intake was associated with poor sleep quality [452]. This is not in total agreement with our study since in our study sample, SFAs is associated with a better quality of sleep.

The mechanisms linking psychological outcomes and DF are not fully understood but it could involve a plethora of pathways and molecules [165]. For example, it has been shown

that fermentable fibers (fructans and galactans) are able to stimulate short chain fatty acid production which can act as neuroactive metabolites and consequently have an impact on the brain and behaviour [338,403,453–455]. This supports the interest to evaluate the microbiome of these patients.

Our results show a great variability in the AUD population. The vast majority of patients have an overall nutritional deficit, but some patients have a nutritional profile close to HS. Nutritional advice should therefore target a sub-population of AUD patients.

In our population of AUD patients those who consumed their meals alone had a lower sociability score but no association was found with nutrient or UPF intakes. Our results are not in line with those of two other studies which showed in a population of Koreans and Australian that eating alone was associated with a lower food quality [81, 82].

We observed that DF intakes, particularly SDF were associated with less gastro-intestinal discomfort in our population of AUD patients. Clinical and experimental studies have demonstrated that SDF may cause bloating, but they also improved functional gastrointestinal disorders [458–460]. The authors stated that their beneficial effect could be explained by their secondary effect on the gut microbiota, inflammation as well as ongut permeability [461]. Increasing fiber intake in alcoholic patients could be a way of reducing their intestinal discomfort. However, DF supplementation has never been tested in the context of AUD.

Our study presents some limitations. The cross-sectional design of the study does not exclude the risk of reverse causality. Indeed, a poor quality diet may be the result of psychological alterations, rather than a causal factor.Then, we used of 3 day-recall which can be subject to memory bias. However, the dietician used the multiple-pass interviewing to limits this bias [348]. In addition, this approach allows us to capture intra-individual variation in dietary intakes and therefore to better estimate the eating habits than with a single 24 hours recall. Our population of healthy subjects is limited; all the subjects have a high level of education which can have an impact on nutritional intake [462]. Nonetheless, the nutrient intakes of those control subjects are comparable to those of a Belgian study conducted in more than 3000 persons, relating protein, fat and carbohydrate intake [463]. Furthermore, the power calculation for the comparison of dietary fiber intakes indicates high association strength to consider our data as valuable in this protocol.

Conclusion

To conclude, our study showed that DF intake is insufficient in AUD patients and is related to anxiety and social impairment. In AUD, depression, anxiety and social impairment are interrelated and can lead to relapse after withdrawal [94,464]. The relapse being a real problem in AUD patients, nutrition – and namely the focus on dietary fiber intake, appears as a key factor to take into account to improve the psychological symptoms of AUD patients and to promote the maintenance of abstinence. Further prospective and interventional studies are needed to evaluate the effects of DF as part of a more balanced diet on metabolic and psychological symptoms of AUD patients, and test whether it may be used as an additional treatment option in this pathology.

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Conflict of interest

The authors declare no competing interests.

Authors' contributions

Conceptualization & design: SL, NMD, PdT, PS, AMN.
Data curation: CA, VT, SL, VC, NMD, AMN, PdT, PS.
Formal analysis: CA, VT, SL, NMD, AMN, LBB.
Funding acquisition: NMD, PdT, PS.
Investigation: CA, SL, PS.
Methodology: SL, NMD, PdT, PS, AMN, HP.
Project administration: SL,AMN, NMD, PdT, PS.
Resources: NMD, PdT, PS.
Software: CA, VT.
Supervision: NMD, PdT, SL, PS.
Validation: SL, NMD, AMN, PdT, PS, LBB.
Writing original draft: CA, SL, NMD.
Writing review & editing: CA, SL, NMD, AMN, PdT, PDC, PS, LBB, HP.

All authors read and approved the final manuscript.

3.6 Supplemental information

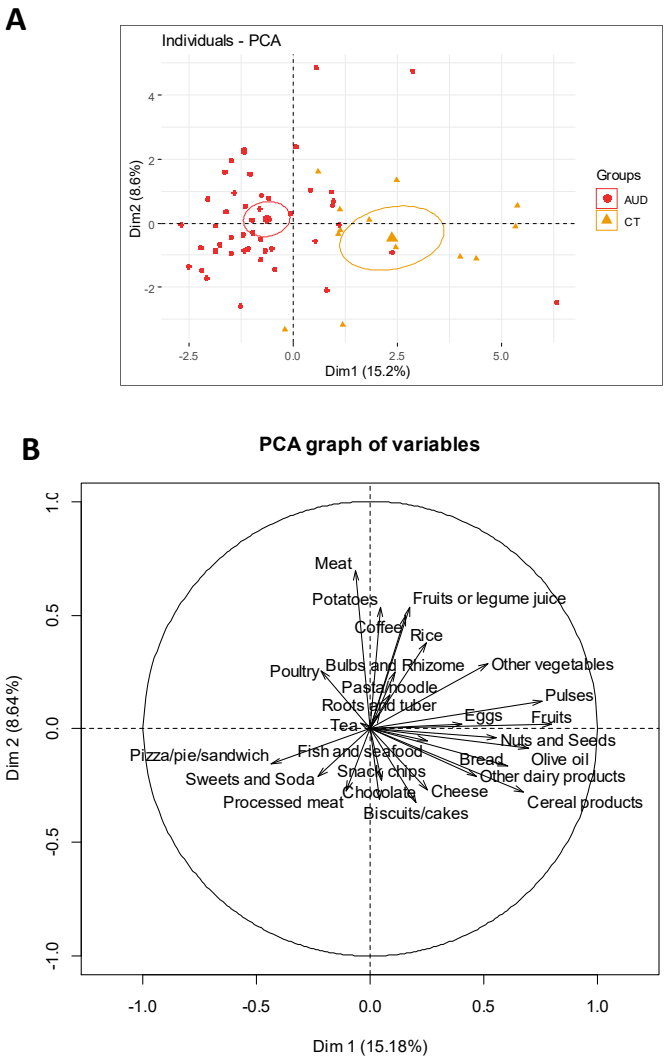

Figure S1: Dietary pattern in AUD patient and healthy subject

N=48 and n=13. Principal component analysis (PCA) with the 27 food products. **A.** Individuals plot with confidence ellipses. **B.** Variable coordinates plot.

Supplementary Table 1: Fatty acid intakes in AUD patients and in healthy subjects

Fatty acids intake/d	HS n=13	AUD N=48	*p* [a]
Proportion of total EI (%)			
Total fat	37.5 ± 6.6	19.4 ± 8.4	**<0.001**
SFA	13.3 ± 3.2	8.4 ± 4.0	**<0.001**
MUFA	17.0 ± 3.6	7.8 ± 4.7	**<0.001**
PUFA	6.8 ± 3.1	3.0 ± 1.8	**<0.001**
n6-PUFA	3.9 ± 1.6	1.3 ± 1.1	**<0.001**
n3-PUFA	1.2 ± 1.4	0.3 ± 0.4	**<0.001**
EPA	0.09 ± 0.10	0.03 ± 0.05	**0.002**
DHA	0.13 ± 0.14	0.04 ± 0.07	**<0.001**
Trans FA	0.4 ± 0.2	0.2 ± 0.2	**<0.001**
Proportion of total FA (%)			
SFA	36.0 ± 7.5	44.6 ± 12.8	**0.02**
MUFA	45.1 ± 4.5	38.5 ± 9.6	**0.003**
PUFA	17.7 ± 5.5	15.7 ± 6.7	0.17
n6-PUFA	10.1 ± 3.2	7.1 ± 5.1	**0.008**
n3-PUFA	2.9 ± 2.9	1.8 ± 1.7	**0.03**
EPA	0.24 ± 0.27	0.13 ± 0.22	0.10
DHA	0.36 ± 0.43	0.25 ± 0.65	**0.04**
Trans FA	1.1 ± 0.4	1.3 ± 0.8	0.92
Cholesterol (g/d)	0.3 ± 0.1	0.2 ± 0.1	**0.001**

Values are means ± standard deviation.

[a] p values were calculated using T-test or Mann Whitney Wilcoxon's test

AUD, Alcohol use disorder; DHA, Docosahexaenoic acid; EI, Energy intake; EPA, Eicosapentaenoic acid; FA, Fatty acid; HS, Healthy subjects; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids.

Supplementary Table 2: Micronutrient intake in AUD patients and in healthy subjects

Micronutrient intake/d	HS n=13	AUD n=48	*P*[a]	Number of HS below RDA[b] (%)	Number of AUD patients below RDA[b] (%)	P [c]
Macro-elements						
Sodium (g/d)	2.3 ± 0.7	2.0 ± 0.8	0.27	-		
Potassium (g/d)	3.0 ± 0.7	2.8 ± 1.1	0.41	5 (38.5)	29 (60.4)	0.21
Calcium (mg/d)	896.6 ± 325.5	705.5 ± 442.9	**0.04**	8 (61.5)	38 (79.2)	0.27
Phosphorus (g/d)	1.3 ± 0.3	1.1 ± 0.5	0.06	1 (7.7)	13 (27.0)	0.43
Mg^{2+} (mg/d)	305.6 ± 69.0	303.0 ± 114.3	0.55	6 (46.1)	22 (45.8)	1.00
Oligo-elements						
Iron (mg/d)	10.9 ± 2.6	11.9 ± 6.5	0.91	11 (92.3)	37 (77.0)	0.71
Copper (mg/d)	0.9 ± 0.3	0.9 ± 0.7	0.25	10 (76.9)	35 (72.9)	1.00
Zinc (mg/d)	9.9 ± 2.2	9.0 ± 3.6	0.18	2 (15.4)	18 (37.5)	0.19
Selenium (µg/d)	109.2 ± 51.6	63.5 ± 37.3	**0.002**	3 (23.0)	28 (58.3)	**0.03**
Vitamins						
Vit. B1 (mg/d)	1.3 ± 0.4	1.1 ± 0.8	0.10	4 (30.8)	30 (62.5)	0.06
Vit. B2 (mg/d)	1.4 ± 0.3	1.6 ± 1.8	0.41	4 (30.8)	23 (47.9)	0.35
Vit. B12 (µg/d)	8.1 ± 13.6	3.2 ± 2.2	**0.02**	7 (53.8)	34 (70.8)	0.32
Folates (µg/d)	316.4 ± 86.2	218.8 ± 139.7	**0.01**	1 (7.7)	27 (56.2)	**0.002**
Vit. C (mg/d)	121.6 ± 59.3	81.8 ± 85.5	**0.01**	5 (38.5)	36 (75.0)	**0.02**
Vit. D (µg/d)	8.0 ± 6.2	2.9 ± 4.0	**<0.001**	9 (69.2)	44 (91.7)	0.05
Vit. E (mg/d)	12.7 ± 2.8	7.0 ± 7.0	**<0.001**	3 (23.1)	41 (85.4)	**<0.001**

Values are means ± standard deviation.

[a]T-test or Mann Whitney's test.

[b] Reference values from the Nutritional recommendations for Belgium– 2016 (Conseil Supérieur de la Santé. Recommandations nutritionnelles pour la Belgique - 2016. Bruxelles: CSS; 2016. Avis n° 9285).

[c]Fisher's exact test to compare the proportion of AUD and HS below the recommendations.

AUD, alcohol use disorder; HS, healthy subjects; Mg^{2+}, magnesium;RDA, recommended daily allowance.

Supplementary Table 3: Distribution of the food products across tertiles of total dietary fibers intake in AUD patients (n=48)

	T1 <8g n=14	T2 [8-15g] n=18	T3>15g n=16	p[a]
Roots and tubers	0.0 ± 0.0	17.79 ± 28.5	28.5 ± 39.4	0.007
BULBES	8.6 ± 30.7	8.96 ± 21.4	22.6 ± 32.7	0.01
Rhizome	0.0 ± 0.0	3.65 ± 11.7	9.3 ± 23.2	0.09
Other vegetables	63.1 ± 65.9	113.36 ± 80.0	280.7 ± 193.4	<0.001
Pulses	0.0 ± 0.0	7.45 ± 20.0	37.9 ± 48.4	<0.001
Potatoes	41.6 ± 47.5	71.97 ± 79.0	123.3 ± 115.6	0.03
Fruits	12.4 ± 37.9	35.33 ± 74.1	114.8 ± 180.1	0.02
Pasta, noodle	27.5 ± 41.6	51.45 ± 71.9	47.4 ± 62.1	0.29
Rice	4.0 ± 10.9	11.36 ± 19.8	19.4 ± 50.7	0.41
Pizza, sandwich, pie	46.6 ± 73.5	46.47 ± 63.4	36.9 ± 60.6	0.65
Chocolate	0.0 ± 0.0	2.78 ± 11.8	0.3 ± 1.3	0.43
Biscuit, cake	8.4 ± 16.7	15.70 ± 30.7	9.8 ± 24.3	0.66
Cereal products	0.0 ± 0.0	2.39 ± 10.13	11.3 ± 27.4	0.06
Bread	24.8 ± 38.6	34.84 ± 34.3	58.3 ± 48.5	0.02
Meat	28.8 ± 38.1	70.66 ± 66.8	62.4 ± 52.3	0.07
Poultry	31.0 ± 53.9	16.80 ± 34.8	30.3 ± 42.3	0.95
Eggs	5.9 ± 18.0	5.75 ± 16.4	3.1 ± 9.1	0.86
Fish and seafood	12.2 ± 38.0	50.86 ± 74.4	8.6 ± 19.5	0.85
Processed meat	23.6 ± 34.9	18.67 ± 40.0	31.2 ± 42.9	0.86
Sweet and soda	294.3 ± 598.8	188.52 ± 467.7	109.7 ± 202.0	0.36
Snack and chips	2.1 ± 5.3	21.17 ± 61.4	3.1 ± 12.5	0.61
Cheese	36.7 ± 34.3	36.60 ± 52.7	38.4 ± 54.5	0.44
Other dairy products	26.9 ± 54.9	12.81 ± 24.8	50.5 ± 79.9	0.12
Spirit	10.9 ± 32.0	147.17 ± 384.3	48.6 ± 131.8	0.93
Wine	519.1 ± 550.7	420.61 ± 577.3	468.0 ± 709.1	0.54

Beer	1361.1 ± 1901.0	858.8 ± 1188.4	1330.7 ± 1440.4	0.37
Coffee	35.6 ± 70.4	174.2 ± 173.2	250.0 ± 363.2	0.02
Fruits or legume juice	0.0 ± 0.0	16.0 ± 36.2	170.3 ± 320.0	0.02
Tea	14.8 ± 55.5	49.7 ± 159.7	15.6 ± 36.9	0.40
Olive oil	0.7 ± 2.7	0.70 ± 1.8	3.1 ± 5.9	0.04

Values are means ± standard dviation. T1: tertile 1, T2: tertile 2, T3: tertile 3.

[a] p for trend obtained with Jonckheere Terpstra test

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Supplementary Table 4: **Associations between fructo-oligosacharide, galacto-oligasacharide, n-6 PUFA, potassium intakes and psychological measurements in AUD patients**

	FOS			GOS			n-6 PUFA			Potassium		
	β	IC	P	β	IC	P	β	IC	P	β	IC	P
Depression												
Model 1	0.61	[-3.09 ; 4.31]	0.74	3.49	[-5.38 ; 12.35]	0.43	-0.44	[-2.14;1.26]	0.60	-0.003	[-0.006 ; 0.001]	0.08
Model 2	0.61	[-3.91 ; 5.13]	0.79	4.24	[-5.85 ; 14.33]	0.40	-0.47	[-2.24;1.29]	0.59	-0.004	[-0.008 ; -0.003]	**0.03**
Model 3	0.78	[-4.09 ; 5.67]	0.75	4.40	[-5.93 ; 14.73]	0.39	-0.47	[-2.28;1.34]	0.60	-0.003	[-0.009 ; -0.001]	**0.03**
Anxiety												
Model 1	1.70	[-2.92 ; 6.33]	0.46	-9.41	[-20.23 ; 1.42]	0.09	-1.89	[-3.95;0.17]	0.07	-0.003	[-0.01;0.001]	0.14
Model 2	2.08	[-3.58 ; 7.74]	0.46	-11.97	[-24.20 ; 0.26]	0.05	-1.89	[-4.04;0.25]	0.08	-0.004	[0.009 ; 0.001]	0.08
Model 3	3.88	[-1.98 ; 9.74]	0.19	-10.97	[-23.26 ; 1.31]	0.08	-1.73	[-3.88 ;0.42]	0.11	-0.004	[-0.008 ; 0.002]	0.17
Sociability												
Model 1	-0.07	[-0.51 ; 0.36]	0.73	1.50	[0.57 ; 2.44]	**0.002**	0.17	[-0.02;0.36]	0.08	0.001	[-0.001 ; 0.006]	0.23
Model 2	-0.10	[-0.63 ; 0.42]	0.69	1.82	[0.79 ; 2.85]	**<0.001**	0.17	[-0.03;0.37]	0.09	0.001	[-0.001 ; 0.001]	0.15
Model 3	-0.24	[-0.79 ; 0.32]	0.39	1.76	[0.72 ; 2.80]	**0.002**	0.16	[-0.04;0.36]	0.12	0.001	[-0.001 ; 0.007]	0.27
Alcohol Craving												
Model 1	-0.11	[-1.85 ; 1.62]	0.89	1.58	[-2.56 ; 5.73]	0.44	0.15	[-0.65 ; 0.95]	0.70	-0.001	[-0.002 ; 0.001]	0.55
Model 2	-0.66	[-2.69 ; 1.36]	0.51	1.68	[-2.87 ; 6.23]	0.99	0.11	[-0.69 ; 0.91]	0.78	-0.001	[-0.003 ; 0.001]	0.21
Model 3	-0.23	[-2.37 ; 1.92]	0.83	2.14	[-2.39 ; 6.67]	0.34	0.18	[-0.61 ; 0.97]	0.65	-0.001	[-0.003 ; 0.001]	0.41

N=48 for all the parameters. FOS, Fructo-oligosaccharide; GOS, Galacto-oligosaccharide; PUFA, Polyunsaturated fatty acids.

[a] Linear model adjusted for age, gender, educational level.

[b] Linear model adjusted for age, gender, educational level, energy intake, BMI, tobacco

[c] Linear model adjusted for age, gender, educational level, energy intake, BMI, Tobacco, alcohol consumption

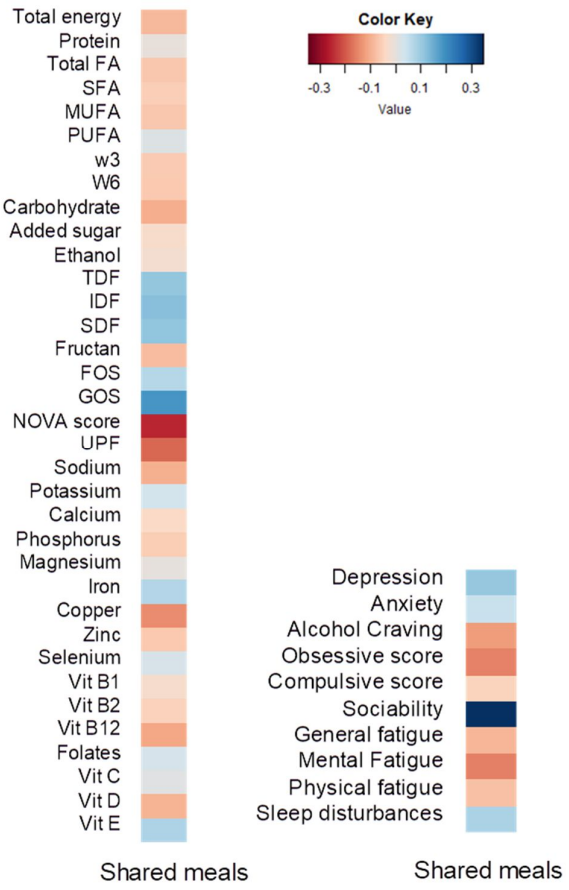

Figure S2: Correlation between the type of meal, nutrients and psychological scores

Pearson correlation n=40. Shared meals represent the number of accompanied meals/total number of meals. The higher the score, the more meals the patient shares with someone

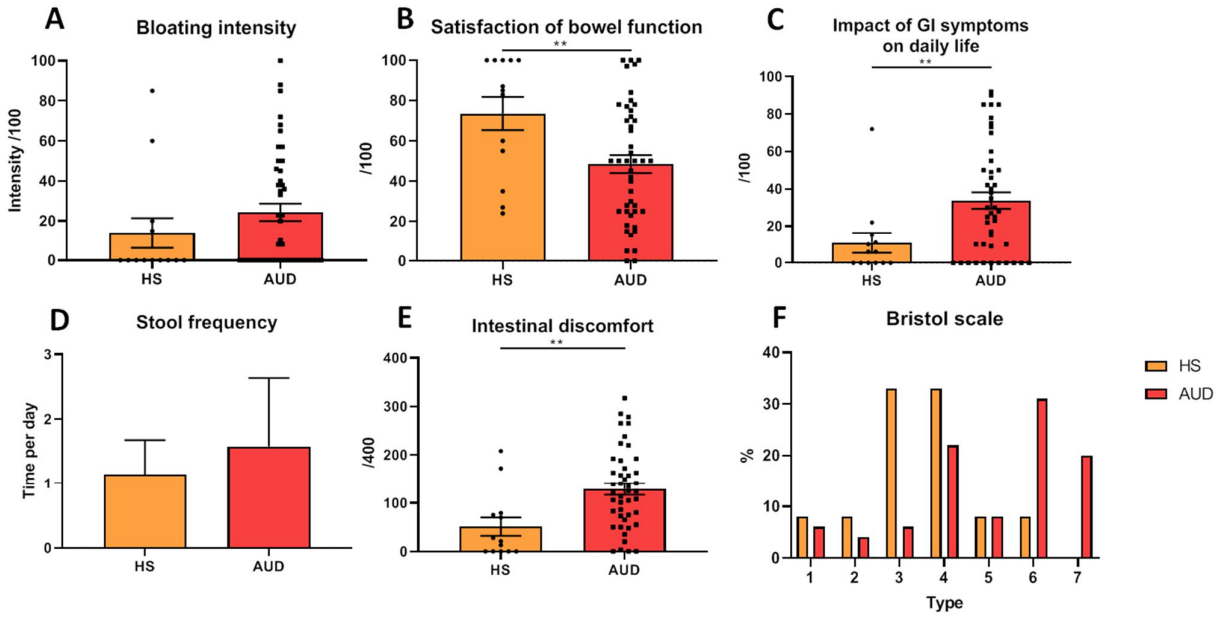

Figure S3: Comparison of gastrointestinal symptoms between HS and AUD patients
*p<0.05, **p<0.01,***p<0.001. N=13 for HS group and n=44 for AUD group.

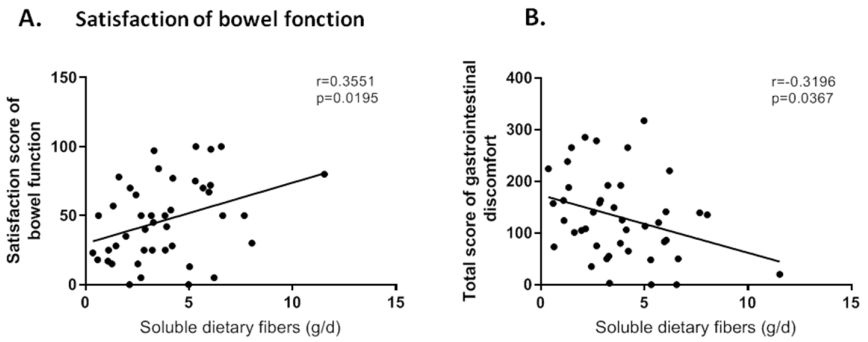

Figure S4: Correlations between soluble dietary fibers and gastrointestinal discomfort in AUD patients

Pearson correlations. N=43

3.7 References

1. Carvalho AF, Heilig M, Perez A, Probst C, Rehm J. Alcohol use disorders. Lancet. 2019;394:781–92.

2. Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci USA. 2014;111:E4485-4493.

3. Buckner JD, Schmidt NB, Lang AR, Small JW, Schlauch RC, Lewinsohn PM. Specificity of Social Anxiety Disorder as a Risk Factor for Alcohol and Cannabis Dependence. J Psychiatr Res. 2008;42:230–9.

4. Baj J, Flieger W, Teresiński G, Buszewicz G, Sitarz E, Forma A, et al. Magnesium, Calcium, Potassium, Sodium, Phosphorus, Selenium, Zinc, and Chromium Levels in Alcohol Use Disorder: A Review. J Clin Med. 2020;9.

5. Lieber CS. The influence of alcohol on nutritional status. Nutr Rev. 1988;46:241–54.

6. Lieber CS. Relationships between nutrition, alcohol use, and liver disease. Alcohol Res Health. 2003;27:220–31.

7. Bebb HT, Houser HB, Witschi JC, Littell AS, Fuller RK. Calorie and nutrient contribution of alcoholic beverages to the usual diets of 155 adults. Am J Clin Nutr. 1971;24:1042–52.

8. Bode C, Bode JC. Alcohol's Role in Gastrointestinal Tract Disorders. Alcohol Health Res World. 1997;21:76–83.

9. Kwok A, Dordevic AL, Paton G, Page MJ, Truby H. Effect of alcohol consumption on food energy intake: a systematic review and meta-analysis. Br J Nutr. 2019;121:481–95.

10. de Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre P-F, et al. The loss of metabolic control on alcohol drinking in heavy drinking alcohol-dependent subjects. PLoS ONE. 2012;7:e38682.

11. Berk M, Jacka FN. Diet and Depression-From Confirmation to Implementation. JAMA. 2019;321:842–3.

12. Adjibade M, Julia C, Allès B, Touvier M, Lemogne C, Srour B, et al. Prospective association between ultra-processed food consumption and incident depressive symptoms in the French NutriNet-Santé cohort. BMC Medicine. 2019;17:78.

13. Molendijk M, Molero P, Ortuño Sánchez-Pedreño F, Van der Does W, Angel Martínez-González M. Diet quality and depression risk: A systematic review and dose-response meta-analysis of prospective studies. J Affect Disord. 2018;226:346–54.

14. Li Y, Lv M-R, Wei Y-J, Sun L, Zhang J-X, Zhang H-G, et al. Dietary patterns and depression risk: A meta-analysis. Psychiatry Res. 2017;253:373–82.

15. Huang T, Xu M, Lee A, Cho S, Qi L. Erratum: Consumption of whole grains and cereal fiber and total and cause-specific mortality: prospective analysis of 367,442 individuals. BMC Med. 2015;13:85.

16. Chen G-C, Tong X, Xu J-Y, Han S-F, Wan Z-X, Qin J-B, et al. Whole-grain intake and total, cardiovascular, and cancer mortality: a systematic review and meta-analysis of prospective studies. The American Journal of Clinical Nutrition. 2016;104:164–72.

17. Miki T, Eguchi M, Kurotani K, Kochi T, Kuwahara K, Ito R, et al. Dietary fiber intake and depressive symptoms in Japanese employees: The Furukawa Nutrition and Health Study. Nutrition. 2016;32:584–9.

18. Stephen AM, Champ MM-J, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev. 2017;30:149–90.

19. Delzenne NM, Olivares M, Neyrinck AM, Beaumont M, Kjølbæk L, Larsen TM, et al. Nutritional interest of dietary fiber and prebiotics in obesity: Lessons from the MyNewGut consortium. Clinical Nutrition. 2019. doi:10.1016/j.clnu.2019.03.002.

20. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99:1877–2013.

21. Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. Microbiota and the social brain. Science. 2019;366:eaar2016.

22. Berding K, Long-Smith CM, Carbia C, Bastiaanssen TFS, Wouw M van de, Wiley N, et al. A specific dietary fibre supplementation improves cognitive performance—an exploratory randomised, placebo-controlled, crossover study. Psychopharmacology. 2020;:1–15.

23. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2012;302:G966–78.

24. Bajaj JS. Alcohol, liver disease and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16:235–46.

25. Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, et al. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. Gut Microbes. 2020;12:1782157.

26. Jeynes KD, Gibson EL. The importance of nutrition in aiding recovery from substance use disorders: A review. Drug and Alcohol Dependence. 2017;179:229–39.

27. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. American Psychiatric Association; 2013. doi:10.1176/appi.books.9780890425596.

28. Beaton GH, Milner J, McGuire V, Feather TE, Little JA. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. Am J Clin Nutr. 1983;37:986–95.

29. Favier J-C, Ireland-Ripert J, Toque C, Feinberg M. Répertoire général des aliments : table de composition = Composition tables. Paris: Technique & Documentation; 1995. http://www.documentation.ird.fr/hor/fdi:010009113. Accessed 25 Jun 2020.

30. Superior Health Council. Dietary guidelines for the Belgian adult population. Brussels; 2019.

31. Samieri C, Jutand MA, Feart C, Capuron L, Letenneur L, Barberger-Gateau P. Dietary patterns derived by hybrid clustering method in older people: association with cognition, mood, and self-rated health. J Am Diet Assoc. 2008;108:1461–71.

32. Kalala G, Kambashi B, Everaert N, Beckers Y, Richel A, Pachikian B, et al. Characterization of fructans and dietary fibre profiles in raw and steamed vegetables. Int J Food Sci Nutr. 2018;69:682–9.

33. Monteiro CA, Cannon G, Levy R, Moubarac J-C, Jaime P, Martins AP, et al. NOVA. The star shines bright. World Nutrition. 2016;7:28–38.

34. Neyrinck AM, Rodriguez J, Vinoy S, Maquet V, Walter J, Bischoff SC, et al. The FiberTAG project: Tagging dietary fibre intake by measuring biomarkers related to the gut microbiota and their interest for health. Nutr Bull. 2020;45:59–65.

35. Strunk KK, Lane FC. The Beck Depression Inventory, Second Edition (BDI-II): A Cross-Sample Structural Analysis. Measurement and Evaluation in Counseling and Development. 2016;:0748175616664010.

36. Spielberger CD, Gorsuch RC, Lushene RE. Manual for the State-Trait Anxiety Inventory (Form Y):(“self-evaluation questionnaire”) Consulting Psychologists Press. Palo Alto, CA. 1983.

37. Ansseau M, Besson J, Lejoyeux M, Pinto E, Landry U, Cornes M, et al. A French Translation of the Obsessive-Compulsive Drinking Scale for Craving in Alcohol-Dependent Patients: A Validation Study in Belgium, France, and Switzerland. EAR. 2000;6:51–6.

38. Whelan DC, Zelenski JM. Experimental Evidence That Positive Moods Cause Sociability. Social Psychological and Personality Science. 2012;3:430–7.

39. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res. 1995;39:315–25.

40. Carpenter JS, Andrykowski MA. Psychometric evaluation of the pittsburgh sleep quality index. Journal of Psychosomatic Research. 1998;45:5–13.

41. Sobell LC, Sobell MB. Timeline Follow-Back. In: Litten RZ, Allen JP, editors. Measuring Alcohol Consumption. Totowa, NJ: Humana Press; 1992. p. 41–72. doi:10.1007/978-1-4612-0357-5_3.

42. Orrell M, Yard P, Handysides J, Schapira R. Validity and reliability of the Health of the Nation Outcome Scales in psychiatric patients in the community. Br J Psychiatry. 1999;174:409–12.

43. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res. 1975;12:189–98.

44. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11:395–402.

45. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther. 2016;44:693–703.

46. González I, Cao K-AL, Davis MJ, Déjean S. Visualising associations between paired 'omics' data sets. BioData Mining. 2012;5:19.

47. Manari AP, Preedy VR, Peters TJ. Nutritional intake of hazardous drinkers and dependent alcoholics in the UK. Addict Biol. 2003;8:201–10.

48. Thomson M, Fulton M, Elton RA, Brown S, Wood DA, Oliver MF. Alcohol consumption and nutrient intake in middle-aged Scottish men. Am J Clin Nutr. 1988;47:139–45.

49. Jerlhag E. Gut-brain axis and addictive disorders: A review with focus on alcohol and drugs of abuse. Pharmacology & Therapeutics. 2018. doi:10.1016/j.pharmthera.2018.11.005.

50. Leggio L, Addolorato G, Cippitelli A, Jerlhag E, Kampov-Polevoy AB, Swift RM. Role of feeding-related pathways in alcohol dependence: A focus on sweet preference, NPY, and ghrelin. Alcohol Clin Exp Res. 2011;35:194–202.

51. Mehta S, Baruah A, Das S, Avinash P, Chetia D, Gupta D. Leptin levels in alcohol dependent patients and their relationship with withdrawal and craving. Asian Journal of Psychiatry. 2020;51:101967.

52. Kiefer F, Jahn H, Jaschinski M, Holzbach R, Wolf K, Naber D, et al. Leptin: a modulator of alcohol craving? Biological Psychiatry. 2001;49:782–7.

53. Badaoui A, De Saeger C, Duchemin J, Gihousse D, De Timary P, Stärkel P. Alcohol dependence is associated with reduced plasma and fundic ghrelin levels: ALCOHOLISM AND GHRELIN. European Journal of Clinical Investigation. 2008;38:397–403.

54. Edington J. Problems of nutritional assessment in the community. Proc Nutr Soc. 1999;58:47–51.

55. Konttinen H, Männistö S, Sarlio-Lähteenkorva S, Silventoinen K, Haukkala A. Emotional eating, depressive symptoms and self-reported food consumption. A population-based study. Appetite. 2010;54:473–9.

56. Kim SY, Breslow RA, Ahn J, Salem N. Alcohol Consumption and Fatty Acid Intakes in the 2001?2002 National Health and Nutrition Examination Survey. Alcoholism Clin Exp Res. 2007;31:1407–14.

57. Borsonelo EC, Galduróz JCF. The role of polyunsaturated fatty acids (PUFAs) in development, aging and substance abuse disorders: Review and propositions. Prostaglandins, Leukotrienes and Essential Fatty Acids. 2008;78:237–45.

58. Liao Y, Xie B, Zhang H, He Q, Guo L, Subramaniapillai M, et al. Efficacy of omega-3 PUFAs in depression: A meta-analysis. Transl Psychiatry. 2019;9:190.

59. Larrieu T, Layé S. Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety. Front Physiol. 2018;9. doi:10.3389/fphys.2018.01047.

60. Hussain G, Schmitt F, Loeffler J-P, Gonzalez de Aguilar J-L. Fatting the brain: a brief of recent research. Front Cell Neurosci. 2013;7:144.

61. Buydens-Branchey L, Branchey M. Long-chain n-3 polyunsaturated fatty acids decrease feelings of anger in substance abusers. Psychiatry Res. 2008;157:95–104.

62. Leclercq S, Le Roy T, Furgiuele S, Coste V, Bindels LB, Leyrolle Q, et al. Gut Microbiota-Induced Changes in β-Hydroxybutyrate Metabolism Are Linked to Altered Sociability and Depression in Alcohol Use Disorder. Cell Rep. 2020;33:108238.

63. Gómez-Donoso C, Sánchez-Villegas A, Martínez-González MA, Gea A, Mendonça R de D, Lahortiga-Ramos F, et al. Ultra-processed food consumption and the incidence of depression in a Mediterranean cohort: the SUN Project. Eur J Nutr. 2020;59:1093–103.

64. Grochowski C, Blicharska E, Baj J, Mierzwińska A, Brzozowska K, Forma A, et al. Serum iron, Magnesium, Copper, and Manganese Levels in Alcoholism: A Systematic Review. Molecules. 2019;24.

65. Barve S, Chen S-Y, Kirpich I, Watson WH, McClain C. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. Alcohol Res. 2017;38:289–302.

66. Torres SJ, Nowson CA, Worsley A. Dietary electrolytes are related to mood. Br J Nutr. 2008;100:1038–45.

67. Thi Thu Nguyen T, Miyagi S, Tsujiguchi H, Kambayashi Y, Hara A, Nakamura H, et al. Association between Lower Intake of Minerals and Depressive Symptoms among Elderly Japanese Women but Not Men: Findings from Shika Study. Nutrients. 2019;11:389.

68. Fawehinmi TO, Ilomäki J, Voutilainen S, Kauhanen J. Alcohol Consumption and Dietary Patterns: The FinDrink Study. PLoS ONE. 2012;7:e38607.

69. Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet. 2019.

70. Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell. 2016;165:1762–75.

71. Degroote S, Hunting DJ, Baccarelli AA, Takser L. Maternal gut and fetal brain connection: Increased anxiety and reduced social interactions in Wistar rat offspring following peri-conceptional antibiotic exposure. Prog Neuropsychopharmacol Biol Psychiatry. 2016;71:76–82.

72. Aslam H, Green J, Jacka FN, Collier F, Berk M, Pasco J, et al. Fermented foods, the gut and mental health: a mechanistic overview with implications for depression and anxiety. Nutritional Neuroscience. 2020;23:659–71.

73. Woo J, Lynn H, Lau WY, Leung J, Lau E, Wong SYS, et al. Nutrient intake and psychological health in an elderly Chinese population. Int J Geriatr Psychiatry. 2006;21:1036–43.

74. Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, et al. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome. 2018;6:133.

75. St-Onge M-P, Roberts A, Shechter A, Choudhury AR. Fiber and Saturated Fat Are Associated with Sleep Arousals and Slow Wave Sleep. Journal of Clinical Sleep Medicine. 2016;12:19–24.

76. Burokas A, Arboleya S, Moloney RD, Peterson VL, Murphy K, Clarke G, et al. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry. 2017;82:472–87.

77. van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol (Lond). 2018;596:4923–44.

78. Sarkar A, Harty S, Johnson KV-A, Moeller AH, Carmody RN, Lehto SM, et al. The role of the microbiome in the neurobiology of social behaviour. Biol Rev Camb Philos Soc. 2020.

79. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the Manipulation of Bacteria–Gut–Brain Signals. Trends in Neurosciences. 2016;39:763–81.

80. Dalile B, Vervliet B, Bergonzelli G, Verbeke K, Van Oudenhove L. Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: a randomized, placebo-controlled trial. Neuropsychopharmacol. 2020. doi:10.1038/s41386-020-0732-x.

81. Chae W, Ju YJ, Shin J, Jang S-I, Park E-C. Association between eating behaviour and diet quality: eating alone vs. eating with others. Nutr J. 2018;17:117.

82. Williams R, Rollo M, Schumacher T, Collins C. Diet Quality Scores of Australian Adults Who Have Completed the Healthy Eating Quiz. Nutrients. 2017;9:880.

83. Roberfroid M. Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. Critical Reviews in Food Science and Nutrition. 1993;33:103–48.

84. Silveira ALM, Ferreira AVM, de Oliveira MC, Rachid MA, da Cunha Sousa LF, dos Santos Martins F, et al. Preventive rather than therapeutic treatment with high fiber diet attenuates clinical and inflammatory markers of acute and chronic DSS-induced colitis in mice. Eur J Nutr. 2017;56:179–91.

85. Fedorak RN, Madsen KL. Probiotics and the Management of Inflammatory Bowel Disease: Inflammatory Bowel Diseases. 2004;10:286–99.

86. Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer R-J. Review article: the role of butyrate on colonic function: REVIEW: ROLE OF BUTYRATE ON COLONIC FUNCTION. Alimentary Pharmacology & Therapeutics. 2007;27:104–19.

87. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and

Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol. 2017;14:491–502.

88. Galobardes B, Morabia A, Bernstein MS. Diet and socioeconomic position: does the use of different indicators matter? Int J Epidemiol. 2001;30:334–40.

89. Temme E, Huybrechts I, Vandevijvere S, De Henauw S, Leveque A, Kornitzer M, et al. Energy and macronutrient intakes in Belgium: results from the first National Food Consumption Survey. Br J Nutr. 2010;103:1823–9.

90. Rustad JK, Stern TA, Prabhakar M, Musselman D. Risk factors for alcohol relapse following orthotopic liver transplantation: a systematic review. Psychosomatics. 2015;56:21–35.

91. Zywiak WH, Westerberg VS, Connors GJ, Maisto SA. Exploratory findings from the Reasons for Drinking Questionnaire. Journal of Substance Abuse Treatment. 2003;25:287–92

CHAPTER 2: Is the gut microbiota related to the social network and fundamental social cognition abilities? The case of alcohol-use-disorder.

It has been highlighted that not all AUD patients have a gut dysbiosis but about 40% of them. Knowing this we wanted to investigate which factors could explain this difference in microbial composition. In the previous chapter we observed that AUD patients consumed less fiber and other nutrients (protein, fat etc) compared to healthy subjects. However, this was not the case for all patients, some of whom had a nutritional profile close to that of healthy subjects. Nutrition being one of the most important factors influencing the gut microbiota, it could be responsible for the observed disparities. In the literature, other factors such as gender, age, stress, medication and more recently social interaction have also been shown to influence the composition of the microbiota.

The aim of the present work was to better understand the relationship between the gut microbiota and social functioning, by segregating AUD patients according to the existence of a gut microbiota dysbiosis and to study its link with sociability. We therefore compared dysbiotic and non-dysbiotic AUD subjects in terms of sociodemographic, biological and psychological characteristics. Finally we also evaluated whether the microbial differences among AUD patients could be related to environmental factors such as, diet, medications, or traumatic events.

Is the gut microbiota related to the social network and fundamental social cognition abilities? The case of alcohol-use-disorder.

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3.1 Abstract

Objective: Social functioning is severely impaired in various psychiatric disorders. Recently, a link between sociability and the gut microbiota has been suggested in both clinical and preclinical studies, including in the context of alcohol use disorders (AUD), but no studies has evaluated the precise nature of the social impairments in humans. This study aims at testing in details the relationship between the gut microbiota and social functioning in a population of actively drinking AUD patients.

Method: The gut microbiota composition and the existence of a gut dysbiosis was assessed in of 50 AUD patients and compared to their levels of inflammation, to psychological symptoms, and to social functioning, i.e sociability measures assessed by questionnaires, social cognition assessed by a visual perspective task and the characteristics of the social networks assessed by mapping techniques. Logistic regression models were used to control for possible confounding factors.

Results: A dysbiotic subset of patients (35%) was identified, who were younger, thinner and had a higher craving score compared to non-dysbiotic patients. Interestingly, we found severe social impairments in the dysbiotic group: they had lower sociability scores, tended to have difficulties to take into account another person's visual perspective and exhibited a smaller and less connected social network. They also displayed a higher level

of IL-8. These differences were not related to differences in nutritional or medication intake.

Conclusion: These results show for the first time an important link between the existence of a dysbiosis and impairments of different facets of sociability, including the importance and richness of the social network in AUD. The gut microbiota appears to be an appealing target to tackle alterations in social behaviour, a central characteristic of psychiatric disorders.

Keywords: Gut-Brain axis, Alcohol use disorder, social cognition, dysbiosis

3.2 Introduction

Social isolation is a dimension related to the expression and severity of mental health disorders and may partially result from deficits in social cognition that are present in most major domains of psychiatry [1–6]. Furthermore, studies in sociology have also shown that the abundance and the quality of the social network of individuals suffering a mental health disorder is associated with less severe psychiatric symptoms [7, 8], better quality of life [9], greater satisfaction, better social functioning [10] and less risks of hospitalization [11]. These social dimensions are probably among the most important factors that participate to the perpetuation of mental health disorders with a real questioning on the nature of the factors involved in these social deficits.

The gut microbiota is a dynamic ecosystem that can be influenced by several factors including stress, diet or the medication [12, 13]. Recent studies conducted mainly in patients presenting with autistic spectrum disorders or in preclinical models [13–15] have supported the possibility of a link between sociability and alterations of the composition of the gut microbiota. For example, germ-free mice displayed deficits in social recognition and social cognition [16]. It has also been shown that *Lactobacillus reuteri* supplementation rescued the social behaviour in an autistic mouse model [17] and that *Bifidobacterium longum* and specific *Lactobacilli* strains supplementation improved antisocial behaviour as well as sociability, in autism spectrum disorders (ASD) children [18]. Human observational studies revealed disturbances in the composition of the gut microbiota (reductions in microbial diversity and reduced abundance of beneficial bacteria) in neuropsychiatric conditions associated with a social deficit such as ASD [19], schizophrenia [20], social anxiety and alcohol use disorders (AUD) [21–23]. AUD is a complex disorder combining behavioural, neurobiological and psychosocial impairments [24], where social and emotional deficits have recently emerged as central to the expression of the disorder [25]. AUD individuals exhibit difficulties in perceiving and interpreting the emotions of others [26–28], a reduced ability to take the perspective of others [29], an impairment of emotional regulation [30] and a vulnerability to social exclusion [31]. The social dimension is central in the management of AUD as social difficulties have been associated with high relapse rates after alcohol withdrawal [32, 33] and the quality of social support is an important factor that can influence drinking rates but also the maintenance of abstinence [34].

Among AUD patients, a more severe form of the disease has been associated to an increase in gut permeability, a gut dysbiosis [35] and to deficits in sociability [36].

Preclinical studies, where mice were transplanted with the gut microbiota of AUD or ASD patients showed that the mice replicated the behavioural phenotype observed in patients, including impaired social behaviour [36, 37], supporting the existence of a causal role of the gut microbiota and social impairments. Altogether these data support the possibility of a role of the gut microbiota in social abilities, with potent consequences for the development of mental health disorders. However, currently, in humans, the only evidence was that of a correlation between the gut microbiota and scores on sociability scales [36].

The aim of the present study was to better understand the relationship between the gut microbiota and social functioning, by segregating AUD patients according to the existence of a gut microbiota dysbiosis and to study their link with sociability. To obtain a broader and complementary evaluation of the social abilities, AUD patients were tested with various questionnaires, but also with a task investigating social cognition abilities and with a precise measurement of the importance and richness of their social network using sociogram.

Finally, we also evaluated whether the microbial differences among AUD patients could be related to environmental factors such as, diet, medications, or traumatic events.

3.3 Materials and Methods

Subjects

The data of this cross-sectional study were obtained at the first time-point of a randomized, double-blind, placebo-controlled study evaluating the impact of prebiotic supplementation on the gut-liver-brain axis (Amadieu C et al, accepted in *Gut Microbes*). A total of 50 AUD patients hospitalized for a 3-week highly standardized alcohol-detoxification program in St-Luc academic hospital (Brussels, Belgium) were enrolled on voluntary basis and for the study were tested at the beginning of alcohol-withdrawal, before any intervention. The severity of AUD was checked by a psychiatrist by using the criteria of the mini *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*.

Inclusion criteria were the following: male or female, 18 to 65 years old, french speaking, and active alcohol consumption until at least 48 hours prior to admission. Patients suffering from another addiction (except tobacco), with inflammatory bowel diseases or other chronic inflammatory diseases (such as rheumatoid arthritis), cancer, metabolic diseases such as obesity (BMI≥ 30 kg/m2), diabetes, bariatric surgery, and severe cognitive impairment (MMSE < 24) were excluded from the study. Patients with known cirrhosis or significant hepatic fibrosis (≥F2) detected by Fibroscan (> 7.6 kPa) at admission were also excluded from the study. Other exclusion criteria were the following: the use of antibiotics, probiotics or prebiotics within 2 months prior to enrolment and the use of non-steroidal anti-inflammatory drugs or glucocorticoids within one month prior to enrolment.

AUD patients were matched for age, gender and BMI with 14 HS with no AUD (Alcohol use disorders test [AUDIT] score <8 in males and <7 in females). They were recruited using flyers posted in Brussel's public setting.

The study was approved by the institutional ethics committee (2017/04JUL/354 and 2014/14AOU/438) and all participants signed informed consent prior to inclusion.

Outcomes

Gut microbiota analyses

Stool samples were collected on day 2 of the detoxification program in forty-six AUD patients. They were stored immediately at -20°C and then transferred to -80°C within 5 to 10 hours. Genomic DNA was extracted from the feces using a QIAamp DNA Stool Mini Kit

(Qiagen, Germany), including a bead-beating step and following the protocol Q [38]. The composition of the gut microbiota was analysed by Illumina sequencing of the 16S rRNA gene as previously described (Amadieu C et al, accepted in *Gut Microbes*).

Psychological symptoms assessment

Self-reported questionnaires were used on day 1 and 2 to assess anxiety (State-Trait Anxiety Inventory [STAI form YA]), depression (the Beck Depression Inventory [BDI]) and alcohol craving (the Obsessive-Compulsive Drinking Scale modified version [OCDS]) as described before [39]. The patients had also completed the Post-traumatic Stress Diagnostic Scale (PDS) questionnaire, a self report measure of post-traumatic stress disorder (PTSD). The questionnaire includes a symptoms severity score which ranges from 0 to 51 [40].

Sociability assessments

The emotional intelligence (EI) was measured using the French version of the TEIque a self-reported questionnaire that consists in 75 items rated on a 7-point scale ranging from 1 (strongly disagree) to 7 (strongly agree) [41]. It assesses a global trait EI score as well as scores on 4 specific factors: well-being, self-control, emotionality and sociability. Sociability was also measured using the social situation test [42, 43].

In order to measure instructed and spontaneous capacities to take into account the perspective of others, AUD patients performed the visual perspective task (VPT) on day 1, as described elsewhere [44]. In short, during the task, the patient saw on computer screen an adult avatar in the middle of a 3-wall room with 0 to 3 red discs hanging on the walls. The patient had to decide whether or not a prompted number (ranging from 0 to 3) matched the number of discs visible on a screen from a prompted target perspective, which could be either the participant's perspective (self-perspective condition) or the perspective of the avatar (other-perspective condition) (Supplementary Figure 1A). The number of visible discs could be the same for both perspectives (congruent perspective condition) or different (incongruent perspective condition).

Reaction times (RTs) and accuracy were collected for all conditions [2 (perspective: self vs. other) × 2 (consistency: consistent vs. inconsistent)]. As in previous studies, only matching trials and accurate RTs were analyzed. Performance is expected to weaken in incongruent perspective trials because either the self-perspective is spontaneously computed in other-perspective trials and interferes at judging what the avatar sees, causing an *egocentric*

effect (or bias or intrusion) or because the avatar's perspective is spontaneously computed in self-perspective trials and interferes at judging what the patient him/herself sees, causing an *altercentric* effect (or bias or intrusion).

On the third week of the program, the patients completed the ego-network survey, using the sociogram technique, during a face-to-face interview. The methodology used has already been described elsewhere [45]. Quickly, participants are interviewed using a simple question: "Who are the people who support you in your daily life?". The respondent is then asked to list the people who support him, and then define the type of support received in four specific areas: finances, housing, activities, and health. Supporters are then placed on Hogan's bullseye map, and the participant is then asked to link those whom he or she believed exchanged information ("who shares information about you?") (Supplementary Figure 1B). The different indicators obtained with the sociogram are described in Table 1.

In summary, in order to have a complete overview of the social impairments of AUD patients, several tools were used including self reported questionnaires (social situation test, TEIque), neuropsychological task (VPT), and the social network.

Table 1: Definition of the main measures used to describe the social networks

	Definition
Density (%)	Proportion of effective ties among all possible ties between alters
Network size	Number of alters (network members) supporting ego (the focal service user)
Number of dyads	Number of dyads in ego's network
Number of triads	Number of triads in ego's network
Degree	Average number of other alters with which all ego's alters are in contact
Betweenness	Frequency where an alter is a crossing point along the shortest path between two other alters
Closeness	Ratio between (n-1) and the distance between pairs of nodes
Modularity	Tendency of nodes to cluster
Number of communities	Number of communities (linked alters) in the network
Diameter	Maximum distance in the graph
Transitivity	Percentage of triples that are triads.
Professional proportion	Proportion of professional (psychiatrist, nurse, social worker, etc.) among the alters supporting ego
Gender homophily	Proportion of gender homophily in the network (link between alters of the same gender)
Ring homophily	Circle homophily on the target, i.e. the fact that alters preferentially have links with other alters in the same circle.
Isolated	Alter which is not in contact with any other alter in ego's network
Isolated dyads	Number of dyads of two isolated alters (without link to another alter) in the network
Number of community with more than 3 alters	Number of communities with more than 3 alters
Cliques	Subset of alters that are all connected to each other (maximum density subnetwork)

Diet anamnesis

On Day 2 of alcohol withdrawal, all participants were interviewed using three non-consecutive 24-h dietary recall (related to the week before hospitalization: week 0) by a trained dietician as previously described [43]. Energy and nutrient intakes were evaluated using the Nubel Pro program (Nubel asbl, Belgium) and the French food composition database (CIQUAL 2017). Dietary fibers (DF) including soluble fibers, insoluble fibers, fructans, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) were evaluated using a specific database from the FiberTAG project [46].

Blood parameters

Fasting blood samples were collected on day 2. Blood samples were centrifuged at 1000g for 15 min at 4°C and the plasma was frozen at -80°C in a biobank. Plasma concentrations of growth factors (Brain derived neurotrophic factor [BDNF]) and inflammatory markers (IL-18, MCP-1, IFN-γ, IL-8, IL-10, TNF-a, IL-6) were determined using the Meso Scale Discovery (MSD) U-PLEX assay (Rockville, MD, USA).

Statistical analysis

Based on β-diversity index, we segregate our population of AUD patients into two groups: non-dysbiotic and dysbiotic patients. To do so, we performed a Principal Coordinate Analysis (PCoA) with the Bray Curtis index and extracted the component scores for each individual (HS and AUD). The separation of the subjects was then calculated according to a deviance criterion at a threshold of 1.65 SD of the mean score of the first component of the HS group.

Assumptions of normality and homoscedasticity were checked with the Shapiro–Wilk and Levene tests, respectively.

To identify more precisely the differences in gut microbiota composition between HS, non-dysbiotic and dysbiotic AUD patients, we used Linear discriminant analysis Effect Size (LEfSE) [47]. The selected genera were then compared between the three groups of subjects using Kruskal–Wallis tests followed by a Dunn's test. P-values of Kruskal–Wallis tests were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure. In this study, the HS group was used exclusively to determine the dysbiosis state of AUD patients and for the microbial comparisons.

In order to compare the non-dysbiotic and the dysbiotic group, Mann-Whitney U test or T-test were performed according to data distribution. Then multivariate associations

between psychological symptoms, biological outcomes, the measures used to describe the social network and the dysbiosis (yes vs no) were estimated. We used logistic regression models adjusted for age, gender and body mass index [BMI] (model 1) and subsequently added the nutritional intake (model 2). In order to take into account the whole nutritional pattern of the subjects for the adjustment, we constructed a summary variable with all nutrients using a principal component analysis (PCA).

Statistical analyses were performed using SAS version 9.4, R studio version 3.5.1 and Graphpad Prism 8.0. p-value or q-value < 0.05 were considered statistically significant.

3.4 Results

Identification and description of dysbiotic and non-dysbiotic AUD patients

The graph of β-diversity allowed us to discriminate AUD patients who clustered together with the healthy subjects (HS), hereafter referred as non-dysbiotic, and AUD patients who clustered outside the ellipse of HS and referred as dysbiotic. The two groups of AUD patients were selected based on the component score obtained with the PCoA of Bray Curtis index (Figure 1A) (see statistical analysis section for more details). Sixteen patients were classified as dysbiotic (35% of patients) and 30 patients were non-dysbiotic.

Regarding α-diversity, dysbiotic AUD patients displayed a lower number of observed species as well as lower Chao-1, Shannon and Simpson indexes compared to HS and non-dysbiotic patients (Figure 1B). The gut microbiota of dysbiotic patients was composed of 157 species on average compared to 203 for non-dysbiotic patients and 211 for HS which represent a reduction of 25%. The LEfSe analysis revealed that 9 genera were different between the 3 groups of patients. *Ruminococcus* as well as *Christensenellaceae R7* group, *Oscillospiraceae NK4A214* group, *Oscillospiraceae UCG 003* and an unknown genus from the family *Eggerthellaceae* were higher in HS and non-dysbiotic patients than in dysbiotic patients (Figure 1C-E). *Parabacteroides*, *Lachnoclostridium*, *Erysipelatoclostridium* and *Flavonifractor* were higher in dysbiotic patients compared to HS and non-dysbiotic patients (Figure 1C-E).

The sociodemographic characteristics and clinical features of AUD patients according to gut dysbiosis are presented in Table 2. Dysbiotic AUD patients were younger and had a lower BMI than non-dysbiotic patients. There was no difference for gender, DSM-5 score, the duration of drinking habit or the quantity of ethanol consumed.

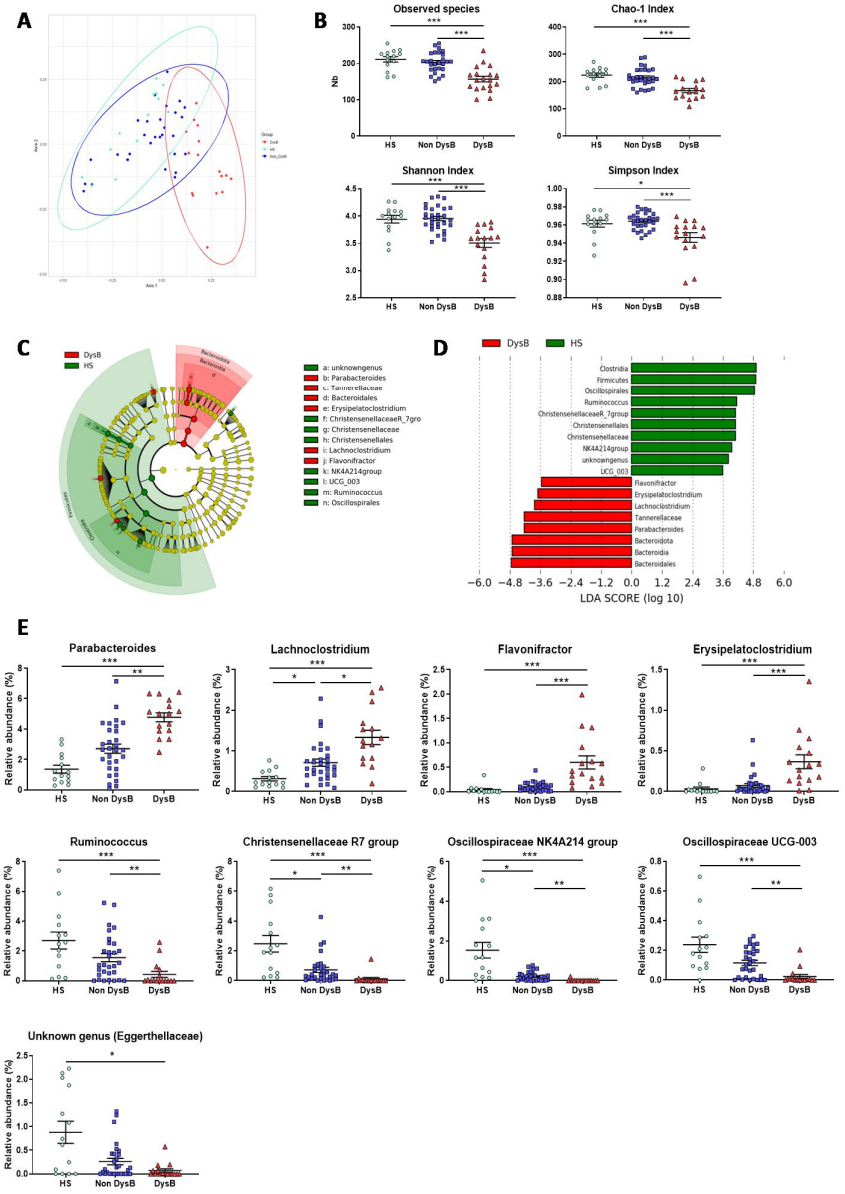

Figure 1 : Gut microbiota composition and microbial diversity according to gut dysbiosis in alcohol use disorder patients (AUD)

Healthy subjects (HS): n=14, Dysbiotic AUD patients (DysB): n=16, Non-Dysbiotic AUD patients (Non-DysB) : n=30. For each panel, data are expressed as mean ± SEM.

(A) Principal coordinate analysis (PCoA) of the Bray Curtis index **(B)** Measure of alpha-diversity indexes: Number of observed species, Chao-1, Shannon, Simpson. (**C**) Total bacteria measured by qPCR. (**D**) Cladogram using LEfSe method indicating the phylogenetic distribution of gut microbiota of HS and AUD patient according to dysbiosis. Each successive circle represents a phylogenetic level. (**E**) Histogram of the LDA scores reveals the most differentially abundant taxa among the three different groups. Taxa enriched in the HS group are highlighted in green, in blue for Non-DysB group and in red for the DysB group in the linear discriminant analysis (LDA). Graphical representation was performed using Galaxy/Hutlab tool (huttenhower.sph.harvard.edu/galaxy). (**F**) Relative abundances of genera those were significantly different between the three groups. Kruskal Wallis tests were performed to compare the three groups followed by a Dunn's test and p-values were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure *p<0.05, **p<0.01,***p<0.001.

Table 2: Socio-demographic characteristics and clinical features of AUD patients according to gut dysbiosis

	Dysbiotic n=16	**Non Dysbiotic n=30**	**p**
Sociodemographic characteristics			
Age (y)	43.6 ± 7.3	50.7 ± 9.2	0.01
Gender (woman)	7 (43.8)	9 (30.0)	0.35
Family status, n (%)			0.08
Single	9 (56.2)	10 (33.3)	
Couple / married	3 (18.8)	16 (53.3)	
Separated/divorced	4 (25.0)	4 (13.4)	
Educational level,n (%)			0.43
Primary	2 (12.5)	3 (10.0)	
Secondary	7 (43.8)	8 (26.7)	
Superior	7 (43.7)	19 (63.33)	
Clinical examination			
Weight, kg	66.7 ± 12.9	75.1 ± 11.8	0.03
BMI, kg/m^2	22.3 ± 3.3	24.8 ± 3.0	0.01
Heart rate, bpm	90.1 ± 11.7	85.1 ± 16.6	0.22
SBP, mm Hg	136.1 ± 27.6	143.0 ± 18.6	0.26
DBP, mm Hg	82.8 ± 19.3	87.4 ± 19.8	0.28
CRP mg/dl	4.3 ± 7.4	2.7 ± 2.2	0.63
Cushman	4.4 ± 2.1	4.7 ± 2.8	0.68
MMSE	28.1 ± 2.1	27.8 ± 2.5	0.78
Alcohol history			
DSM-5 score	8.9 ± 1.7	8.2 ± 1.7	0.23
Age of loss of control (y)	29.4 ± 7.4	32.9 ± 11.9	0.26
Numbers of withdrawal	2.6 ± 2.2	2.1 ± 2.3	0.07
Duration of drinking habit (y)	14.2 ± 7.6	17.7 ± 11.9	0.56
Alcohol consumption (g/d)	135.8 ± 63.9	127.1 ± 50.7	0.65

Values are means ± SD. n=46. p values were calculated using a T-test or Mann Whitney Wilcoxon's test and Chi 2 test or Fisher's test for categorical variables

AUD: alcohol use disorder; BMI: body mass index; CRP: C-Reactive Protein; DBP: diastolic blood pressure; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; MMSE; Mini-Mental State Examination; SBP: systolic blood pressure.

Dysbiotic AUD patients had a higher alcohol craving and a lower sociability score

We then compared the mood, sociability score, and alcohol craving of both groups of patients, since we have previously shown that AUD with an altered gut microbiota had higher scores of depression, anxiety and alcohol craving [35]. Dysbiotic and non-dysbiotic patients had similar scores of anxiety and depression (Table 3). However, the compulsive subscale of craving score was higher in dysbiotic patients than in non-dysbiotic, even after adjustment for potential confounders (Table 3). The sociability evaluated with the TEIque was also associated with gut dysbiosis after adjustment for age, gender, BMI and nutritional intake (Model 2 OR= 0.34, p=0.04, Table 3). The score was higher in non-dysbiotic compared to dysbiotic AUD patients meaning that dysbiotic AUD patients displayed alteration of sociability (Table 3).

Since stress and traumatic events could influence the gut microbiota composition [48, 49], we investigated in both subgroups of AUD patients whether the score of PTSD was different according the dysbiosis status. No difference was observed for the symptoms severity score (Table 3).

Table 3: Psychological parameters of AUD patients according to gut dysbiosis

	Dysbiotic n=16	Non Dysbiotic n=30	p[a]	Model 1[b]		Model 2[c]	
	Mean± SD	Mean± SD		OR	p	OR	p
Mood							
Anxiety	48.2 ± 16.7	46.7 ± 13.8	0.67	0.99 [0.94 ; 1.04]	0.75	0.99 [0.93 ; 1.04]	0.59
Depression	27.4 ± 14.1	25.1 ± 11.0	0.63	1.00 [0.94 ; 1.06]	0.95	1.01 [0.94 ; 1.06]	0.97
Craving							
Total score	27.3 ± 4.6	23.2 ± 5.7	0.03	1.13 [0.97 ; 1.33]	0.10	1.14 [0.97 ; 1.34]	0.12
Obsessive score	11.7 ± 3.4	10.0 ± 3.7	0.18	1.07 [0.88 ;1.32]	0.55	1.06 [0.85 ; 1.32]	0.59
Compulsive score	15.6 ± 2.2	13.2 ± 2.8	0.007	1.64 [1.03 ; 2.62]	0.037	1.69 [1.05 ; 2.71]	0.03
Sociability							
Social high pleasant activities	4.0 ± 1.0	3.6 ± 1.5	0.47	1.26 [0.71 ; 2.22]	0.43	1.34 [0.75 ; 2.42]	0.32
Social medium pleasant activities	3.8 ± 1.1	3.6 ± 1.5	0.38	1.28 [0.72 ; 2.27]	0.39	1.32 [0.74 ; 2.36]	0.34
Social low pleasant activities	3.2 ± 1.1	3.0 ± 1.6	0.38	1.38 [0.79 ; 2.40]	0.26	1.37 [0.79 ; 2.40]	0.26
Emotionnal inteligence							
Well-being	4.6 ± 1.0	4.7 ± 0.9	0.87	1.41 [0.53 ; 3.72]	0.49	1.63 [0.48 ; 3.34]	0.63
Self-control	4.2 ± 0.9	4.3 ± 0.8	0.77	1.43 [0.49 ; 4.12]	0.51	1.27 [0.41 ; 3.90]	0.67
Sociability	3.5 ± 1.0	4.3 ± 1.1	0.03	0.44 [0.17 ; 1.15]	0.09	0.34 [0.12; 0.97]	0.04
Motivation	4.7 ± 0.7	4.6 ± 1.1		0.94 [0.41 ; 2.12]		0.87 [0.37 ; 2.05]	0.75
Emotionality	4.6 ± 0.7	4.9 ± 0.9	0.38	0.81 [0.25 ; 2.60]	0.72	0.90 [0.39; 2.02]	0.79
PTSD							
Symptoms severity	18.0 ± 13.9	17.3 ± 11.4	0.90	0.97 [0.88 ; 1.06]	0.48	0.97 [0.88 ; 1.07]	0.52

[a]p values were calculated using a T-test or Mann Whitney Wilcoxon's test.
[b] Logistic regression adjusted for age, gender and body mass index
[c] Logistic regression adjusted for age, gender, body mass index and nutritional intakes
AUD: alcohol use disorder; OR: Odd ratio; PTSD: Post-traumatic stress disorder.

Dysbiotic AUD patients displayed a smaller and less connected social network

We then checked if the dysbiotic group displayed difficulties to take the perspective of others, compared to the non-dysbiotic group, by using a visual perspective-taking task. A 2 (Consistency: Congruent vs. incongruent perspectives) x 2 (Perspective: Self- vs. other-perspective) x 2 (Group: Dysbiotic vs. Non-Dysbiotic) ANOVA was conducted on the mean reaction time (RT). One patient was not included in the analyses for outlying performance (accuracy at chance level). The ANOVA revealed a significant main effect of Congruency, $F(1, 43) = 19.16$, $p < 0.001$, with a slower RT in the incongruent condition (mean = 1.47s) than in the congruent condition (mean = 1.27s). The analyses showed no significant main effect of Perspective, $F(1, 43) < 1$, $p = 0.82$ and a significant Consistency x Perspective interaction, $F(1, 43) = 8.89$, $p=0.005$. These results replicate the results from the original study [44]. A trend toward a significant interaction between Congruency x Perspective x Group was observed, $F(1, 43)= 3.07$, $p=0.087$. This latter interaction is driven by absence of altercentric effect among dysbiotic patients ($p=0.781$) in comparison to a significant altercentric effect in non-dysbiotic patients ($p=0.026$) and in all previous studies conducted in healthy adults [44]. The ANOVA conducted on accuracy shows only a significant main effect of Congruency, $F(1, 43) = 5.70$, $p=0.021$, in line with previous studies. The triple interaction with Group is non-significant, $F(1, 43) = 2.09$, $p=0.155$, but nevertheless shows an identical pattern, with no altercentric effect among dysbiotic patients ($p=0.790$) and a trend towards a significant altercentric effect in non-dysbiotic patients ($p=0.090$). These converging trends strongly suggest that dysbiotic patients tended not to spontaneously take into account the perspective of other (Figure 2).

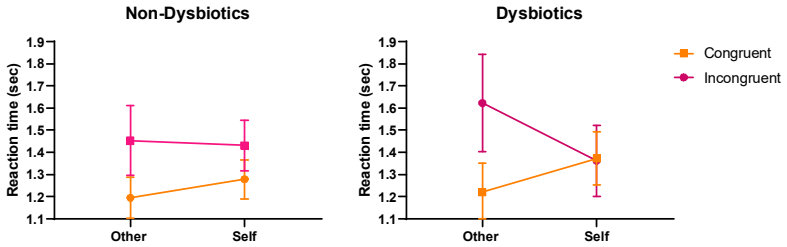

Figure 2: Mean reaction times (sec) according to perspective and congruency in the visual perspective task (VPT) in non-dysbiotic and dysbiotic AUD patients

Slower performance in incongruent perspectives trials is caused by the interference between the instructed perspective to take and the irrelevant and conflicting perspective to not take but nevertheless spontaneously computed. The notable exception is the avatar's perspective among dysbiotic patients that is not spontaneously computed (far right side).

On average, dysbiotic patients had a smaller social network with a lower number of alters and communities compared to non-dysbiotic when we adjusted for potential confounders (Table 4). The dysbiotic network was composed of 7 alters on average compared to 10 in the non-dysbiotic group. Among these alters, 3 were isolated in each group meaning that they were not in contact with any other alter in the network. Twenty-two percent of the alters belonged to the health care community in non-dysbiotic group and 13% in dysbiotic group. However, the result did not reach significance after adjustment for potential confounders (Table 4). The networks appeared to be less cohesive in the dysbiotic group since the transitivity index was almost twice as high in the non-dysbiotic group as in the dysbiotic group ($p=0.02$ in model 1 and 2; Table 4). Modularity was also associated with gut dysbiosis ($p=0.03$ in model 1 and 2; Table 4).

No significant difference in the composition of the social network was observed between dysbiotic and non-dysbiotic groups (Supplementary Figure 2). About 50% of the network members were from the family sphere in the non-dysbiotic group versus 42% in the dysbiotic group. Friends represented 21% and 26% of the network for non-dysbiotics and dysbiotics respectively. Mental health care personnel represented approximately 10% and 7% of the social network of non-dysbiotics and dysbiotics respectively (Supplementary Figure 2).

Table 4: Structure of the social network of AUD patients according to gut dysbiosis

	Dysbiotic n=15	Non-Dysbiotic n=25		Model 1[b]		Model 2[c]	
	Mean ± SD	Mean ± SD	p[a]	OR	p	OR	p
Density (%)	19.9 ± 16.3	20.0 ± 16.3	0.99	0.99 [0.95 ; 1.04]	0.79	0.99 [0.94 ; 1.05]	0.82
Network size (no.)	7.53 ± 4.24	9.72 ± 3.69	0.09	0.76 [0.59 ;0.98]	0.03	0.73 [0.55 ;0.98]	0.03
Number of dyads (no.)	5.93 ± 7.06	7.32 ± 5.22	0.17	0.92 [0.80 ; 1.05]	0.22	0.92 [0.80 ; 1.06]	0.25
Number of triads (no.)	4.60 ± 9.70	3.68 ± 4.38	0.25	0.99 [0.89 ; 1.11]	0.94	1.01 [0.90 ; 1.13]	0.89
Number of communities (no.)	3.60 ± 3.11	5.40 ± 2.06	0.01	0.65 [0.43 ; 0.98]	0.04	0.63 [0.41 ; 0.98]	0.04
Number of community with more than 3 alters (no.)	0.67 ± 0.72	1.16 ± 0.69	0.037	0.10 [0.01 ; 0.63]	0.01	0.06 [0.01 ; 0.60]	0.01
Cliques (no.)	0.73 ± 0.70	1.44 ± 1.29	0.11	0.41 [0.16 ; 0.99]	0.03	0.41 [0.16 ; 0.99]	0.04
Degree (%)	5.7 ± 10.2	6.5 ± 8.3	0.91	1.01 [0.94 ; 1.09]	0.79	1.02 [0.94 ; 1.09]	0.70
Betweenness (%)	8.1 ± 13.9	11.3 ± 23.2	0.67	0.99 [0.95 ; 1.03]	0.58	0.99 [0.94 ; 1.03]	0.51
Closeness (%)	42.2 ± 28.1	43.6 ± 19.5	1.00	1.01 [0.97 ; 1.04]	0.74	1.01 [0.98 ; 1.05]	0.49
Modularity (ranges from −1 to 1)	0.14 ± 0.20	0.28 ± 0.26	0.13	0.01 [0.01 ; 0.68]	0.03	0.01 [0.01 ; 0.61]	0.03
Diameter (ranges from 0 to 6)	1.40 ± 1.06	1.64 ± 0.76	0.40	0.63 [0.27 ; 1.49]	0.29	0.62 [0.25 ; 1.55]	0.31
Transitivity (%)	33.0 ± 36.6	58.9 ± 41.5	0.057	0.97 [0.95 ; 0.99]	0.02	0.97 [0.95 ; 0.99]	0.02
Professional proportion (%)	13.1 ± 26.7	22.2 ± 17.2	0.02	0.98 [0.95 ; 1.03]	0.54	0.99 [0.96; 1.04]	0.83
Gender homophily (%)	30.3 ± 30.0	49.4± 25.2	0.06	0.97 [0.94 ; 1.00]	0.08	0.97 [0.931 ; 1.01]	0.11
Ring homophily (%)	46.1 ± 40.7	68.4 ± 29.9	0.09	0.99 [0.96 ; 1.01]	0.22	0.99 [0.96 ; 1.01]	0.33
Largest full mesh (no.)	1.20 ± 1.93	1.64 ± 1.89	0.39	0.86 [0.57 ; 1.29]	0.45	0.88 [0.59 ; 1.33]	0.55
Isolated (no.)	3.13 ± 2.90	3.16 ± 1.80	0.40	0.98 [0.69 ; 1.40]	0.92	1.01 [0.67 ; 1.43]	0.90
Isolated dyads(no.)	0.47 ± 0.64	0.80 ± 0.96	0.33	0.57 [0.18 ; 1.83]	0.35	0.62 [0.19 ; 2.05]	0.43

[a]p values were calculated using a T-test or Mann Whitney Wilcoxon's test.
[b] Logistic regression adjusted for age, gender and body mass index
[c]Logistic regression adjusted for age, gender, body mass index and nutritional intakes
AUD: alcohol use disorder; OR: Odd ratio.

Dysbiotic AUD patients displayed a higher IL-8 level compared to non-dysbiotic patients

Inflammation being one of the main communication pathways between the gut and the brain, we measured several inflammatory parameters. IL-8 only was associated with dysbiosis adjusted for age, gender and BMI (model 1 OR=1.21, p=0.048, Supplementary Table 1). After adjustment for nutritional intake, only a trend was observed (model 2 OR=1.20, p=0.06, Supplementary Table 1).

BDNF is a key regulator of synaptic plasticity. It has been shown to be altered in depression and anxiety disorders and modulated by gut microbiota. However, no difference was observed between dysbiotic and non-dysbiotic patients (Supplementary Table 1).

The difference in gut microbiota composition was not explained by nutritional intake nor medication use

It is now well established that nutrition is one of the main factors influencing the composition of the gut microbiota [50]. The comparison of the nutritional profile of dysbiotic *versus* non-dysbiotic patients was assessed by PCA including all nutrients. Supplementary Figure 3 shows that the diet of the two groups of AUD patients was similar as the two ellipses overlapped. To confirm this result we compared the intake of each nutrient individually between the two groups (Supplementary Table 2). The analysis revealed no difference between the dysbiotic and non-dysbiotic groups in terms of energy, macronutrient or DF intakes. The consumption of beer, wine or spirit was similar between both groups of AUD patients (Supplementary Figure 3).

Commonly used non-antibiotic drugs could alter the composition and function of the gut microbiota [51, 52]. We shown no difference between dysbiotic and non-dysbiotic patients in terms of medication use (Supplementary Table 3).

Social network position is linked to gut microbiota diversity and composition

Finally, we wanted to study the link between the microbial alterations observed in dysbiotic patients and the social indexes (Figure 3). The size of the network was associated with an increased microbial richness (Figure 3A). Indeed, the number of observed species and the Chao-1 index were both significantly and positively correlated with the number of alters and the number of communities in the network (Figure 3A). A positive correlation

was also observed between the alpha-diversity indices (number of species, Shannon and Simpson) and the modularity (Figure 3A).

We then looked at correlations with specific bacteria, the ones that were significantly different between the dysbiotic and non-dysbiotic patients. We found that *Lachnoclostridium*, *Flavonifractor* and *Erysipelatoclostridium*, three bacteria that were increased in dysbiotic patients, were negatively correlated with the network size (number of alters and number of communities) while *Oscillospiraceae NK4A214* group and *Oscillospiraceae UCG_003* were positively correlated with the size of the network (Figure 3B). Concerning visual perspective task, *Oscillospiraceae UCG_003*, *Oscillospiraceae Nk4A214 group* and *Christensenellaceae R7 group* were associated with an increased alter bias while *Lachnoclostridium* and *Flavonifractor* were associated with a decrease (Figure 3B).

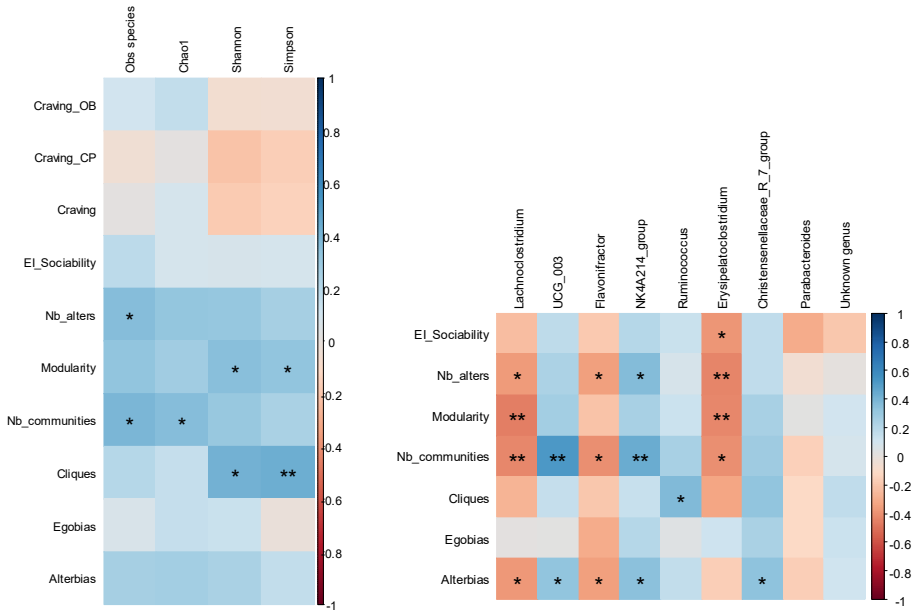

Figure 3: Partial correlations between sociability indexes and microbial data

Spearman partial correlations between the sociability indexes and A. α diversity and B. microbial genera which were different between dysbiotic and non dysbiotic AUD subjects. Correlations were adjusted for age, gender and body mass index. *p<0.05, **p<0.01,***p<0.001. EI, emotional intelligence.

3.5 Discussion

This work dissected the link between gut dysbiosis and various social parameters in AUD patients. We and other have previously shown that only a subgroup of patients had alterations in gut microbiota composition, without having unravelled which factors could explain microbiome variations in this context. In the population of AUD patients studied in this paper, those with an altered gut microbiota were younger, thinner and had a higher alcohol craving score than patients with a gut microbiota similar to HS, the so-called non-dysbiotic AUD patients. Regarding the biological parameters, only IL-8 levels were significantly different between dysbiotic and non-dysbiotic AUD patients. We also demonstrated that dysbiotic patients displayed a lower sociability score, a less spontaneous tendency to adopt the point of view of others, and a smaller and less connected social network. No differences were observed between the two groups of patients in terms of nutritional intake or medication use.

In our population of AUD patients, we observed that approximately 35% of patients were dysbiotics. This proportion was consistent with the proportion of dysbiotic AUD patients described in the literature [35, 53]. A lower bacterial diversity was observed in subjects with an altered microbiota compared to non-dysbiotic or healthy subjects. Decreased alpha-diversity was considered an indicator of an unhealthy microbiota and has been associated with various chronic diseases such as obesity and diabetes but also with unhealthy diet habits [54]. Regarding the latter, no differences in dietary habits were observed between the two groups of patients. We also found that dysbiotic patients exhibited a higher relative abundance of *Parabacteroides*, *Lachnoclostridium*, *Flavonifractor* and *Erysipelatoclostridium* compared to non-dysbiotic and HS groups. Despite contradictory results between studies, *Parabacteroides*, *Flavonifractor* and *Lachnoclostridium* have been shown to be increased in ASD, bipolar disorders, schizophrenia or major depressive disorders compared to HS [55–61]. On the contrary, dysbiotic patients displayed a gut microbiota poor in *Ruminococcus*, *Christensenellaceae R7 group*, *Oscillospiraceae NK4A214 group* and *Oscillospiraceae UCG-003* compared to healthy and non-dysbiotic subjects. The decrease in *Ruminococcus* was already observed in AUD patients with high intestinal permeability [35]. *Ruminococcus* is a butyrate producer which is known to contribute to upregulation of tight-junction protein expression, promotes the intestinal barrier function and exerts an antiinflammatory effect [62, 63] . Butyrate can also cross the blood brain barrier and influence the brain. It has been shown that butyrate has a beneficial effect on social and repetitive behaviour in an

autistic mouse model [64]. We observed that *Christensenellaceae* was almost depleted in dysbiotic subjects. This observation is particularly interesting since this bacterium family has been associated with better metabolic health [65]. It has been shown that this bacterium is more important in centenarians and could represent a marker of healthy aging [65, 66]. Furthermore, affective disorders have been associated with a higher prevalence of *Flavonifractor* genus and a lower abundance of *Christensenellaceae* that were associated with an increased oxidative stress and low-grade systemic inflammation [67, 68].

Interestingly, we demonstrated that AUD patients with an altered gut microbiota displayed a higher alcohol craving score and a lower sociability score (TEI questionnaire) meaning that they described themselves as having more difficulties in asserting themselves socially, managing the emotions of others, and being effective in communication. We also observed that dysbiotic patients had a smaller and less connected social network than non-dysbiotic AUD subjects. Furthermore, concerning the visual perspective taking, dysbiotic and non-dysbiotic patients both presented with a normal egocentric bias (i.e. the patient's viewpoint is computed and interferes at judging what the other person sees) but, surprising, the altercentric bias (i.e., the avatar's viewpoint is spontaneously computed and interferes at judging what the patient him/herself sees) was absent in the dysbiotic group whereas present in non-dysbiotic patients and in all studies involving healthy adults [44]. This highlights the loss of the spontaneous tendency of these dysbiotic patients to take into account another person's visual perspective.

The social network of alcohol-dependent patients has already been studied using the Social Network Index. Authors showed that the social network size and diversity was smaller among individuals with alcohol dependence, compared to individual with alcohol abuse or no AUD [69]. However, these findings were only based on questionnaires, while we used the sociograms methodology that proposes a much deeper description of the social network. It has already been shown that AUD patients exhibit alterations in social cognition, particularly in the recognition of emotions, which persisted after 3 months of abstinence [70–72]. AUD patients also display reduced abilities for taking the perspective of others [73]. These deficits may be at the origin of social integration problems or difficulties in maintaining satisfactory interpersonal relationships and could promote social isolation [28]. Some studies report that AUD patients have an increased sensitivity to social rejection [31]. These aspects play a prominent role in the management of AUD, as

Zywiak *et al* have shown that over 60% of relapses after detoxification can be directly attributed to emotional or interpersonal difficulties [32].

The link between gut microbiota and sociability is recent and has been highlighted by convincing results of FMT where the behavioural phenotype was transferred from donor to recipient mice [74, 75]. In our study we observed that AUD patients with lower microbial diversity have alterations in sociability as well as a smaller social network. Our observation is consistent with a similar one made in a population of 600 adults [76]. This result is difficult to interpret considering the bidirectional communication that exists between the gut microbiota and behaviour. Indeed we have seen that microbial diversity was correlated to different indices of sociability as well as some bacterial genera namely members of *Oscillospiracae* family, *Lachnoclostridium*, *Flavonifractor*, *Erysipelatoclostridium* or *Christensenellaceae R7* group. It is possible that some bacterial genera influence the brain through neural, immune or endocrine pathways [12]. In an autistic mouse model the ability of *Lactobacillus reuteri* to restore social behaviour was mediated by the vagus nerve [17]. Recently, the role of the hypothalamic-pituitary axis has been shown to be pivotal in the regulation of social behaviour in mice [77]. Some neuroactive metabolites produced by gut bacteria could also modulate social behaviour. For example p-cresol, a byproduct of bacterial fermentation of tyrosine, induces social behaviour deficit and microbial changes in mice [78] and is increased in urine or feces of autistic children [79, 80]. SCFAs or certain bile acids could also induce neurobiological changes and affect behaviour [81–83].

Studies in primates and even in humans have shown that social contact impacts the composition of the gut microbiota and that family members have a closed gut microbiota composition [84–87]. Therefore, we cannot exclude that the social behaviour and the size of the social network themselves influence the composition of the gut microbiota in AUD dysbiotic patients.

Our study presents some limitations. As previously mentioned the cross sectional data did not allow us to assess the causality. Indeed, gut microbiota can affect behaviour and behaviour can in turn influence gut microbiome composition. Our sample size is also limited and therefore further studies are needed to confirm these results. However, our study is the first to investigate the link between sociability and gut microbiota in AUD patients. One strength of our approach is the use of different complementary approaches to measure sociability including the mapping of the social support network which is original in our context. Another interest of our study is the evaluation of the influence of

medication or diet, two factors known to have a strong impact on the composition of the gut microbiota [50, 51]. Logistic regression models allowed us to exclude the influence of diet.

In conclusion, we showed that impaired social behaviour in AUD patients was associated with gut dysbiosis. These results are novel and are in agreement with recent preclinical studies suggesting a link between the gut microbiome and sociability. Knowing that social difficulties may influence the relapse in AUD patients, these results, if confirmed by other studies, could contribute to the development of strategies to modulate gut microbiota or improve social cognition in AUD patients.

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Conflict of interest

The authors declare no competing interests.

Data availability statement

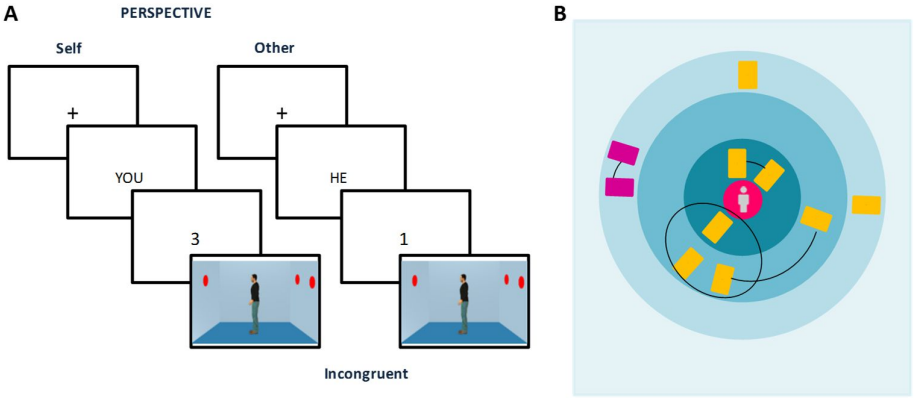
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The accession number for the raw data generated with the 16S rRNA gene sequencing reported in this paper is BioProject PRJNA745947 (SRA) and are available here https://www.ncbi.nlm.nih.gov/bioproject/PRJNA745947/.

Authors' contributions

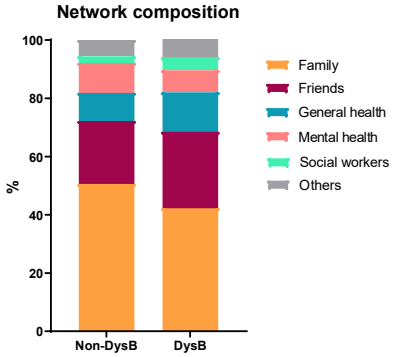
Conceptualization & design: PS, SL, NMD, PdT, AMN.
Data curation: CA, SL, PdT, NMD, AMN, PS.
Formal analysis: CA, SL, HB, HG.
Funding acquisition: NMD, PdT, PS.
Investigation: CA, SL, PS.

Methodology: SL, NMD, PdT, PS, AMN, CA, DS, HB, HG, QL, VL, PN.
Project administration: AMN, PS, SL, NMD, PdT.
Resources: NMD, PdT, PS.
Software: CA, HB, HG, QL.
Supervision: PdT, NMD, SL ,PS.
Validation: SL, PdT, NMD, PS.
Writing original draft: CA, SL, PdT, QL, HB.
Writing review & editing: CA, SL, PdT, NMD, HB, DS, HG, PN, VL, PS, QL, AMN.

3.6 Supplemental information



Supplementary Figure 1: Illustration of visual perspective-taking task and sociogram.
A. Illustration of two trials of the incongruent perspectives/ self or other perspective conditions in the visual perspective-taking task. In the first trial, participants had to judge whether or not they saw 3 discs. In the second trial, participants had to judge whether the avatar did or did not see 1 disc.
B. Illustration of the sociogram. The pink circle in the center represents the patient. The different concentric circles represent the different levels of support (from most important [closest to the patient] to least important).

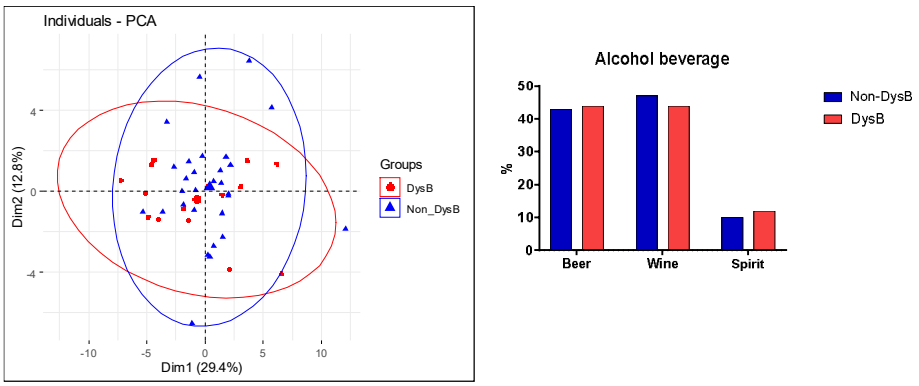


Supplementary Figure 2: Social network composition of Dysbiotic and non-dysbiotic AUD patients

Supplementary Table 1: Biological parameters of AUD patients according to gut dysbiosis

	Dysbiotic n=16	Non Dysbiotic n=30		Model 1[b]		Model 2[c]	
	Mean± SD	Mean± SD	p [a]	OR	p	OR	p
Inflammation							
TNFα (ng/ml)	2.8 ± 1.4	3.5 ± 2.1	0.25	0.93 [0.61 ; 1.41]	0.74	1.02 [0.67 ; 1.56]	0.92
IL-6 (ng/ml)	3.8 ± 2.2	5.0 ± 3.3	0.22	0.94 [0.68 ; 1.29]	0.70	0.99 [0.70 ; 1.39]	0.94
IL-18 (ng/ml)	547.0 ± 289.0	543.1 ± 230.4	0.73	1.00 [0.99 ; 1.00]	0.64	1.00 [0.99 ; 1.00]	0.69
IFNγ(ng/ml)	123.4 ± 121.2	172.4 ± 152.5	0.14	1.00 [0.99 ; 1.00]	0.55	1.00 [0.99 ; 1.01]	0.98
IL-8 (ng/ml)	8.0 ± 5.0	6.3 ± 3.4	0.41	1.21 [1.01 ; 1.46]	0.048	1.20 [0.99 ; 1.46]	0.06
MCP-1 (ng/ml)	231.9 ± 70.3	277.2 ± 90.5	0.07	0.99 [0.98 ; 1.01]	0.39	1.00 [0.98 ; 1.01]	0.57
IL-10 (ng/ml)	1.8 ± 1.3	2.0 ± 1.3	0.62	1.06 [0.56 ; 2.01]	0.85	1.41 [0.67 ; 2.98]	0.36
Growth factor							
BDNF (ng/ml)	38.7 ± 16.1	43.7 ± 25.4	0.84	0.98 [0.95 ; 1.02]	0.40	0.98 [0.95 ; 1.02]	0.42

[a]p values were calculated using a T-test or Mann Whitney Wilcoxon's test.
[b] Logistic regression adjusted for age, gender and body mass index.
[c]Logistic regression adjusted for age, gender, body mass index and nutritional intakes.
ALT: alanine aminotransferase; AST:aspartate aminotransférase; AUD: alcohol use disorder; BDNF: brain-derived neurotrophic factor; CAP: control attenuation parameter; CK18-M65; Serum cytokeratin 18 ; sCD14: soluble CD14; GLP-1: Glucagon-like peptide-1; LBP: Lipopolysaccharide Binding Protein; OR: Odd ratio; PGRP: peptidoglycan recognition proteins; PYY: peptide YY.



Supplementary Figure 3: Principal component analysis (PCA) with the macronutrients.
N=46. (A) Individuals plot with confidence ellipses. (B) Comparison of the principal types of alcohol beverage consumed according to gut dysbiosis. Alcohol consumption was calculated by using the time-line follow-back approach and is based on the week prior to the hospitalization.

Supplementary Table 2: Energy and nutritional intake of AUD patients according to gut dysbiosis

	Dysbiotic patients n=16	Non-Dysbiotic patients n=30	p[a]	Model 1[b]	
	Mean± SD	Mean± SD		OR	P
Energy intake (Kcal/d)	2396.6 ± 989.6	2343.0 ± 623.2	0.50	1.00 [0.99 ; 1.01]	0.29
Macronutrients (g/d)					
Alcohol	133.6 ± 78.6	124 ± 51.3	0.78	1.01 [0.99 ; 1.02]	0.11
Protein	57.6 ± 20.2	67.5 ± 19.8	0.11	0.96 [0.91 ; 1.01]	0.09
Carbohydrates	201 ± 113.9	188.9 ± 98.2	0.62	1.00 [0.99 ; 1.01]	0.69
Added suggar	114.1 ± 87.9	103.4 ± 82.7	0.83	1.00 [0.99 ; 1.01]	0.78
Fats	47.5 ± 31.1	49.9 ± 20.5	0.50	0.99 [0.97 ; 1.03]	0.91
Cholesterol	135.4 ± 76.4	189.0 ± 103.0	0.07	0.99 [0.98 ; 1.01]	0.07
Ratio n-6/n-3 PUFAs	7.7 ± 5.6	6.1 ± 4	0.30	1.06 [0.90 ; 1.24]	0.47
Fiber intake					
Total DF (g/d)	10.8 ± 8.3	14.2 ± 7.8	0.11	0.92 [0.82 ; 1.03]	0.16
Insoluble DF (g/d)	5.5 ± 4	7.3 ± 4.8	0.13	0.86 [0.70 ; 1.06]	0.16
Soluble DF (g/d)	3.2 ± 2.1	4.1 ± 2.4	0.33	0.75 [0.50 ; 1.12]	0.16
FOS (g/d)	0.97 ± 1.03	0.92 ± 0.97	0.92	1.24 [0.55; 2.78]	0.60
GOS (g/d)	0.24 ± 0.49	0.21 ± 0.29	0.40	2.82 [0.92; 1.06]	0.35
Transformed food					
NOVA score	2.3 ± 0.8	2 ± 0.7	0.25	1.43 [0.47; 4.39]	0.53
TF	14.3 ± 12.9	14.5 ± 11.2	0.75	0.99 [0.92; 1.06]	0.15
UTF	33.9 ± 24.5	24.2 ± 21.1	0.17	1.01 [0.98; 1.05]	0.97

[a]p values were calculated using a T-test or Mann Whitney Wilcoxon's test.

[b] Logistic regression adjusted for age, gender and BMI.

AUD: alcohol use disorder; DF: dietary fiber; FOS: fructo-oligosaccharide; GOS: galacto-oligosaccharide; PUFAs: polyunsaturated fatty acids; TF: transformed food; UTF: ultra-transformed food

Supplementary Table 3: Description of medication use according to gut dysbiosis

	Dysbiotic n=16	Non Dysbiotic n=30	
	n (%)	n (%)	p
Anxiolytics	5 (31.3)	7 (23.3)	0.73
Antidepressants	4 (25.0)	12 (40.0)	0.31
Neuroleptics	0 (0.0)	6 (20.0)	0.08
Hypnotics	2 (12.5)	2 (6.7)	0.60
Antihypertensives	1 (6.3)	6 (20.0)	0.39
Beta-blockers	1 (6.3)	0 (0.0)	0.35
Hypolipidemic drugs	0 (0.0)	4 (13.3)	0.28
Myorelaxants	1 (6.3)	2 (6.7)	1.00
Proton pump inhibitors	2 (12.5)	7 (23.3)	0.46
Medications to decrease ethanol drinking in AUD patients	1 (6.3)	3 (10.0)	1.00

p values were calculated using Fisher's test

3.7 Reference

1. Smith KJ, Victor C. Typologies of loneliness, living alone and social isolation, and their associations with physical and mental health. Ageing & Society. 2019;39:1709–30.
2. Green MF, Horan WP, Lee J. Nonsocial and social cognition in schizophrenia: current evidence and future directions. World Psychiatry. 2019;18:146–61.
3. Weightman MJ, Air TM, Baune BT. A review of the role of social cognition in major depressive disorder. Front Psychiatry. 2014;5:179.
4. Uekermann J, Kraemer M, Abdel-Hamid M, Schimmelmann BG, Hebebrand J, Daum I, et al. Social cognition in attention-deficit hyperactivity disorder (ADHD). Neurosci Biobehav Rev. 2010;34:734–43.
5. Bora E, Pantelis C. Meta-analysis of social cognition in attention-deficit/hyperactivity disorder (ADHD): comparison with healthy controls and autistic spectrum disorder. Psychol Med. 2016;46:699–716.
6. Uekermann J, Daum I. Social cognition in alcoholism: a link to prefrontal cortex dysfunction? Addiction. 2008;103:726–35.
7. Degnan A, Berry K, Sweet D, Abel K, Crossley N, Edge D. Social networks and symptomatic and functional outcomes in schizophrenia: a systematic review and meta-analysis. Soc Psychiatry Psychiatr Epidemiol. 2018;53:873–88.
8. Kawachi I, Berkman LF. Social ties and mental health. J Urban Health. 2001;78:458–67.
9. Cobb S. Social support as a moderator of life stress. Psychosomatic Medicine. 1976;38:300–14.
10. Liebke L, Bungert M, Thome J, Hauschild S, Gescher DM, Schmahl C, et al. Loneliness, social networks, and social functioning in borderline personality disorder. Personal Disord. 2017;8:349–56.
11. Albert M, Becker T, McCrone P, Thornicroft G. Social networks and mental health service utilisation--a literature review. Int J Soc Psychiatry. 1998;44:248–66.
12. Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev. 2019;99:1877–2013.
13. Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. Microbiota and the social brain. Science. 2019;366:eaar2016.
14. Dill-McFarland KA, Tang Z-Z, Kemis JH, Kerby RL, Chen G, Palloni A, et al. Close social relationships correlate with human gut microbiota composition. Sci Rep. 2019;9:703.
15. Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell. 2016;165:1762–75.
16. Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Mol Psychiatry. 2014;19:146–8.
17. Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, et al. Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. Neuron. 2019;101:246-259.e6.
18. Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-Feki HSA, Saad K, et al. The role of probiotics in children with autism spectrum disorder: A prospective, open-label study. Nutr Neurosci. 2018;21:676–81.

19. Li Q, Han Y, Dy ABC, Hagerman RJ. The Gut Microbiota and Autism Spectrum Disorders. Frontiers in Cellular Neuroscience. 2017;11:120.
20. Szeligowski T, Yun AL, Lennox BR, Burnet PWJ. The Gut Microbiome and Schizophrenia: The Current State of the Field and Clinical Applications. Frontiers in Psychiatry. 2020;11:156.
21. Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. Biol Psychiatry. 2014;76:725–33.
22. Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology. 2011;54:562–72.
23. Bjørkhaug ST, Aanes H, Neupane SP, Bramness JG, Malvik S, Henriksen C, et al. Characterization of gut microbiota composition and functions in patients with chronic alcohol overconsumption. Gut Microbes. 2019;10:663–75.
24. WHO | Global status report on alcohol and health 2018. WHO. http://www.who.int/substance_abuse/publications/global_alcohol_report/en/. Accessed 11 Jan 2019.
25. Carbia C, Lannoy S, Maurage P, López-Caneda E, O'Riordan KJ, Dinan TG, et al. A biological framework for emotional dysregulation in alcohol misuse: from gut to brain. Mol Psychiatry. 2021;26:1098–118.
26. Le Berre A-P. Emotional processing and social cognition in alcohol use disorder. Neuropsychology. 2019;33:808–21.
27. Freeman CR, Wiers CE, Sloan ME, Zehra A, Ramirez V, Wang G-J, et al. Emotion Recognition Biases in Alcohol Use Disorder. Alcohol Clin Exp Res. 2018. https://doi.org/10.1111/acer.13802.
28. D'Hondt F, Campanella S, Kornreich C, Philippot P, Maurage P. Below and beyond the recognition of emotional facial expressions in alcohol dependence: from basic perception to social cognition. Neuropsychiatr Dis Treat. 2014;10:2177–82.
29. Maurage F, de Timary P, Tecco JM, Lechantre S, Samson D. Theory of Mind Difficulties in Patients with Alcohol Dependence: Beyond the Prefrontal Cortex Dysfunction Hypothesis. Alcoholism: Clinical and Experimental Research. 2015;39:980–8.
30. Coskunpinar A, Dir AL, Cyders MA. Multidimensionality in impulsivity and alcohol use: a meta-analysis using the UPPS model of impulsivity. Alcohol Clin Exp Res. 2013;37:1441–50.
31. Maurage P, Joassin F, Philippot P, Heeren A, Vermeulen N, Mahau P, et al. Disrupted regulation of social exclusion in alcohol-dependence: an fMRI study. Neuropsychopharmacology. 2012;37:2067–75.
32. Zywiak WH, Westerberg VS, Connors GJ, Maisto SA. Exploratory findings from the Reasons for Drinking Questionnaire. Journal of Substance Abuse Treatment. 2003;25:287–92.
33. Morris EP, Stewart SH, Ham LS. The relationship between social anxiety disorder and alcohol use disorders: A critical review. Clinical Psychology Review. 2005;25:734–60.
34. Brooks AT, Lòpez MM, Ranucci A, Krumlauf M, Wallen GR. A Qualitative Exploration of Social Support during Treatment for Severe Alcohol Use Disorder and Recovery. Addict Behav Rep. 2017;6:76–82.

35. Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci USA. 2014;111:E4485-4493.
36. Leclercq S, Le Roy T, Furgiuele S, Coste V, Bindels LB, Leyrolle Q, et al. Gut Microbiota-Induced Changes in β-Hydroxybutyrate Metabolism Are Linked to Altered Sociability and Depression in Alcohol Use Disorder. Cell Rep. 2020;33:108238.
37. Sharon G, Cruz NJ, Kang D-W, Gandal MJ, Wang B, Kim Y-M, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell. 2019;177:1600-1618.e17.
38. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, et al. Towards standards for human fecal sample processing in metagenomic studies. Nat Biotechnol. 2017;35:1069–76.
39. Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, Mikolajczak M, et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. Brain Behav Immun. 2012;26:911–8.
40. Foa EB, Cashman L, Jaycox L, Perry K. The validation of a self-report measure of posttraumatic stress disorder: The Posttraumatic Diagnostic Scale. Psychological Assessment. 1997;9:445–51.
41. Mikolajczak M, Luminet O, Leroy C, Roy E. Psychometric properties of the Trait Emotional Intelligence Questionnaire: factor structure, reliability, construct, and incremental validity in a French-speaking population. J Pers Assess. 2007;88:338–53.
42. Whelan DC, Zelenski JM. Experimental Evidence That Positive Moods Cause Sociability. Social Psychological and Personality Science. 2012;3:430–7.
43. Amadieu C, Leclercq S, Coste V, Thijssen V, Neyrinck AM, Bindels LB, et al. Dietary fiber deficiency as a component of malnutrition associated with psychological alterations in alcohol use disorder. Clinical Nutrition. 2021;40:2673–82.
44. Samson D, Apperly IA, Braithwaite JJ, Andrews BJ, Bodley Scott SE. Seeing it their way: evidence for rapid and involuntary computation of what other people see. J Exp Psychol Hum Percept Perform. 2010;36:1255–66.
45. Wyngaerden F, Tempels M, Feys J-L, Dubois V, Lorant V. The personal social network of psychiatric service users. Int J Soc Psychiatry. 2020;66:682–92.
46. Neyrinck AM, Nazare J-A, Rodriguez J, Jottard R, Dib S, Sothier M, et al. Development of a Repertoire and a Food Frequency Questionnaire for Estimating Dietary Fiber Intake Considering Prebiotics: Input from the FiberTAG Project. Nutrients. 2020;12:2824.
47. Segata N, Izard J, Waldron L, Gevers D, Miropolsky L, Garrett WS, et al. Metagenomic biomarker discovery and explanation. Genome Biol. 2011;12:R60.
48. Bajaj JS, Sikaroodi M, Fagan A, Heuman D, Gilles H, Gavis EA, et al. Posttraumatic stress disorder is associated with altered gut microbiota that modulates cognitive performance in veterans with cirrhosis. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2019;317:G661–9.
49. Leclercq S, Forsythe P, Bienenstock J. Posttraumatic Stress Disorder: Does the Gut Microbiome Hold the Key? Can J Psychiatry. 2016;61:204–13.

50. Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol. 2019;16:35–56.
51. Vich Vila A, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Nat Commun. 2020;11:362.
52. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, et al. Extensive impact of non-antibiotic drugs on human gut bacteria. Nature. 2018;555:623–8.
53. Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. American Journal of Physiology-Gastrointestinal and Liver Physiology. 2012;302:G966–78.
54. Fan Y, Pedersen O. Gut microbiota in human metabolic health and disease. Nat Rev Microbiol. 2021;19:55–71.
55. Spichak S, Bastiaanssen TFS, Berding K, Vlckova K, Clarke G, Dinan TG, et al. Mining microbes for mental health: Determining the role of microbial metabolic pathways in human brain health and disease. Neuroscience & Biobehavioral Reviews. 2021;125:698–761.
56. Zhai Q, Cen S, Jiang J, Zhao J, Zhang H, Chen W. Disturbance of trace element and gut microbiota profiles as indicators of autism spectrum disorder: A pilot study of Chinese children. Environmental Research. 2019;171:501–9.
57. Hu S, Li A, Huang T, Lai J, Li J, Sublette ME, et al. Gut Microbiota Changes in Patients with Bipolar Depression. Advanced Science. 2019;6:1900752.
58. Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, Natale A, et al. Gut Microbiota Features in Young Children With Autism Spectrum Disorders. Frontiers in Microbiology. 2018;9:3146.
59. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain, Behavior, and Immunity. 2015;48:186–94.
60. Laue HE, Korrick SA, Baker ER, Karagas MR, Madan JC. Prospective associations of the infant gut microbiome and microbial function with social behaviors related to autism at age 3 years. Sci Rep. 2020;10:15515.
61. Ding X, Xu Y, Zhang X, Zhang L, Duan G, Song C, et al. Gut microbiota changes in patients with autism spectrum disorders. Journal of Psychiatric Research. 2020;129:149–59.
62. Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol. 2019;16:461–78.
63. Louis P, Flint HJ. Formation of propionate and butyrate by the human colonic microbiota. Environmental Microbiology. 2017;19:29–41.
64. Kratsman N, Getselter D, Elliott E. Sodium butyrate attenuates social behavior deficits and modifies the transcription of inhibitory/excitatory genes in the frontal cortex of an autism model. Neuropharmacology. 2016;102:136–45.
65. Waters JL, Ley RE. The human gut bacteria Christensenellaceae are widespread, heritable, and associated with health. BMC Biol. 2019;17:83.
66. Biagi E, Franceschi C, Rampelli S, Severgnini M, Ostan R, Turroni S, et al. Gut Microbiota and Extreme Longevity. Curr Biol. 2016;26:1480–5.

67. Coello K, Hansen TH, Sørensen N, Ottesen NM, Miskowiak KW, Pedersen O, et al. Affective disorders impact prevalence of Flavonifractor and abundance of Christensenellaceae in gut microbiota. Progress in Neuro-Psychopharmacology and Biological Psychiatry. 2021;110:110300.
68. Vinberg M, Ottesen NM, Meluken I, Sørensen N, Pedersen O, Kessing LV, et al. Remitted affective disorders and high familial risk of affective disorders associate with aberrant intestinal microbiota. Acta Psychiatrica Scandinavica. 2019;139:174–84.
69. Mowbray O, Quinn A, Cranford JA. Social networks and alcohol use disorders: findings from a nationally representative sample. Am J Drug Alcohol Abuse. 2014;40:181–6.
70. Monnot M, Lovallo WR, Nixon SJ, Ross E. Neurological basis of deficits in affective prosody comprehension among alcoholics and fetal alcohol-exposed adults. J Neuropsychiatry Clin Neurosci. 2002;14:321–8.
71. Maurage P, Campanella S, Philippot P, Charest I, Martin S, de Timary P. Impaired emotional facial expression decoding in alcoholism is also present for emotional prosody and body postures. Alcohol Alcohol. 2009;44:476–85.
72. Foisy M-L, Kornreich C, Fobe A, D'Hondt L, Pelc I, Hanak C, et al. Impaired emotional facial expression recognition in alcohol dependence: do these deficits persist with midterm abstinence? Alcohol Clin Exp Res. 2007;31:404–10.
73. Maurage F, de Timary P, Tecco JM, Lechantre S, Samson D. Theory of Mind Difficulties in Patients with Alcohol Dependence: Beyond the Prefrontal Cortex Dysfunction Hypothesis. Alcoholism: Clinical and Experimental Research. 2015;39:980–8.
74. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. Gastroenterology. 2011;141:599-609.e3.
75. Kelly JR, Borre Y, O' Brien C, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res. 2016;82:109–18.
76. Johnson KV-A. Gut microbiome composition and diversity are related to human personality traits. Human Microbiome Journal. 2020;15:100069.
77. Wu W-L, Adame MD, Liou C-W, Barlow JT, Lai T-T, Sharon G, et al. Microbiota regulate social behaviour via stress response neurons in the brain. Nature. 2021;595:409–14.
78. Bermudez-Martin P, Becker JAJ, Caramello N, Fernandez SP, Costa-Campos R, Canaguier J, et al. The microbial metabolite p-Cresol induces autistic-like behaviors in mice by remodeling the gut microbiota. Microbiome. 2021;9:157.
79. Gabriele S, Sacco R, Cerullo S, Neri C, Urbani A, Tripi G, et al. Urinary p-cresol is elevated in young French children with autism spectrum disorder: a replication study. null. 2014;19:463–70.
80. Altieri L, Neri C, Sacco R, Curatolo P, Benvenuto A, Muratori F, et al. Urinary p-cresol is elevated in small children with severe autism spectrum disorder. Biomarkers. 2011;16:252–60.
81. Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Frontiers in Endocrinology. 2020;11:25.

82. van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol (Lond). 2018;596:4923–44.
83. McMillin M, DeMorrow S. Effects of bile acids on neurological function and disease. FASEB J. 2016;30:3658–68.
84. Tung J, Barreiro LB, Burns MB, Grenier J-C, Lynch J, Grieneisen LE, et al. Social networks predict gut microbiome composition in wild baboons. eLife. 2015;4:e05224.
85. Grieneisen LE, Livermore J, Alberts S, Tung J, Archie EA. Group Living and Male Dispersal Predict the Core Gut Microbiome in Wild Baboons. Integrative and Comparative Biology. 2017;57:770–85.
86. Song SJ, Lauber C, Costello EK, Lozupone CA, Humphrey G, Berg-Lyons D, et al. Cohabiting family members share microbiota with one another and with their dogs. eLife. 2013;2:e00458.
87. Schloss PD, Iverson KD, Petrosino JF, Schloss SJ. The dynamics of a family's gut microbiota reveal variations on a theme. Microbiome. 2014;2:25.

CHAPTER 3: Investigation of the prebiotic approach in the management of AUD patients during alcohol withdrawal

In the first chapter we have shown that AUD patients have a low dietary fiber intake that was associated with an increased anxiety and lower sociability score. We also demonstrated that patients presenting gut microbiota alterations had a higher craving score and a less complex social network.

Futhermore, we and others have previously demonstrated that inulin type fructans (ITF) supplementation was beneficial in the context of obesity and metabolic disorders. For instance, ITF supplementation improves gut barrier function, decreases serum LPS and inflammatory cytokines in genetic and nutritional models of obesity.

We hypothesised that the gut microbiota, when altered in patients with AUD, may be responsible for the behavioural impairments associated with alcohol addiction and liver alteration. We therefore wanted to modulate the gut microbiota of patients in order to regulate gut dysbiosis and to improve psychological symptoms and alcohol related liver disease.

A mix of inulin and FOS in obese women has been shown to increase *Bifidobacterium* and *Faecalibacterium prausnitzii,* two bacteria known to be decreased in AUD patients with an altered gut microbiota and more severe psychological symtoms. These bacteria were negatively correlated with serum LPS levels in obeses women. Knowing that this dietary fiber with prebiotic properties could be a interesting way to restaure dysbalance of the gut microbiota composition reported in AUD patients and could be a promising strategy to control inflammation and therefore behavioural and liver alterations in AUD patients.

This chapter is divided in two parts and has the following objectives:

1°) to evaluate the impact of inulin supplementation on gastrointestinal tolerance, mood, alcohol craving and sociability and biological markers of satiety, lipid and glucose homeostasis.

The results related to the first part of this chapter were adapted from an article accepted in Gut Microbes.

2°) to investigate the effect of inulin supplementation on liver parameters and systemic inflammation in AUD subjects. Because it has been shown that patients with more severe liver disease have a more altered gut microbiota we performed a stratified analysis in order to study the effect of inulin in this subgroup of patient with progressive alcohol liver disease.

The results related to the second part are under review in EBiomedicines.

To do so, a randomized, double-blinded, placebo-controlled trial was performed. This pilot study included 50 AUD patients assigned to receive either inulin or maltodextrin during three weeks.

A. Restoring an adequate dietary fiber intake by inulin suplementation: a pilot study showing an impact on gut microbiota and behaviour in AUD patients

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3.1 Abstract

Objective: Alcohol use disorder (AUD) is a chronic relapsing disease associated with malnutrition, metabolic disturbances and gut microbiota alterations which are correlated with the severity of psychological symptoms. This study aims at supplementing AUD patients with prebiotic fiber during alcohol withdrawal, in order to modulate the gut microbiota composition and to evaluate its effect on gastrointestinal tolerance, metabolism and patient's behavior.

Methods: A randomized, double-blind, placebo-controlled study included 50 AUD patients assigned to inulin *versus* maltodextrin daily supplementation for 17 days. Biological measurements (fecal microbial 16S rDNA sequencing, serum biology), dietary intake, validated psychological questionnaires and gastrointestinal tolerance assessment were performed before and after the intervention.

Results: Inulin significantly decreased the richness and evenness and induced changes of 8 genera ($q < 0.1$) including *Bifidobacterium* and *Bacteroides.* Prebiotic had minor effects on gastrointestinal symptoms and nutritional intakes compared to placebo. All patients showed an improvement in depression, anxiety and craving scores during alcohol withdrawal regardless of the intervention group. Interestingly, only patients treated with inulin significantly improved the sociability score and had an increased serum level of brain-derived neurotrophic factor.

Conclusions: This pilot study shows that inulin is well tolerated and modulates the gut microbiota and the social behavior in AUD patients, without further improving other psychological and biological parameters as compared to placebo.
Gut2Brain study, clinicaltrial.gov: NCT03803709
, https://clinicaltrials.gov/ct2/show/NCT03803709

Keywords: Gut-Brain axis, Alcohol use disorder, prebiotics, inulin, psychological symptoms, sociability

3.2 Introduction

Alcohol use disorder (AUD) is a major public health problem affecting 5 to 10% of the population in developed countries. AUD is associated with metabolic disturbances, nutritional imbalance and has deleterious effects on mental health [1,2]. AUD patients are prone to develop emotional and cognitive symptoms that contribute to the persistence of addictive behavior and to the risk of relapse [2]. Chronic alcohol consumption induces alterations in neurotransmission and it has been shown that alcohol consumption and appetite regulation share common neurobiological mechanisms with hormones (leptin, ghrelin) and neuromodulators (dopaminergic, opioidergic system) being involved in both eating and addictive behaviours [3–6]. However, chronic ethanol exposure impacts other systems that could interact with the brain and therefore also influence behavior. Indeed, chronic alcohol consumption is associated with alterations in the composition and function of the gut microbiota [7–9]. These changes include increased abundance of Lachnospiraceae while there is a decrease of some specific bacteria like *Bifidobacterium* and *Faecalibacterium prausnitzii* [7,10,11] . Several studies demonstrated that alterations of gut function could have an impact on cognition, mood and behaviour [12–15]. We have previously established a link between gut dysbiosis, intestinal permeability and the severity of psychological symptoms such as depression, anxiety, alcohol craving but also social impairments, suggesting the involvement of the gut-brain axis in the aetiology of AUD [7,16].

Diet is one of the main modulators of the gut microbiota composition and function [17]. AUD patients have reduced carbohydrate, protein and fat intakes, and their dietary fiber (DF) intake is also well below the recommended value [18–20]. Among DF, inulin-type fructans are interesting as they go along with the definition of prebiotics: “substrates that are selectively used by host microorganisms conferring a health benefit” meaning that they promote the growth of some specific bacteria.[21]. Inulin-type fructans are natural components present in several fruits and vegetables including wheat, onion, banana, garlic, jerusalem artichoke, chirory and leek [22]. Inulin is fermented in the colon and has been shown to promote the growth of *Bifidobacterium* and *Faecalibacterium prausnitzii* [23,24]. The effects of inulin on gut health and metabolism have been widely studied in the context of obesity and metabolic disorders. For instance, fructan supplementation improves gut barrier function, decreases serum LPS and inflammatory cytokines in preclinical and clinical studies[23,25,26]. We have also shown that DF deficiency in AUD patients is associated with gastrointestinal discomfort and psychological alterations [18].

Therefore, inulin supplementation could be an interesting approach to increase dietary fiber intake and to modulate the gut microbiota in order to improve psychological symptoms of AUD patients.

The objective of the study was to test in a randomized, placebo-controlled design the effect of enhanced dietary fiber intake, through inulin supplementation, on gut microbiota composition gastrointestinal tolerance, mood, alcohol craving and biological markers of satiety, lipid and glucose homeostasis.

3.3 Results

Study population

Among the 50 enrolled patients, 4 dropped out of the study in the placebo group and 3 in the inulin group (Figure 1). The population therefore consisted of 21 patients with complete data in the placebo group and 22 in the inulin group at T2. Compliance with the study treatment was 96% in placebo and 98% in inulin group.

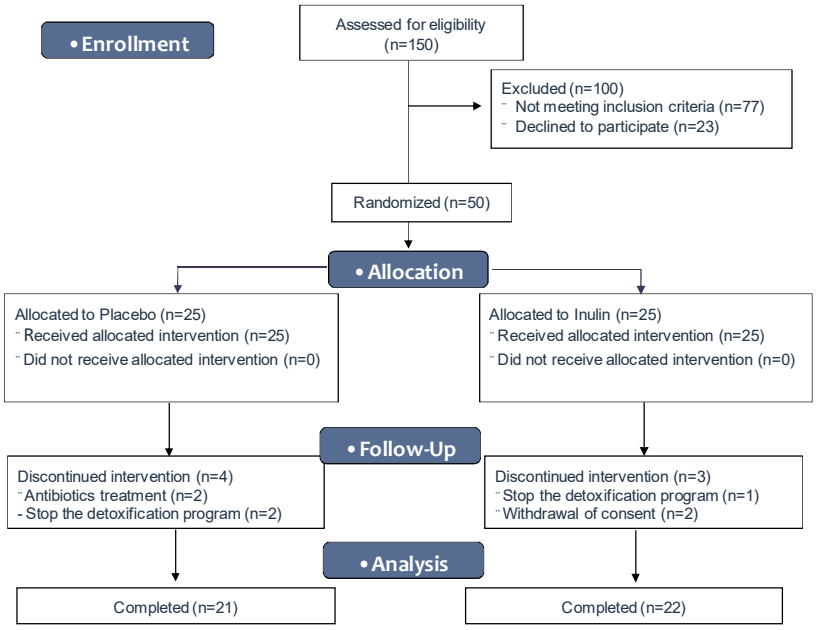

Figure 1: Flow chart of the Gut2Brain study

The sociodemographic and clinical comparisons of the two groups are presented in Table 1. Patients from both groups were similar except for the DSM-5 score (p=0.01) and the number of alcohol withdrawal cures (p=0.04). The inulin group had on average 1 more criteria in the DSM-5 classification and underwent less previous alcohol withdrawal cures (2.6 ± 2.4 in placebo *vs* 1.4 ± 0.80 in inulin group). The patients in both groups were characterized by severe AUD (DSM-5 ≥ 6 criteria) and no difference was found in terms of alcohol consumption, duration of drinking habits and age of loss of control. A gender imbalance was observed between the groups although it did not reach significance (24% of women in placebo group vs 50% in inulin group; p=0.11). Eight patients relapsed during

the second week of the program (at home) in placebo group vs 12 in inulin group (32% *vs* 48% respectively p=0.25). The subjects who relapsed had consumed alcohol on 3 out of 7 days (2.8 ± 2.1 in placebo *vs* 3.4 ± 1.9 in inulin group, p=0.45). Patients who relapsed in the placebo group consumed 79 g/d of ethanol on average *vs* 76 g/d in the inulin group (p=0.96).

Table 1: Baseline characteristics of study participants

	Placebo n=21	**Inulin n=22**	**p**
Sociodemographic characteristics			
Age (y)	48.0 ± 9.0	48.4 ± 9.8	0.90
Gender , n (%)			0.11
Male	16 (76.2)	11 (50.0)	
Female	5 (23.8)	11 (50.0)	
Marital status, n (%)			0.56
Couple/ married	9 (42.9)	7 (32.0)	
Single	8 (38.1)	12 (52.0)	
Separated/divorced	4 (19.0)	3 (16.0)	
Educational level. n (%)			0.73
Primary	2 (9.5)	2 (9.1)	
Secondary	8 (38.1)	6 (27.3)	
Superior	11 (52.4)	14 (63.6)	
Clinical examination			
Weight (kg)	71.5 ± 10.4	73.4 ± 14.7	0.64
BMI (kg/m^2)	23.5 ± 3.5	24.4 ± 3.1	0.34
MMSE score	28.8 ± 1.2	27.7 ± 2.9	0.33
Smoking, n (%)	17 (81.0)	16 (72.7)	0.72
Alcohol history			
DSM-5 AUD score	7.9 ± 2.0	9.3 ± 1.4	0.02
Age of loss of control (y)	31.6 ± 10.6	31.9 ± 12.0	0.93
Number of alcohol withdrawal cures	2.6 ± 2.4	1.4 ± 0.80	0.04
Duration of drinking habit (y)	15.7 ± 10.2	16.5 ± 11.9	0.95
Alcohol consumption (g/d)	127.9 ± 59.6	152.7 ± 90.7	0.54

Values are means ± standard deviation.N=43.

p values were calculated using a T-test or Mann Whitney Wilcoxon's test and Chi2 test or Fisher's test for categorical variables. AUD, Alcohol use disorders; Alcohol Use Disorders Test; BMI, Body mass index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; MMSE, Mini Mental State Examination.

Inulin supplementation is well tolerated by AUD patients

It has been shown that fermentable DF intake, such as inulin, could lead to bloating and discomfort in some individuals [27,28]. We therefore carefully monitored the gastrointestinal tolerance of patients throughout the intervention. Gastrointestinal pain was assessed from the first day of hospitalization and then every other day from the beginning of treatment (day 3 to day 18). The results are presented in Figure 2. There was no difference for abdominal pain, bloating, satisfaction of transit or the impact of the symptoms on daily life between placebo and inulin groups (Figure 2A-D). The frequency and the consistency of stools were comparable between both groups of treatment (Figure 2E-F). Because it has been shown that functional gastrointestinal disorders are more prevalent in women than in men, we also took into account the gender [29]. Gender adjustment did not affect the results (Supplementary Table 1).

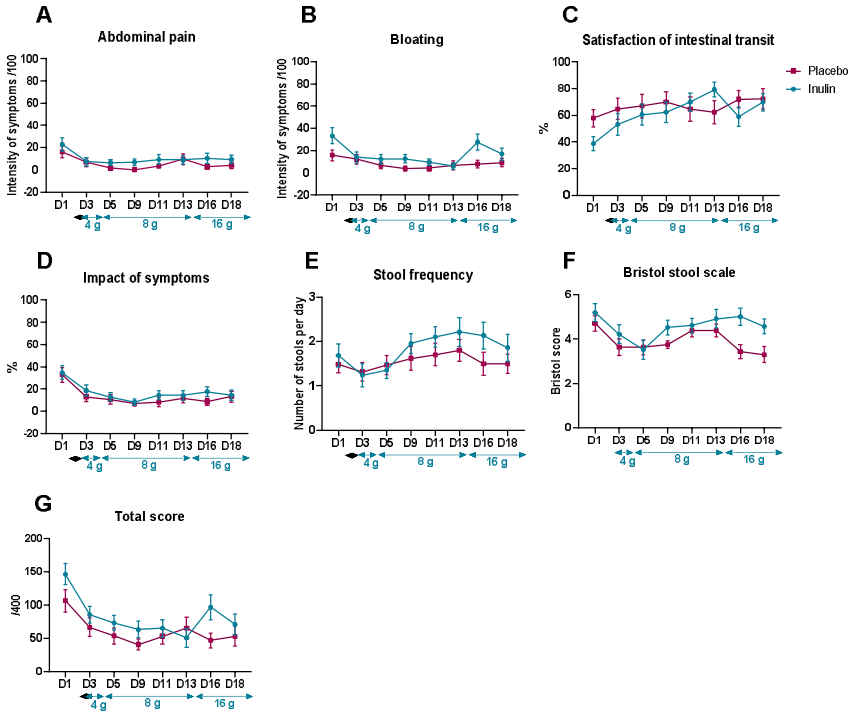

Figure 2: Changes in gastrointestinal symptoms after inulin supplementation in AUD patients

Values are mean ± SEM. Gastrointestinal symptoms including abdominal pain (A), bloating (B) and satisfaction of intestinal transit (C), impact of the symptoms on daily life (D), stool frequency (E), Bristol stool form scale (F) and (G) total tolerance score. Linear mixed models were performed for detecting the treatment effect throughout

the study. D1 represents the baseline score before the supplementation. D3 represents the first day of inulin or placebo treatment.

Inulin supplementation induces changes in gut microbiota composition at phylum, family, and genus level in AUD patients

Fecal samples were collected in 24 patients at T1 and in 19 at T2 for the placebo group and 22 patients at T1 and 19 at T2 for the inulin group. MANOVA with 9999 permutations performed on 4 beta-diversity indices (Bray-Curtis, Jaccard, Unweighted UniFrac and Weighted UniFrac) returned a non-significant p-value (Figure 3A). However, the α-diversity indexes highlighted that inulin induced a decrease in richness and evenness. Indeed, inulin supplementation decreased significantly the number of observed species as well as Chao1 and Shannon indexes (Figure 3B). The total bacteria amount, measured by qPCR, was not impacted by inulin supplementation (Figure 3C). Phylum and family levels of bacteria revealed changes in the inulin group (Figure 3D). Indeed, in this group, we observed a significant increase in Actinobacteriota phylum ($q < 0.05$) and Bifidobacteriaceae family ($q<0.05$). We also observed a significant decrease in the Bacteroidaceae family in the inulin group. At the genus level, prebiotic treatment largely increased *Bifidobacterium* and decreased *Bacteroides, Dorea* and *Ruminococcus* torques group ($q < 0.05$; Table 2).We also observed a trend towards an increase in *Faecalibacterium* relative abundance (4.8 ± 3.2% at T1 vs 6.3 ± 4.4% at T2; $p=0.055$; data not shown) after inulin supplementation. Inulin supplementation induced a significant increase in the abundance of *Bifidobacterium adolescentis* and *Bifidobacterium longum* (Supplementary Figure 1).

In the placebo group, some changes occurred (none of them at the q value), with a decrease of *Acidaminococcus*, *Sutterella*, *Oscillibacter*, *Escherichia-Shigella*, *Flavonifractor* and *Bifidobacterium* and an increase in *Lachnospiraceae ND3007* group*, Lachnospiraceae NK4A136* group*, Gordonibacter*, *Monoglobus*, *Oscillospiraceae_UCG-002* and *Oscillospiraceae_UCG-003* ($p<0.05$ and $q>0.1$; Table 2).

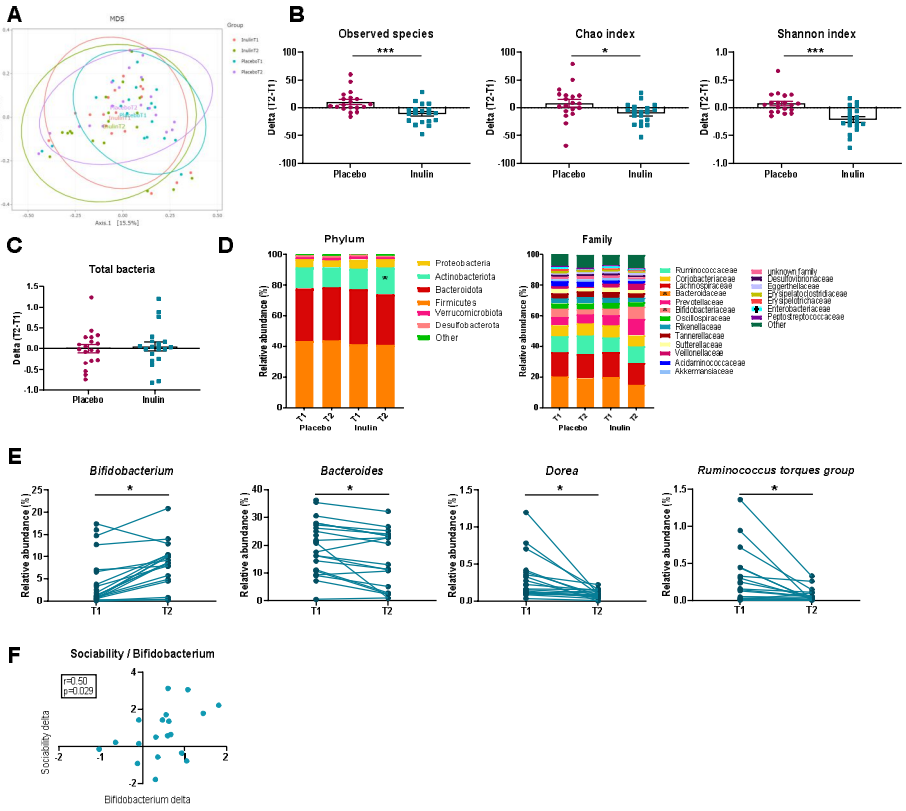

Figure 3: Effect of inulin supplementation on gut microbiota composition in alcohol use disorder patients (**A**) Principal coordinate analysis (PCoA) of the Bray Curtis index (**B**) Changes in alpha-diversity indexes: Number of observed species, Chao-1 and Shannon. *p<0.05, **p<0.01,***p<0.001 (**C**) Changes in total bacteria measured by qPCR. (**D**) Relative abundances of bacterial taxa accounting for more than 1%, at the phylum and family levels, assessed using Illumina 16S rRNA gene sequencing in AUD patients supplemented with placebo (n=19) or inulin (n=19). Wilcoxon paired tests were performed to compare the evolution from baseline in each group. P-values were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure *q<0.10 in inulin group, † q<0.10 in placebo group. (**E**) Relative abundances of genera that were significantly change between T1 and T2 in inulin group. Wilcoxon paired tests were performed and p-values were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure *q<0.1 (F) Spearman correlation between the change in sociability score (T2-T1) and the change of *Bifidobacterium* levels (T2-T1) measured by qPCR in inulin group.

Table 2: Significant changes in relative abundance of gut bacteria at the genus level in AUD patient receiving inulin or placebo for 3 weeks*

		Placebo		Inulin		p	q	p	q
		T1	T2	T1	T2	Placebo	Placebo	Inulin	Inulin
Changed in placebo	Acidaminococcus	1.059 ± 1.460	0.660 ± 1.031	0.630 ± 1.523	0.857 ± 1.854	0,039	0,203	0,418	0,617
	Lachnospiraceae ND3007 group	0.162 ± 0.154	0.210 ± 0.228	0.133 ± 0.155	0.135 ± 0.132	0,016	0,148	0,670	0,785
	Lachnospiraceae NK4A136 group	0.043 ± 0.112	0.125 ± 0.305	0.089 ± 0.259	0.124 ± 0.344	0,017	0,148	1,000	1,000
	Monoglobus	0.158 ± 0.202	0.450 ± 0.815	0.183 ± 0.241	0.157 ± 0.221	0,005	0,148	0,977	1,000
	Oscillibacter	0.344 ± 0.344	0.206 ± 0.177	0.283 ± 0.277	0.222 ± 0.196	0,049	0,218	0,077	0,268
	Escherichia Shigella	1.038 ± 2.391	0.220 ± 0.498	0.406 ± 0.825	0.464 ± 1.769	0,011	0,148	0,134	0,321
	Eubacterium siraeum group	0.351 ± 0.577	0.822 ± 1.130	0.137 ± 0.267	0.260 ± 0.524	0,026	0,148	0,754	0,850
	Sutterella	1.998 ± 1.512	1.377 ± 1.169	2.945 ± 2.086	2.086 ± 1.419	0,009	0,148	0,055	0,241
	Oscillospiraceae UCG-002	1.852 ± 2.348	2.316 ± 2.411	1.829 ± 2.166	1.291 ± 1.646	0,021	0,148	0,098	0,284
	Oscillospiraceae UCG-003	0.069 ± 0.083	0.138 ± 0.155	0.100 ± 0.106	0.061 ± 0.068	0,041	0,203	0,060	0,241
	Gordonibacter	0.056 ± 0.085	0.221 ± 0.460	0.110 ± 0.281	0.047 ± 0.075	0,025	0,148	0,295	0,495
Changed in inulin	Dorea	0.261 ± 0.228	0.212 ± 0.174	0.289 ± 0.279	0.087 ± 0.057	0,651	0,781	<0,001	0,007
	Bacteroides	20.39 ± 10.40	19.34 ± 9.77	19.83 ± 9.48	14.83 ± 10.33	0,073	0,278	0,002	0,028
	Ruminococcus torques group	0.456 ± 0.612	0.279 ± 0.330	0.255 ± 0.352	0.064 ± 0.092	0,296	0,460	0,001	0,027
	Lachnospiraceae unknown genus	0.693 ± 0.438	0.622 ± 0.348	0.787 ± 0.527	0.370 ± 0.321	0,275	0,460	<0,001	0,007
	Haemophilus	0.015 ± 0.039	0.034 ± 0.066	0.197 ± 0.792	0.601 ± 0.974	0,575	0,743	0,021	0,180
	Butyricicoccus	0.371 ± 0.243	0.460 ± 0.277	0.567 ± 0.418	0.481 ± 0.380	0,418	0,616	0,029	0,194
	Desulfovibrio	0.816 ± 1.195	0.811 ± 1.004	0.786 ± 1.233	0.552 ± 1.092	0,737	0,825	0,030	0,194
	Dialister	0.821 ± 1.976	0.434 ± 1.026	0.847 ± 1.504	2.445 ± 3.050	0,488	0,686	0,017	0,159
Opposite Changes	Bifidobacterium	5.275 ± 5.232	3.556 ± 3.263	4.300 ± 5.621	8.177 ± 4.967	0,026	0,148	<0,001	0,007
	Oscillospiraceae unknown genus	0.227 ± 0.123	0.241 ± 0.111	0.192 ± 0.114	0.151 ± 0.094	0,047	0,217	0,005	0,054
	Flavonifractor	0.259 ± 0.301	0.171 ± 0.293	0.344 ± 0.537	0.359 ± 1.060	0,019	0,148	0,029	0,194
Similar changes	Colidextribacter	0.253 ± 0.183	0.137 ± 0.132	0.244 ± 0.247	0.132 ± 0.103	0,003	0,148	0,003	0,040
	Erysipelotrichaceae UCG 003	0.985 ± 0.926	0.682 ± 0.962	0.728 ± 0.962	0.358 ± 0.560	0,026	0,148	0,011	0,118
	Lachnoclostridium	1.051 ± 0.666	0.774 ± 0.654	0.981 ± 1.162	0.791 ± 0.962	0,026	0,148	0,032	0,194

*Genus significantly modified after 17 days of treatment were identified using Wilcoxon paired test. P-values were adjusted to control the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure (q value).

Inulin treatment marginally modulates food and drink intakes upon alcohol withdrawal in AUD patients

Globally, inulin supplementation had only a minor effect on food intake (Table S2). Inulin reduced the consumption of roots and tubers despite a lack of significance in the within group comparison (β= -33.2, p=0.02; β= -34.4, p=0.03 in model 1 and 2 respectively; Table S2). Patients in inulin group increased their fruit or vegetable juice consumption compared to placebo (β= 185.8, p=0.03; β= 202.5, p=0.03 in model 1 and 2 respectively; Table S2). The placebo group increased bread (p=0.001), biscuit and cakes (p=0.02) and cheese (p=0.009) consumption while the inulin group increased dairy products (excluded cheese) consumption (p=0.03). Both groups significantly increased sweets and soda intake (+170 g/d, p=0.001 for the placebo group and +254 g/d, p=0.04 for the inulin group), the difference between groups was not significant (Table S2). The consumption of raw fruits and coffee also increased, independently of the treatment group, during the withdrawal period (fruits: +48 g/d, p=0.03 for placebo group and +42 g/d, p=0.02 for inulin group; Coffee: +0.18 L/d, p=0.006 for placebo group and +0.16 L/d, p=0.008 for inulin group; Table S2).

The Supplementary Table 3 presents the total energy and macronutrient intake of AUD patients. During the second week of the program, at home, 20 patients relapsed (8 in placebo group vs 12 in inulin group) but ethanol consumption was comparable between the two groups. Inulin had no significant effect on food related- energy intake which increased in both groups during the withdrawal period (by 61% in placebo group and by 34% in inulin group; Table S3).

Protein and fat intakes (in grams per day) significantly increased by 24% and 50%, respectively, in the placebo group, whereas those changes were not significant in the inulin group (Table S3). The detailed fat intake is presented in Table S4. Subjects in the inulin group consumed significantly less monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) than patients in the placebo group when expressed in g/d (Model 1). Only the effect on PUFA was maintained when we adjusted for the quantity of ethanol consumed during the second week of the program (Model 2; Table S4). When the results were expressed in % of fatty acids, the intake of saturated fatty acids (SFA) increased significantly in the inulin group compared to placebo while MUFA intake decreased regardless of the model considered for the analysis (Table S4).

We have already shown in a previous study that fiber intake was very low in actively drinking AUD patients [18]. During the second week of the program, at home, the two

groups increased significantly their TDF intake to reach, on average, 19 grams per day (Table S3). Twenty eight percent of patients in the placebo group had an intake of more than 25g/d during the second week of the program (Figure S2 A-B). The supplementation with 8 grams of inulin was not sufficient to reach the recommended 25 grams of fiber per day since only 38% of the patients in the inulin group had an intake higher than 25 grams when the supplementation was taken into account (Figure S2B).

Fructan, FOS and GOS intakes (in g/d) increased significantly in the placebo group and fructan and FOS intakes tend to be lower in the inulin group than in placebo (β=-0.97, p=0.08 and β= -0.76, p=0.06 respectively in model 2; Table S3). This observation is consistent with the fact that inulin-treated AUD patients ate less roots and tubers than the placebo (Table S2). Taking into account the inulin supplementation, the total amount of fructan intake averaged 9.4 g per day (1.4g from food and 8g from supplementation) in the inulin group and 2.1 g per day in the placebo group during the second week of the program.

The effect of inulin supplementation on micronutrient intakes is presented in supplementary table 5. Zinc was differentially modulated by inulin supplementation when we take into account the gender and the quantity of ethanol consumed during the second week of the program (Model 2). Indeed, zinc intake, decreased significantly in the inulin group (β= -2.7, p=0.03, Table S5). Inulin supplementation had no impact on the intake of other micronutrients.

Biological outcomes, except BDNF, are not modulated by inulin supplementation

It has been shown that inulin supplementation could affect lipid and glucose homeostasis [30,31]. We did not observe any difference between the placebo and the inulin group concerning the change (T2-T1) in plasma levels of glucose, cholesterol, triglycerides and non-esterified fatty acids (Table 3).

Gut hormones are known to be regulated by DF intake including inulin [32]. Moreover, it has been shown that AUD patients display altered levels of some gut hormones that could be related to psychological symptoms [33–35]. We therefore investigated the effect of inulin on the levels of gut hormones and gut peptides. Inulin supplementation did not affect the levels of gut peptides and gut hormones (Table 3). We did not observe any effect of prebiotic treatment on glucose metabolism as glucagon levels were not modified by the supplementation (Table 3).

We next studied the effect of inulin supplementation on BDNF, a neurotrophic factor that has been associated with various neuropsychiatric disorders [36]. Inulin increased the serum BDNF level (Model 2: β=12.7, p=0.04; Table 3). Negative correlations between the BDNF level at T2 and the alcohol craving score (presented below) were observed in the global population and in the inulin group (r= -0.37, p= 0.02 in global AUD population and r=-0.67, p<0.001 in inulin group; data not shown).

Table 3: Effect of inulin supplementation on biological parameters

	Placebo		Inulin		Difference in change from baseline M1	Difference in change from baseline M2
	T1	T2	T1	T2	β [95% CI]	β [95% CI]
Metabolism						
Glucagon(pM)	21.5 ± 11.0	17.1 ± 6.7	23.8 ± 11.2	18.9 ± 8.3	2.44 [-1.95 ; 6.83] p=0.27	1.67 [-2.52 ; 5.87] p=0.42
Glucose (mg/dL)	73.6 ± 8.2	71.7 ± 9.5	75.2 ± 12.1	74.0 ± 9.2	2.13 [-2.92 ; 7.18] p=0.40	1.96 [-3.16 ; 7.08] p=0.44
Cholesterol mmol/L	4.8 ± 0.9	4.5 ± 0.8	5.2 ± 1.1	4.6 ± 1.0	-0.14 [-0.55 ; 0.28] p=0.50	-0.17 [-0.57 ; 0.24] p=0.40
Triglycerides mmol/L	1.4 ± 1.2	1.1 ± 0.4	1.5 ± 1.1	1.4 ± 0.9	0.31 [-0.01 ; 0.63] p=0.06	0.26 [-0.03 ; 0.56] p=0.08
NEFA mmol/L	0.8 ± 0.5	0.5 ± 0.2	0.9 ± 0.5	0.7 ± 0.5	0.13 [-0.11 ; 0.38] p=0.28	0.08 [-0.16 ; 0.31] p=0.52
Gut Hormones/peptides						
Active GLP-1 (pM)	0.69 ± 0.57	0.38 ± 0.27*	0.65 ± 0.39	0.57 ± 0.47	0.11 [-0.12;0.35] p=0.34	0.12 [-0.12 ; 0.36] p=0.31
GLP-1(pM)	29.2 ± 15.6	23.6 ± 11.8	28.6 ± 13.6	26.0 ± 11.1	-0.34 [-6.9;6.3] p=0.92	-1.58 [-7.81 ; 4.64] p=0.61
Active Ghrelin (pg/ml)	189.1 ± 124.5	152.2 ± 73.2	206.6 ± 143.6	228.8 ± 172.1	49.0 [-16.59;114.58] p=0.14	46.40 [-20.5 ; 113.3] p=0.17
Ghrelin total (pg/ml)	594.0 ± 367.8	499.7 ± 235.1	621.3 ± 346.3	564.2 ± 333.4	14.90 [-145.3 ;175.1] p=0.85	16.79 [-147.1 ; 180.71] p=0.84
Leptin/BMI	544.2 ± 545.6	490.3 ± 427.9	861.4 ± 506.3	722.9 ± 476.2*	21.43 [-142.7 ;185.6] p=0.79	2.66 [-149.8 ; 155.2] p=0.97
PYY (pg/ml)	94.4 ± 61.6	80.2 ± 35.7	79.2 ± 32.2	71.6 ± 28.7	-5.19 [-26.8 ;16.4] p=0.63	-7.73 [-29.07 ; 13.62] p=0.47
Growth factors						
BDNF (pg/ml)	41.29 ± 26.47	39.9 ± 17.6	44.7 ± 24.2	51.3 ± 17.8	10.32 [-2.2 ; 22.9] p=0.10	12.72 [0.89 ; 24.54] 0.04

*** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison. β: regression coefficient.

M1: model 1 adjusted for gender and the parameter at baseline

M2: model 2 adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program.

Inulin can modulate social behavior but does not impact mood and alcohol craving in AUD patients

Figure 4 shows the evolution of psychological symptoms between baseline and the end of the study. Depression, anxiety and alcohol craving scores decreased during alcohol withdrawal regardless of the group. The linear regression models revealed that there were no differences in changes from baseline between the placebo and the inulin group for depression, anxiety, craving and fatigue (Table 4). While the sociability score remained stable during alcohol withdrawal in the placebo group, the patients in the inulin group had an increase in the medium pleasant social activity score ($p<0.05$) which remained significant after adjustment for potential confounders ($\beta= 0.68$, $p=0.039$ in model 1 and $\beta=0.71$, $p=0.03$ in model 2; Table 4). A significant positive correlation was observed between the change of *Bifidobacterium* and the change of sociability score (Figure 3F). Inulin supplementation had no effect on fatigue (Table S6).

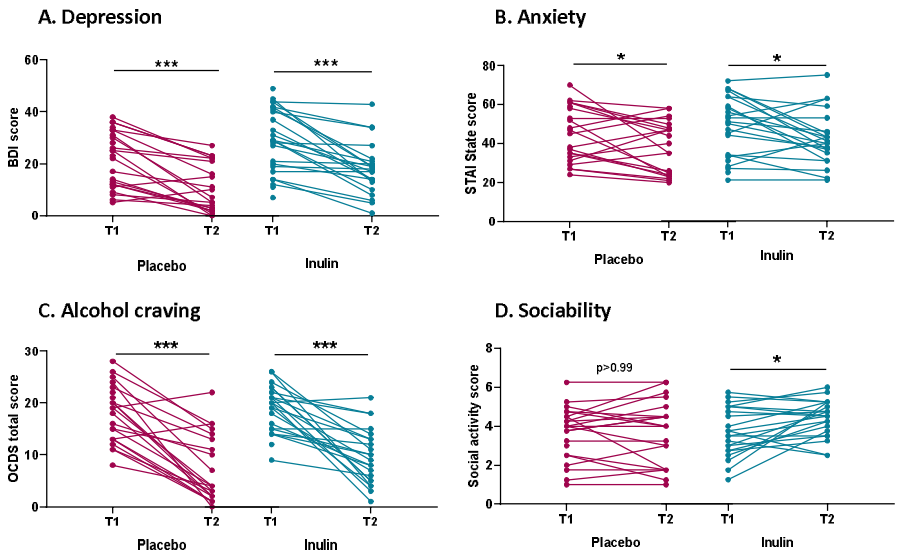

Figure 4. Effect of inulin supplementation on psychological parameters in Alcohol use disorder patients.

(A) Score of depression measured by the Beck Depression Inventory. (B) Anxiety measured by the State-Trait Anxiety Inventory (form YA). (C) Alcohol craving (total score) measured by the Obsessive-Compulsive Drinking Scale. (D) Sociability score (social medium pleasant activity score) measure by the social activity test. Wilcoxon signed ranks tests or paired T-tests were performed to analyze changes from baseline according to the distribution *p<0.05, **p<0.01,***p<0.001.

Table 4: Effect of inulin supplementation on psychological parameters

	Placebo		Inulin		Difference in change from baseline M1 β [95% CI]	Difference in change from baseline M2 β [95% CI]
	T1	T2	T1	T2		
Depression						
BDI	22.0 ± 10.7	10.6 ± 9.2***	29.0 ± 12.5	18.0 ± 10.0***	2.04 [-3.19; 7.27] p=0.44	1.56 [-3.64 ; 6.75] p=0.55
BDI suicide	1.1 ± 1.0	0.5 ± 0.9	1.6 ± 1.4	0.9 ± 1.0**	0.06 [-0.49 ; 0.61] p=0.82	0.05 [-0.53 ; 0.63] p=0.85
BDI fatigue	2.6 ± 1.4	1.0 ± 1.3***	3.4 ± 1.32	1.6 ± 1.6***	0.01 [-0.93; 0.94] p=0.99	-0.04 [-0.99; 0.91] p=0.93
Anxiety						
STAI-State	46.0 ± 13.9	38.1 ± 13.5*	47.2 ± 15.9	42.4 ± 13.6*	1.18 [6.32 ; 8.68] p=0.75	0.45 [-6.98 ; 7.89] p=0.90
Craving						
OCDS	24.3 ± 6.4	7.2 ± 6.5***	25.1 ± 5.2	9.8 ± 5.3***	2.29 [-1.55 ; 6.13] p=0.23	1.39 [-1.69 ; 4.48] p=0.37
OCDS OT	10.6 ± 3.74	4.4 ± 4.4***	10.6 ± 3.9	5.7 ± 3.6***	1.13 [-1.34 ; 3.60] p=0.36	0.54 [-1.42 ; 2.51] p=0.58
OCDS CT	13.7 ± 3.1	2.8 ± 2.3***	14.5 ± 2.4	4.0 ± 2.3***	1.10 [-0.48 ; 2.69] p=0.17	0.76 [-0.62 ; 2.14] p=0.27
Sociability						
Social high pleasant	3.8 ± 1.5	4.2 ± 1.4	3.8 ± 1.4	4.1 ± 1.2	0.23 [-0.52 ; 0.99] p=0.54	0.32 [-0.43 ; 1.08] p=0.39
Social medium pleasant	3.4 ± 1.5	3.7 ± 1.7	3.9 ± 1.3	4.4 ± 1.0*	0.68 [0.04; 1.33] p=0.039	0.71 [0.07; 1.35] p=0.03
Social low pleasant	3.0 ± 1.42	4.0 ± 1.3**	3.2 ± 1.5	4.0 ± 1.4***	0.17 [-0.50; 0.84] p=0.60	0.23 [-0.44 ; 0.91] p=0.49

Values are means ± standard deviation. β: regression coefficient.
M1:Linear regression model adjusted for gender and the parameter at baseline
M2: Linear regression model adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program.
*** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison
AUD, alcohol use disorder; BDI, Beck Depression Inventory; CT: Compulsive Thoughts; OCDS, Obsessive compulsive drinking scale; OT, Obsessive Thoughts; STAI: State-trait anxiety inventory.

3.4 Discussion

The aim of the present study was to promote dietary fiber intake prone to modulate the gut microbiota in AUD patients, by an intervention of inulin versus placebo performed during alcohol withdrawal period. Indeed, from our previous studies, we know that AUD patients are characterized by gut microbial dysbiosis, and, among nutritional disorders, by an intake of DF below the recommendation of the European Food Safety Authority and of the Belgian Health Council (25 to 30 g per day for health) [19,20].

The results obtained with the food survey, carried out during the second week of withdrawal, showed that 8 grams of inulin were not sufficient to reach the recommended 25 g/day, which reinforces the coherence of the study design that gradually increased the amount of inulin up to 16 grams per day. Sixteen grams of inulin supplementation were achieved without significant gastrointestinal side effects. Indeed we showed that inulin was well tolerated by AUD patients with no significant differences compared with placebo concerning abdominal pain, bloating or stool frequency. It has been shown in healthy individuals that inulin increased softening of feces and flatulence episodes [28,37]. In our study, AUD patients supplemented with inulin had a mean Bristol score between 4 and 5 at day 18, which corresponds to a normal score 38. Flatulence episodes were not measured in our study.

Seventeen days of inulin supplementation lead to selective modifications of the gut microbiota in AUD patients. First, we observed a decreased in α-diversity in inulin subjects compared to placebo. While several observational studies showed a positive correlation between dietary fiber intake and microbial diversity [39,40], a meta-analysis reported that dietary fiber supplementation had no effect on alpha diversity [41], likely due to the short duration of the trials (between 3 and 4 weeks). By contrast, a small number of studies have reported a decrease in species richness with inulin supplementation [42,43]. In our study, we also found a decrease in microbial diversity upon inulin exposure in AUD patients. We can conclude that supplementation with only one type of fiber in AUD patients with a poorly varied diet induces a loss of diversity. Since dietary diversity has been shown to correlate positively with microbiota diversity [44], it is likely that long-term adherence to a varied diet is more important in determining microbial diversity than supplementation with an isolated nutrient for a short period. Future long-term studies should be conducted with a combination of different fibers to expect a beneficial effect on

microbial diversity [43]. We also observed a significant increase in the relative abundance of Actinobacteria, *Bifidobacteriaceae* and *Bifidobacterium*, especially *B. adolescentis* and *B. longum*. The other *Bifidobacterium* species were either absent or marginally present, in our AUD population. *Bacteroidaceae* and *Bacteroides* decreased significantly after 17 days of inulin supplementation. These results are in line with a recent systematic review that highlights a modification of these two genera with inulin supplementation in human studies, and is in accordance with our previous data of intervention study performed in obese patients [24,45]. As Healey et al, upon inulin intervention, we found a trend toward an increase in *Faecalibacterium* and a significant decrease in *Dorea* [46]. *Ruminococcus torques* was decreased with inulin supplementation in our study. This bacterium, known to be more abundant in intestinal bowel disease (IBD) patients, is a potent mucus degrader and has been associated with a decrease in gut barrier integrity in previous studies [47]. In the placebo group, we observed changes but none of them reached the q value ($q>0.10$) meaning that 17 days of abstinence alone was not able to induce strong alterations of the gut microbiota composition. This is in line with our previous work showing a relative stability of the gut microbiota after 3 weeks of withdrawal [48,49].

Inulin supplementation had no strong impact on nutrient intake. This is probably due to the duration of the supplementation which was barely one week at the time of the nutritional survey. Abstinence alone induces an increase of all macronutrients regardless of the group. However, patients in placebo group increased their fructans, FOS and GOS intake while there were no significant changes in inulin group. Subjects in the placebo group increased their consumption of roots and tuber and bread during week 2, which could explain this result. Patients in the placebo group significantly increased their lipid intake without an increase in a specific type of lipid when looking at the intake as a percentage of total FA.

We also observed a decrease in zinc intake in the inulin group, which could be explained by a decrease in meat intake in the inulin group even if this later result was not significant. How prebiotics might affect food preferences is still unclear but it has been hypothesized that DF with prebiotic properties could act on the microbiota causing the growth of some specific bacteria [23,50] which can in turn affect eating behavior. Indeed, it has been suggested that bacteria are submitted to selective and evolutionary pressure and are therefore capable of inducing preferences for certain foods to promote their own growth [51]. Daud et al. found that oligofructose supplementation had an impact on the desire to

eat fatty, sweet and salty foods in overweight and obese population [52]. We showed the same effects on food-related behaviour upon an inulin-rich diet intervention for two weeks in healthy volunteers [28]. It is well known that AUD patients have a craving for sweetie food during the withdrawal [53,54]. However, in the present study, inulin supplementation did not reveal any impact on sweet intake in AUD patients since we observed an increase in the consumption of sweets and soda in both groups of patients. It is likely that inulin supplementation or the duration of the supplementation are not sufficient to counteract disturbances in the sensory and reward systems that control both alcohol and palatable food craving [55].

We hypothesized that the altered gut-microbiota-brain axis in AUD patients can be improved by modulating the gut microbiota composition with inulin known to promote beneficial bacteria, like Bifidobacteria. The scores of depression, anxiety and craving decreased significantly in the two groups of treatment. We did not observe any additional effect of inulin supplementation. It is well known that ethanol has a direct effect on the brain and on negative reinforcement processes [56]. Therefore, stopping alcohol has a beneficial effect on negative emotions but we have previously shown that the recovery could also be affected by gut dysbiosis [49]. Although inulin increased the level of *Bifidobacterium*, it was not associated with further improvement in psychological symptoms. No study has investigated yet the effect of inulin on cognitive symptoms or mental health of AUD patients but it has been studied in other contexts. Smith and colleagues highlighted that the acute administration of 5 g of oligofructose-enriched inulin improved wellbeing and episodic memory in healthy volunteers [57]. In obese patients, 3 months of inulin supplementation improved emotional competence and cognitive flexibility [58]. A recent study in patients suffering from coronary artery diseases has shown that the co-supplementation with 15g of inulin and *Lactobacillus rhamnosus GG* during 2 months decreased significantly depression and anxiety scores as well as lipopolysaccharide and inflammatory markers [59].

Interestingly, the subjects had an increase in serum BDNF levels when supplemented with inulin compared to placebo. An impact of prebiotics on BDNF levels has already been demonstrated in several mouse studies [60,61]. BDNF is an important neurotrophin involved in brain plasticity, the levels of which are decreased in anxiety and depression [62] and preclinical studies have highlighted that the gut microbiota could directly modulate the

brain expression of BDNF [63,64]. Even though we did not measure BDNF levels in the brain, it has been shown that peripheral blood BDNF levels are positively correlated with BDNF in the brain [65,66].

Despite the change in BDNF, we did not observe further positive effects of inulin as compared to placebo on psychological symptoms in our AUD population. This can be attributed to several factors. First, the duration of the supplementation, 17 days of supplementation may not be sufficient to observe a significant effect on behaviour. Furthermore, in this study, two factors likely modulate the behaviour of the patients: inulin supplementation and alcohol withdrawal. We have seen that abstinence alone has a strong impact on depressive symptoms, anxiety and craving, and therefore could mask an additional positive effect of prebiotics. It is also possible that the sample size was not large enough to observe a significant effect of inulin on psychological symptoms, as the study was designed to observe a bifidogenic effect. Other studies with a larger sample size are needed to confirm these results.

However, we observed an increase in the sociability subscore (social medium pleasant score) in the inulin group compared to placebo. A 6-week randomized, double blind placebo-controlled study demonstrated that a combination of Bimuno® galactooligosaccharide with a casein/gluten free diet, which increased *B. longum*, improved behavioural symptoms including sociability score in autistic children [67]. Interestingly, in our study the improvement of the sociability score was correlated with the increased *Bifidobacterium* level. The link between the gut microbiota and sociability has been demonstrated in preclinical studies [15] and we have previously shown a link between leaky gut and social impairment in AUD patients [16]. It remains unclear how microbial changes may induce some of the behavioural effects, but it has been shown that *Bifidobacterium longum NCC3001* restores anxiety-like behaviour through the vagus nerve in mice [63]. However, our results should be interpreted with caution as only one out of the six sociability sub-scores was significantly modified by inulin supplementation.

One of the limitations of our study, that may have hidden changes related to the prebiotic intervention, was the higher severity of the AUD DSM5 scores observed at baseline in the treatment group as well as the higher proportion of females. Gender is known to influence the biological parameters but also the recovery from psychological symptoms during abstinence that are usually less rapid in female than male patients [68]. Furthermore,

almost half of the patients relapsed during the intermediate week. This parameter may also modify the trajectory of symptom recovery as well as changes in the gut microbiota composition. It would have been interesting to stratify the population according to these two parameters which was impossible with our small sample size. However, fitting linear models on these two variables limited bias. Finally, the time point of the dietary data collection did not match perfectly with the fecal sample collection at T2. This makes it more difficult to interpret microbial changes in relation to nutritional intake.

In conclusion, our pilot work is the first showing that inulin supplementation is able to modulate the gut microbiota of AUD patients, although it had only a limited impact on biological outcomes or mental health. Inulin supplementation did not promote the expected effects on depression, anxiety and craving probably due to 1) the small sample size 2) the short duration of supplementation 3) the fact that alcohol withdrawal already has a strong impact on psychological symptoms. However, we have shown that bacteria modulated with inulin supplementation could potentially be involved in sociability. Other studies involving longer treatment and larger sample size are needed to investigate whether inulin could be an appropriate nutritional approach to improve psychological symptoms and the biological outcomes of patients with alcohol use disorder.

3.5 Materials and Methods

Study design

This randomized, double blind, placebo-controlled study was conducted from October 2018 to December 2019. Each subject was randomly assigned to daily intake of inulin (Inulin group) or maltodextrin (Placebo group) using the method of randomly permuted blocks (50 subjects randomized into 5 blocks). The randomization was performed via the website http://www.randomization.com by a person not involved in the study in order to ensure the double blind.
Compliance was assessed by counting the bags that were returned by subjects. Participants with a compliance of less than 80% were considered to be non-compliant.

Participant selection

A total of 50 AUD patients hospitalized for a 3-week highly standardized alcohol-detoxification program in St-Luc academic hospital, Brussels, Belgium, were enrolled on voluntary basis. This program consists in 2 weeks at the hospital (weeks 1 and 3), separated by one week outpatient care (week 2). The severity of AUD was checked by a psychiatrist using the criteria of the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)*.
Inclusion criteria were as follows: male or female, 18 to 65 years old, French speaking, and active alcohol consumption until at least 48 hours prior to admission. Patients suffering from another addiction (except tobacco), with inflammatory bowel disease or other chronic inflammatory diseases (such as rheumatoid arthritis), cancer, metabolic diseases such as obesity (BMI≥ 30 kg/m2), diabetes, bariatric surgery, and severe cognitive impairment (MMSE < 24) were excluded from the study. Patients with known cirrhosis or significant hepatic fibrosis (≥F2) detected by Fibroscan (> 7.6 kPa) at admission were also excluded from the study. Other exclusion criteria were the following: the use of antibiotics, probiotics or prebiotics within 2 months prior to enrolment and the use of non-steroidal anti-inflammatory drugs or glucocorticoids within one month prior to enrolment.
The trial protocol was published on protocols.io (dx.doi.org/10.17504/protocols.io.bvs2n6ge). The study was approved by the institutional ethics committee (N°2017/04JUL/354). All participants signed informed consent prior to

inclusion and the trial was registered in the clinicaltrials.gov registry (ClinicalTrials.gov identifier: NCT03803709).

The primary outcome of this trial was the effect of the prebiotic intervention on the gut microbiota composition. The secondary outcomes were the effect of inulin supplementation on gastrointestinal tolerance, nutritional intake, biological markers of satiety, lipid and glucose homeostasis and psychological parameters.

Dietary intervention

Inulin (Fibruline®) and maltodextrin (placebo) were kindly provided in similar opaque packaging by Cosucra (Warcoing, Belgium) to ensure the double-blind procedure. The patients were asked to dilute the powder in a hot drink (tea, coffee) or yoghurt. According to previous studies on the effect of inulin and knowing that is a digestible non fermentable carbohydrate, maltodextrin has been selected as placebo [23,45]. Inulin and maltodextrin had the same taste, odor and texture.

In order to reduce the gastrointestinal side effects, the dose of inulin or maltodextrin increased gradually from 4 to 16 g per day during the treatment (4 g from Day 3 to Day 4; 8 g from Day 5 to Day 14 and 16 g from Day 15 to Day 19 of the detoxification program). Indeed we have previously shown that 16g of inulin per day was well tolerated and had a bifidogenic effect in obese patients [23,45].

Outcomes

Gut microbiota composition

Stool samples were collected at Day 2 (T1) and at the end of the intervention (Day 19 – T2). They were collected in a sterile container and stored immediately at -20°C and then transferred to -80°C within 5 to 10 hours. Genomic DNA was extracted from the feces using a QIAamp DNA Stool Mini Kit (Qiagen, Germany), including a bead-beating step and following the protocol Q [69]. After extraction, dsDNA concentration was measured using the NanoPhotometer® Spectrophotometer (Implen, CA, USA). The composition of the gut microbiota was analysed by Illumina sequencing of the 16S rRNA gene. The V3-V4 region of the 16S rRNA gene was PCR-enriched using the primer pairs V3F_Nextera (CCTACGGGAGGCAGCAG) and Meta_V4_806R (GGACTACHVGGGTWTCTAAT). The amplicons were purified, quantified and sequenced using an Illumina Miseq to produce 2x300-bp sequencing products at the University of Minnesota Genomics Center. During the sequencing run, a quality score is assigned to each base call, using the Illumina's quality scoring methodology. The mean quality score for each sample was > 33.8. Then, the sequence reads are converted automatically to FASTQ using a bcl2fastq converter.. 16S rDNA amplicon sequences were analysed using FROGS pipeline [70]. Amplicons were filtered according to their size then clustered into OTUs using Swarm (aggregation parameter d = 1 + d = 3). Chimera were removed using VSEARCH combined with an innovative chimera cross-validation and OTUs were kept when representing more than 0.005% of the total number of sequences [71]. OTUs were classified using the reference database Silva138 16S with a pintail quality of 100 [72]. Relative abundance of each OTU was calculated after data normalization using a threshold of 33133 reads per sample.

qPCR of 16S rDNA was used to quantify the abundance of total bacteria (F: ACT-CCT-ACG-GGA-GGC-AGC-AG, R: ATT-ACC-GCG-GCT-GCT-GG) and *Bifidobacterium* spp (F: GAT-TCT-GGC-TCA-GGA-TGA-ACG-C, R:CTG-ATA-GGA-CGC-GAC-CCC-AT). PCR amplification was carried out as follows: 10 min at 95 °C, followed by 45 cycles of 3 s at 95 °C, 26 s at 58 °C or 60 °C, and 10 s at 72 °C. Detection was achieved with the QuantStudio3 instrument and software (Applied Biosystems) using the GoTaq qPCR MasterMix Plus for SYBR Assay (Promega). BSA was added to samples. Each assay was performed in duplicate in the same run. For construction of standard curves, fivefold dilution series from target species genomic DNA preparations (DSMZ, Braunshweig, Germany) were applied to the PCR.

Gastrointestinal tolerance

Gastrointestinal symptoms were measured using a French version of a self-reported questionnaire initially used to evaluate the symptoms of irritable bowel syndrome developed by gastroenterologists at the St-Luc hospital as described before [18,73]. Patients completed this questionnaire at baseline and then every other day after the beginning of supplementation.

Dietary intake

On Day 2 of alcohol withdrawal, all participants were interviewed using three non-consecutive 24-h dietary recall (related to the week before hospitalization: week 0) by a trained dietician as previously described [18]. During the second week of the program (week 2: at home) patients were asked to complete a food diary in which they registered all the food and drinks consumed during 3 defined days (two weekdays and one weekend day). The participants were instructed to specify all ingredients per eating moment: breakfast, morning snack, lunch, afternoon snack, dinner, and evening snack. Detailed guidance notes, including ingredients most often omitted (e.g. fats, added sugars, beverages) and their unit of measurement (weight and household units), were provided in the diary. To avoid bias, participants did not receive any advice from the dietician regarding their eating habits. Advice was provided "on demand" at the end of study. At the beginning of week 3, careful analysis of the food diary was performed by the dietician during a face-to-face interview with the patient. Energy and nutrient intakes were evaluated using the Nubel Pro program (Nubel asbl, Belgium) and the French food composition database (CIQUAL 2017). Dietary fibers including soluble fibers, insoluble fibers, fructans, fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS) were evaluated using a specific database from the FiberTAG project [74]. The results were expressed in quantities and in proportion of total energy intake (EI). The lipid intakes were also expressed in proportion of total fatty acids (FA).

Blood parameters

Fasting blood samples were collected at T1 and T2. Blood samples were centrifuged at 1000g for 15 min at 4°C and the plasma was frozen at -80°C in a biobank. Plasma

concentrations of gut hormones (GLP-1, leptin, ghrelin and PYY) and growth factors (Brain derived neurotrophic factor [BDNF]) were determined using the Meso Scale Discovery (MSD) U-PLEX assay (Rockville, MD, USA). Plasma triglycerides, total cholesterol and glucose were dosed by enzymatic colorimetric test (Diasys Diagnostic and System, Holzheim, Germany). Plasma non esterified fatty acid levels were assessed using a commercially available enzymatic assay (Randox Laboratories, Crumlin, UK).

Psychological symptoms assessment

At baseline (T1: day 1-2) and at the end of the supplementation (T2: day 19) all patients were tested for anxiety, depression and alcohol craving with self-reported questionnaires (French versions): the State-Trait Anxiety Inventory [STAI form YA], the Beck Depression Inventory [BDI] and the Obsessive-Compulsive Drinking Scale modified version [OCDS] as described previously [75]. The OCDS can be divided into two subscales, an 'obsessive' subscale and a 'compulsive' subscale. We used a modified version adapted to withdrawal that excluded items related to drinking. Fatigue was assessed using the Multidimensional Fatigue Inventory-20 [76] and sociability using the social situation test [77]. All these questionnaires have been described previously [18].

Statistical analysis

Statistical analyses were performed using SAS version 9.4, R studio version 3.5.1 and Graphpad Prism 8.0.

Data were presented as mean ± standard error of the mean (SEM) or mean ± standard error deviation (SD). Normality was assessed by the Shapiro–Wilk test. According to data distribution, Mann-Whitney U test or T-test were performed to compare the baseline characteristics of placebo and inulin groups.

We calculated the total dietary fiber intake for each patient and we added the 8g of inulin in the inulin group at week 2. The evolution between T1 and week 2 in each group was assessed using a paired T-test or a Wilcoxon signed-rank test. Then we calculated the proportion of patients who achieved a fiber intake of at least 25 g per day using a Fisher test.

The effect of inulin supplementation on gastrointestinal symptoms was studied using a linear mixed model with time and treatment as fixed effects and patient as random effect.

A second model adjusting for gender was performed. The gastrointestinal scores at day 1 and day 18 were then compared in each group in order to study the evolution of the symptoms between baseline and the end of the supplementation. To do so, we used paired T-test or Wilcoxon signed-rank test. Finally, the changes from baseline (D18-D1) were compared between groups using Mann-Whitney U test or T-test.

For gut microbiota analysis, phyla, families and genus with an average relative abundance superior to 0.1% were analysed. We used a Mann–Whitney U test in R to compare the relative abundance between the placebo and inulin group and the within group analyses were evaluated using a Wilcoxon paired test. The p-values were adjusted to control for the false discovery rate for multiple testing according to the Benjamini and Hochberg procedure. $q < 0.10$ was considered statistically significant.

For each psychological and biological outcome, a change variable was calculated as the difference between end-of-study (T2) and baseline measurements (T1). For nutritional data the changes variables were calculated as the difference between week 2 and week 0. As there was an imbalance between genders (24% of women in placebo group vs 50% in the inulin group) and knowing that gender can influence the evolution of psychological symptoms [68], we adjusted the linear regression models for gender and/or alcohol consumption to avoid potential bias. In order to study the effect of the withdrawal period alone, within group analyses were evaluated using a Wilcoxon signed-rank test. P values <0.05 were considered statistically significant.

Sample size was estimated using G*Power based on the bifidogenic effect of inulin [23,78]. Therefore, we estimated that a total sample size of 50 participants, with a 20% drop out during the study and 20 patients in each group completing the study provides 80% power to observe an effect size of 0.34 for the relative abundance of *Bifidobacterium* genus using a 0.05 two-sided significance level.

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Conflict of interest

The authors declare no competing interests.

Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The accession number for the raw data generated with the 16S rRNA gene sequencing reported in this paper is BioProject PRJNA745947 (SRA) and are available here https://www.ncbi.nlm.nih.gov/bioproject/PRJNA745947/ .

Authors' contributions

Conceptualization & design: SL, NMD, PdT, PS, AMN.
Data curation: CA, SL, NMD, AMN, PdT, PS.
Formal analysis: CA, VT, SL, VC, QL, NMD, AMN.
Funding acquisition: NMD, PdT, PS.
Investigation: CA, SL, PS.
Methodology: SL, NMD, PdT, PS, AMN, HP, LBB.
Project administration: SL, AMN, NMD, PdT, PS.
Resources: NMD, PdT, PS.
Software: CA, VT, VC.
Supervision: NMD, PdT, SL, PS.
Validation: SL, NMD, AMN, PdT, PS, LBB.
Writing original draft: CA, SL
Writing review & editing: CA, SL, NMD, PdT, PS, AMN, QL, LBB, HP.

All authors read and approved the final manuscript.

3.6 Supplemental information

Table S1: Evolution of gastrointestinal symptoms after inulin supplementation in AUD patients

	Placebo	Inulin	Time	Group	Time x Group
Abdominal pain			<0.001	0.09	0.90
D1	16.0 ± 25.5	22.9 ± 28.3			
D3	7.1 ± 17.1	7.3 ± 16.8			
D5	1.5 ± 4.2	6.5 ± 12.1			
D9	0.3 ± 1.3	6.9 ± 12.1			
D11	3.2 ± 8.4	9.4 ± 19.3			
D13	9.6 ± 21.9	8.9 ± 15.4			
D16	2.6 ± 8.4	10.4 ± 21.2			
D18	3.9 ± 11.5	9.4 ± 16.5			
Bloating			<0.001	0.04	0.18
D1	15.7 ± 23.2	33.4 ± 32.7			
D3	12.1 ± 18.7	14.0 ± 22.4			
D5	6.4 ± 11.3	12.1 ± 18.1			
D9	3.8 ± 10.8	12.6 ± 17.0			
D11	4.2 ± 13.2	9.5 ± 12.9			
D13	6.6 ± 18.0	5.9 ± 11.7			
D16	8.1 ± 15.7	27.7 ± 35.0			
D18	8.6 ± 14.8	16.8 ± 25.1			
Satisfaction of intestinal transit			0.004	0.65	0.13
D1	57.9 ± 31.4	38.6 ± 24.8			
D3	64.9 ± 34.2	53.1 ± 37.4			
D5	67.0 ± 36.4	60.6 ± 35.1			
D9	70.1 ± 34.0	62.5 ± 34.6			
D11	64.8 ± 40.9	70.1 ± 28.8			
D13	62.5 ± 39.9	79.4 ± 23.9			
D16	72.1 ± 27.7	59.0 ± 33.1			
D18	72.4 ± 33.0	69.9 ± 30.3			
Impact of the symptoms			<0.001	0.35	0.96
D1	32.8 ± 31.7	34.8 ± 28.4			
D3	13.1 ± 19.5	18.8 ± 22.0			
D5	10.6 ± 17.4	12.9 ± 17.2			
D9	7.0 ± 9.7	8.1 ± 13.7			
D11	8.3 ± 18.8	14.4 ± 16.7			
D13	11.5 ± 17.5	14.3 ± 19.1			
D16	8.4 ± 13.1	17.7 ± 21.4			
D18	13.1 ± 20.2	14.4 ± 22.1			
Stool frequency			0.001	0.21	0.30

D1	1.5 ± 0.9	1.7 ± 1.2			
D3	1.3 ± 0.9	1.2 ± 1.2			
D5	1.5 ± 0.9	1.4 ± 0.8			
D9	1.6 ± 1.2	2.0 ± 1.0			
D11	1.7 ± 1.1	2.1 ± 1.0			
D13	1.8 ± 1.1	2.2 ± 1.4			
D16	1.5 ± 1.1	2.1 ± 1.4			
D18	1.5 ± 0.9	1.9 ± 1.4			
Bristol score			<0.001	0.04	0.36
D1	4.7 ± 1.6	5.2 ± 1.9			
D3	3.6 ± 1.5	4.2 ± 1.6			
D5	3.6 ± 1.3	3.5 ± 1.8			
D9	3.8 ± 0.8	4.5 ± 1.5			
D11	4.4 ± 1.2	4.6 ± 1.4			
D13	4.4 ± 1.3	4.9 ± 1.7			
D16	3.4 ± 1.2	5.0 ± 1.7			
D18	3.3 ± 1.5	4.6 ± 1.4			
Total tolerance score			<0.001	0.13	0.23
D1	106.6 ± 80.5	152.5 ± 78.7			
D3	67.3 ± 60.0	84.3 ± 62.3			
D5	51.5 ± 50.4	73.5 ± 49.5			
D9	40.9 ± 39.0	63.2 ± 55.3			
D11	51.1 ± 56.2	65.1 ± 57.1			
D13	65.2 ± 75.0	50.8 ± 61.0			
D16	47.0 ± 46.5	96.8 ± 89.1			
D18	53.2 ± 62.1	70.7 ± 74.4			

Linear mixed model included the time, treatment and the interaction time x treatment as fixed effects and a random intercept. The models were adjusted for gender.

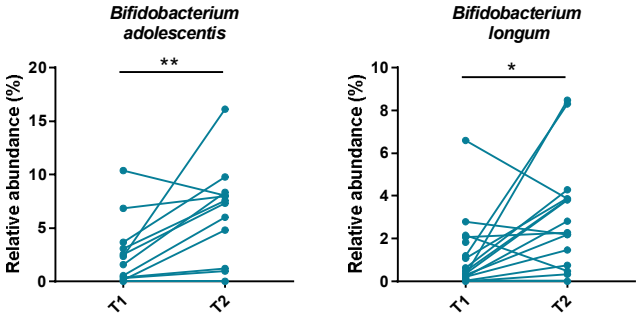

Figure S1: Evolution of *Bifidobacterium* species in inulin group after 17 days of supplementation

Relative abundance of *Bifidobacterium adolescentis* and *Bifidobacterium longum* at baseline (T1) and after 17 days of inulin supplementation (T2). Wilcoxon signed ranks tests or Paired T-tests were performed to analyze changes from baseline according to the distribution *p<0.05, **p<0.01,***p<0.001.

Table S2: Effect of inulin supplementation on food intake in AUD patients during the withdrawal period

	Placebo		Inulin		Difference in change from baseline M1	Difference in change from baseline M2
	Week 0	Week 2	Week 0	Week 2	β [95% CI]	β [95% CI]
Roots and tubers (g/d)	10.3 ± 22.5	39.0 ± 58.1	22.0 ± 36.1	9.2 ± 19.4	-33.2 [-61.7 ; -4.8] p=0.02	-34.4 [-64.4 ; -4.4] p=0.03
Bulbes and rhizome (g/d)	14.24 ± 29.3	59.5 ± 132.4	21.4 ± 40.8	25.0 ± 44.4	-16.5 [-39.4 ; 6.4] p=0.15	-14.9 [-38.8 ; 9.0] p=0.21
Other vegetables(g/d)	140.2 ± 134.9	162.0 ± 136.0	168.7 ± 174.5	163.9 ± 105.0	1.6 [-72.1 ; 75.2] p=0.97	-6.1 [-80.6 ; 68.5 p=0.87
Pulses (g/d)	8.0 ± 23.3	10.9 ± 28.5	22.8 ± 41.3	33.0 ± 81.1	10.4 [-21.9 ; 42.7] p=0.52	7.9 [-25.6 ; 41.4] p=0.63
Potatoes (g/d)	83.5 ± 87.1	74.5 ± 79.2	76.9 ± 96.1	87.9 ± 153.2	28.0 [-50.1 ; 106.0] p=0.47	19.6 [-60.2 ; 99.5] p=0.62
Fruits (g/d)	41.3 ± 73.8	89.0 ± 117.5*	69.0 ± 155.2	111.0 ± 181.1*	-2.3 [-51.8 ; 47.2] p=0.85	2.0 [-49.2 ; 53.1] p=94
Pasta, noodle (g/d)	37.2 ± 59.7	89.7 ± 88.6	49.1 ± 62.4	76.9 ± 84.3	1.09 [-53.1 ; 55.3] p=0.97	-4.71 [-57.4 ; 48.0] p=0.86
Rice (g/d)	7.5 ± 18.3	9.9 ± 31.2	16.3 ± 41.6	23.9 ± 59.8	8.3 [-15.2 ; 31.8] p=0.48	6.9 [-17.4 ; 31.2] p=0.57
Pizza, sandwich, pie (g/d)	42.2 ± 59.5	51.8 ± 60.7	44.4 ± 70.1	60.3 ± 110.5	0.39 [-57.7 ; 58.4] p=0.95	1.95 [-58.3 ; 62.2] p=0.95
Chocolate (g/d)	2.1 ± 10.2	3.0 ± 9.5	0.2 ± 1.1	1.5 ± 4.8	-0.76 [-5.7 ; 4.2] p=0.76	-0.39 [-5.6 ; 4.8] p=0.88
Biscuit, cake (g/d)	15.4 ± 30.0	53.2 ± 59.4*	7.9 ± 18.2	23.9 ± 38.3	-21.0 [-53.6 ; 11.6] p=0.20	-22.3 [56.8 ; 12.1] p=0.20
Cereal products (g/d)	1.8 ± 8.8	2.7 ± 6.8	7.5 ± 22.7	8.0 ± 12.6	3.1 [-3.1 ; 9.2] 0.32	3.9 [-2.4 ; 10.3] p=0.22
Bread (g/d)	28.3 ± 31.3	71.4 ± 61.8**	51.1 ± 48.9	59.9 ± 61.8	-9.2 [-47.5 ; 29.1] p=0.63	-6.6 [46.3 ; 33.1] p=0.74
Meat (g/d)	50.7 ± 53.1	67.6 ± 48.5	60.7 ± 60.7	42.5 ± 55.5	-24.9 [58.6 ; 8.7] p=0.14	-21.2 [56.0 ; 13.7] p=0.22
Poultry (g/d)	24.7 ± 37.2	21.3 ± 33.7	26.2 ± 48.7	27.3 ± 51.2	5.2 [-22.9 ; 33.4] p=0.71	0.60 [-27.8 ; 29.0] p=0.97
Eggs (g/d)	2.2 ± 6.0	8.3 ± 12.8	7.6 ± 19.7	5.6 ± 18.1	-5.7 [-15.1 ; 3.7] p=0.23	-6.4 [-16.3 ; 3.5] p=0.20
Fish and seafood (g/d)	25.0 ± 60.4	27.9 ± 48.6	26.0 ± 48.0	32.8 ± 46.2	6.9 [-17.3 ; 31.0] p=0.57	6.5 [-18.7 ; 31.8] p=0.60
Processed meat (g/d)	25.8 ± 44.4	40.1 ± 34.5	22.8 ± 33.9	45.1 ± 69.2	8.9 [-26.3 ; 44.0] p=0.61	8.0 [28.7 ; 44.7] p=0.66
Sweet and soda (g/d)	164.9 ± 416.1	334.7 ± 505.9**	221.4 ± 476.7	475.4 ± 792.3*	69.5 [-330.0 ; 469.0] p=0.73	25.9 [-3.81.4 ; 433.3] p=0.90
Snack and chips (g/d)	14.3 ± 53.2	5.1 ± 10.3	4.9 ± 13.9	0.8 ± 2.4	2.7 [6.6 ; 1.2] 0.17	-2.9 [-7.0 ; 1.1] 0.15
Cheese (g/d)	26.7 ± 47.5	41.6 ± 32.2**	47.7 ± 46.5	57.1 ± 54.1	3.6 [-27.6 ; 34.8] p=0.82	7.4 [-24.9 ; 39.7] p=0.65
Other dairy products (g/d)	27.6 ± 48.5	69.2 ± 110.8	31.4 ± 67.0	101.0 ± 134.4*	53.4 [-22.3 ; 129.2] p=0.16	60.7 [-17.7 ; 139.2] p=0.12
Coffee (ml/d)	218.0 ± 309.7	398.6 ± 327.6**	100.1 ± 151.9	259.6 ± 339.5**	-59.7 [-272.6 ; 153.2] p=0.74	-36.0 [-254.2 ; 182.2] p=0.57
Fruits or vegetable juice (ml/d)	66.7 ± 195.4	80.4 ± 117.8	58.9 ± 204.2	230.5 ± 378.7*	185.8 [16.1 ; 355.6] p=0.03	202.5 [26.3 ; 378.7] p=0.03
Tea (ml/d)	40.8 ± 139.1	121.1 ± 219.6	15.6 ± 48.7	40.2 ± 72.0	-44.6 [-126.5 ; 37.3] p=0.28	-39.6 [-125.0 ; 45.8] p=0.35
Olive oil (ml/d)	1.0 ± 3.2	3.5 ± 5.8†	2.0 ± 4.5	2.7 ± 3.3	-1.9 [-4.5 ; 0.7] p=0.14	-1.9 [-4.7 ; 0.8] p=0.16

Values are means ± standard deviation. . β: regression coefficient.
M1: model 1 adjusted for gender and the parameter at baseline
M2: model 2 adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program.
*** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison

Table S3: Effect of inulin supplementation on energy and macronutrient intakes in AUD patients during the withdrawal period.

	Placebo		Inulin		Difference in change from baseline M1	Difference in change from baseline M2
	Week 0	Week 2	Week 0	Week 2	β [95% CI]	β [95% CI]
Total EI (kcal/d)	2402 ± 589	2012 ± 718	2315 ± 919	1836 ± 698*	-64.6 [-488.44 ;359.22] p=0.76	-66.1 [-508.4 ; 376.2] p=0.76
Total EI (kcal/d without alcohol beverage)	1191 ± 497	1932 ± 713***	1211 ± 548	1685 ± 679**	-189.6 [-586.6 ;207.5] p=0.34	-118.7 [-538.7 ; 301.3] p=0.57
Nutrient intake (g/d)						
Alcohol	129.3 ± 63.7	8.9 ± 25.9***	125.2 ± 64.5	15.8 ± 31.1***	5.10 [-13.70 ; 23.90] p=0.59	-
Proteins	60.6 ± 18.3	75.4 ± 20.9**	65.1 ± 22.5	73.6 ± 25.5	1.02 [-14.69 ; 16.73] p=0.90	1.49 [-15.47 ; 18.46] p=0.86
Carbohydrates	205.0 ± 96.1	240.8 ± 119.3	186.2 ± 108.1	216.5 ± 100.4	1.47 [-56.31; 59.26] p=0.96	8.69 [-54.08; 71.46] p=0.78
Added sugars	124.2 ± 80.3	112.6 ± 78.0	98.2 ± 83.3	103.8 ± 60.8	9.60 [-29.68 ; 48.88] p=0.62	12.80 [-30.14 ; 55.75] p=0.55
Total dietary fibers	12.4 ± 7.9	19.2 ± 11.1**	14.3 ± 9.2	19.1 ± 9.9*	0.34 [-5.29 . 5.96] p=0.90	0.00 [-6.21; 6.22] p=0.99
Solubles	3.9 ± 2.3	5.4 ± 2.8*	4.0 ± 2.8	6.1 ± 4.1*	1.02 [-0.98 ; 3.02] p=0.31	0.54 [-1.63 ; 2.70] p=0.62
Fructans	1.0 ± 0.9	2.1 ± 2.1*	1.4 ± 1.2	1.4 ± 1.1	-0.89 [-1.93 ; 0.15] p=0.09	-0.97 [-2.07 ; 0.13] p=0.08
FOS	0.8 ± 0.7	1.7 ± 1.4*	1.0 ± 1.1	1.0 ± 0.8	-0.72 [-1.46 ; 0.02] p=0.05	-0.76 [-1.57 ; 0.05] p=0.06
GOS	0.1 ± 1.2	0.3 ± 0.5*	0.4 ± 0.5	0.3 ± 0.3	0.01 [-0.26 ; 0.27] p=0.96	-0.03 [-0.30 ; 0.24] p=0.83
Insolubles	6.5 ± 4.7	10.1 ± 6.0**	7.4 ± 5.1	10.3 ± 6.3	0.30 [-3.05 ; 3.65] p=0.86	-0.37 [-4.02 ; 3.27] p=0.84
Total fat	48.3 ± 25.2	72.6 ± 30.1***	48.1 ± 23.3	62.7 ± 31.2	-13.32 [-31.29 ; 4.65] p=0.14	-11.38 [-31.10 ; 8.34] p=0.25
Proportion of total EI (%)						
Alcohol	37.3 ± 14.2	2.6 ± 6.9***	36.7 ± 14.2	5.4 ± 10.1***	2.20 [-3.46 ; 7.86] P=0.44	-
Proteins	10.8 ± 4.3	16.0 ± 4.6***	11.9 ± 3.6	16.8 ± 4.0***	-0.26 [-2.82 ; 2.29] p=0.84	-0.27 [-2.87 ; 2.32] p=0.83
Carbohydrates	33.2 ± 12.7	46.7 ± 10.8***	31.3 ± 8.1	46.2 ± 9.7***	2.33 [-3.25 ; 7.91] p=0.40	3.85 [-1.59 ; 9.29] p=0.16
Added sugars	19.7 ± 11.0	21.7 ± 10.6	14.2 ± 8.0	22.5 ± 10.1**	3.08 [-3.39 ; 9.55] p=0.34	3.99 [-2.67 ; 10.65] p=0.23
Total fat	18.7 ± 8.3	34.7 ± 6.1***	20.0 ± 8.6	31.6 ± 9.6***	-4.62 [-9.56 ; 0.32] p=0.07	-3.76 [-8.35 ; 0.84] p=0.11

Values are means ± standard deviation. β: regression coefficient.
M1:Linear regression model adjusted for gender and the parameter at baseline
M2: Linear regression model adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program. *** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison. AUD, alcohol use disorder; EI, energy intake; FOS, fructo-oligosaccharide; GOS, galacto-oligosaccharide

Table S4: Effect of inulin supplemen tation on fatty acids intake in AUD patients during the withdrawal (n=48)

	Placebo		Inulin		Difference in change from baseline M1	Difference in change from baseline M2
	Week 0	Week 2	Week 0	Week 2	β [95% CI]	β [95% CI]
Intake (g/d)						
Total fat	48.3 ± 25.2	76.1 ± 26.0***	48.1 ± 23.3	62.7 ± 31.2	0.34 [-5.3 . 5.96] p=0.90	0.0 [-6.2; 6.2] p=0.99
SFA	19.0 ± 9.1	33.4 ± 11.9***	23.5 ± 15.0	32.5 ± 19.8	-1.17 [-12.1 ; 9.7] p=0.83	0.84 [-10.8 ; 12.5] p=0.88
MUFA	21.5 ± 17.2	30.6 ± 12.5**	17.0 ± 8.1	21.6 ± 11.3	-7.9 [-15.4 ; -0.3] p=0.04	-8.1 [-15.4; 0.3] p=0.06
PUFA	7.1 ± 3.2	11.0 ± 6.0**	6.9 ± 3.6	7.6 ± 3.1	-3.8 [-6.8 ; -0.8] p=0.02	-4.1 [-7.5 ; -0.7] p=0.02
n6-PUFA	2.9 ± 2.1	5.1 ± 4.2	3.3 ± 2.2	4.0 ± 2.5	-1.1 [-3.4 ; 1.2] p=0.35	-1.1 [-3.7 ; 1.4] p=0.37
n3-PUFA	0.7 ± 0.8	1.0 ± 1.0	0.8 ± 0.8	1.0 ± 0.7	0.02 [-0.6 ; 0.6] p=0.93	0.02 [-0.6 ; 0.7] p=0.94
EPA	0.06 ± 0.13	0.09 ± 0.10	0.06 ± 0.09	0.07 ± 0.09	-0.02 [-0.08 ; 0.04] p=0.53	-0.02 [-0.09 ; 0.04] p=0.50
DHA	0.08 ± 0.17	0.11 ± 0.14	0.08 ± 0.15	0.17 ± 0.40	0.03 [-0.16 ; 0.22] p=0.73	0.06 [-0.14 ; 0.26] p=0.55
n-6/n-3 Ratio	6.6 ± 5.1	6.3 ± 3.5	6.2 ± 3.9	4.4 ± 1.7	-1.9 [-3.7 ; -0.04] p=0.04	-1.9 [-3.9 ; 0.18] p=0.07
Trans FA	0.7 ± 0.8	1.1 ± 0.6***	0.7 ± 0.5	0.9 ± 0.7	-0.06 [-0.5 ; 0.3] p=0.77	0.02 [-0.4 ; 0.5] p=0.92
Cholesterol	0.2 ± 0.1	0.2 ± 0.1**	0.2 ± 0.1	0.2 ± 0.1	-28.3 [-94.2 ; 37.6] p=0.39	-32.7 [-105.4 ; 40.0] p=0.37
Proportion of total FA (%)						
SFA	41.4 ± 11.0	44.5 ± 8.0	47.8 ± 14.2	51.2 ± 10.3	8.8 [0.7 ;13.0] p=0.03	9.5 [3.4 ;15.6] p=0.003
MUFA	41.4 ± 9.5	39.8 ± 5.3	35.4 ± 9.2	34.1 ± 7.2	-5.3[-9.7 ; -0.9] p=0.02	-7.7 [-12.0 ; -3.4] p=<0.001
PUFA	15.9 ± 6.4	14.1 ± 3.6	15.6 ± 7.2	13.2 ± 4.6	-1.4 [-4.0 ; 1.2] p=0.29	-2.2 [-4.9 ; 0.5] p=0.10
n6-PUFA	6.7 ± 4.7	6.5 ± 3.6	7.7 ± 5.7	6.7 ± 3.6	0.19 [-2.1 ; 2.5] p=0.87	-0.42 [-2.9 ; 0.6] p=0.73
n3-PUFA	1.7 ± 1.6	1.3 ± 0.9	1.9 ± 1.8	1.8 ± 1.1	0.49 [-0.2 ; 1.2] p=0.15	0.39 [-0.3 ; 1.1] p=0.29
EPA	0.14 ± 0.25	0.11 ± 0.12	0.12 ± 0.18	0.13 ± 0.18	0.02 [-0.07 ; 0.12] p=0.63	0.01 [-0.09 ; 0.11] p=0.79
DHA	0.17 ± 0.33	0.14 ± 0.16	0.33 ± 0.86	0.33 ± 0.80	0.15 [-0.19 ; 0.49] p=0.38	0.20 [-0.16 ; 0.57] p=0.27
Trans FA	1.3 ± 0.9	1.5 ± 0.7*	1.2 ± 0.7	1.4 ± 0.5	-0.89 [-0.32 ; 0.53] p=0.63	0.05 [-0.34 ; 0.44] p=0.80

Values are means ± standard deviation. β: regression coefficient.
M1: model 1 adjusted for gender and the parameter at baseline
M2: model 2 adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program.*** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison. AUD, alcohol use disorder; DHA, Docosahexaenoic acid; EI, energy intake; EPA, Eicosapentaenoic acid; FA, Fatty acid; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids; SFA, Saturated fatty acids.

Table S5: Effect of inulin supplementation on micronutrient intake in AUD patients during the withdrawal period.

	Placebo		Inulin		Difference in change from baseline M1	Difference in change from baseline M2
	Week 0	Week 2	Week 0	Week 2	β [95% CI]	β [95% CI]
Macro-elements						
Sodium (mg/d)	2032.3 ± 932.6	2802.4 ± 899.0**	2038.2 ± 774.6	2442.9 ± 1116.3	-281.0 [-938.4;376.4] p=0.39	-255.4 [-971.5;460.7] p=0.47
Potassium (mg/d)	2976.7 ± 1002.9	2676.5 ± 1167.0	2690.1 ± 1240.0	2581.1 ± 1399.6	37.9 [-718.7;794.4] p=0.92	-107.9[-924.8 ;708.9] p=0.79
Calcium (mg/d)	649.0 ± 425.7	752.6 ± 385.1	762.1 ± 461.5	801.1 ± 529.5	-63.3 [-353.0 ;226.5] p=0.66	-0.92 [-315.8;314.0] p=0.99
Phosphorus (mg/d)	1123.5 ± 401.3	1068.5 ± 363.0	1157.2 ± 575.0	1101.2 ± 501.0	66.4 [-225.6 ; 358.4] p=0.65	67.2 [-247.1; 381.5] p=0.67
Magnesium (mg/d)	310.0 ± 97.8	270.5 ± 94.5	296.0 ± 130.5	249.9 ± 115.0	-18.1 [-87.8 ; 47.5] p=0.58	-31.3 [-100.1 ; 37.4] P=0.36
Oligo-elements						
Iron (mg/d)	11.1 ± 6.8	10.1 ± 5.1	12.7 ± 6.7	9.7 ± 8.2*	-1.5 [-5.7 ; 2.8] p=0.49	-2.4 [-5.2 ; 0.3] p=0.08
Copper (mg/d)	0.9 ± 0.7	1.0 ± 0.6	0.9 ± 0.7	0.9 ± 0.7	-0.11 [-0.5 ; 0.3] p=0.59	-0.15 [-0.6 ; 0.3] P=0.48
Zinc (mg/d)	7.9 ± 3.2	9.2 ± 3.4	10.2 ± 3.7	8.0 ± 4.1*	-2.3 [-4.8 ; 0.11] p=0.06	-2.7 [-5.1 ; -0.3] p=0.03
Selenium (µg/d)	67.3 ± 41.6	69.4 ± 30.8	59.8 ± 32.8	57.1 ± 30.5	-8.7 [-28.6 ; 11.1] p=0.38	-13.5 [-34.5 ; 7.57] p=0.20
Vitamins						
Vit. B1 (mg/d)	1.1 ± 0.7	1.1 ± 0.5	1.1 ± 0.8	1.1 ± 0.6	-0.01 [-0.3 ; 0.3] p=0.95	-0.05 [-0.42 ; 0.31] p=0.77
Vit. B2 (mg/d)	2.0 ± 2.4	1.2 ± 0.7	1.2 ± 0.7	1.4 ± 0.9	0.32 [-0.2 ; 0.8] p=0.22	0.4 [-0.2 ; 0.9] P=0.22
Vit. B12 (µg/d)	3.5 ± 2.6	3.6 ± 1.6	2.8 ± 1.7	3.1 ± 1.7	-0.5 [-1.6 ; 0.5] p=0.32	-0.7 [-1.8 ; 0.4] p=0.20
Folates (µg/d)	219.5 ± 118.3	255.8 ± 134.8	218.1 ± 160.8	261.9 ± 177.1	14.1 [-88.9 ; 117.0] p=0.78	-0.25 [-112.0 ; 111.5] p=0.99
Vit. C (mg/d)	92.4 ± 94.5	91.7 ± 83.6	71.3 ± 76.0	126.0 ± 108.2*	44.6 [-12.9 ; 102.1] p= 0.12	36.8 [-26.1 ; 99.6] p=0.24
Vit. D (µg/d)	2.6 ± 3.2	4.9 ± 6.9	4.9 ± 6.9	5.1 ± 6.7	-0.50 [-4.0 ; 3.0] p=0.77	-0.03 [-3.9 ; 3.8] p= 0.99
Vit. E (mg/d)	8.5 ± 8.2	8.6 ± 5.3	5.5 ± 5.2	8.5 ± 7.1	0.19 [-4.0 ; 4.3] p=0.93	0.50 [-4.2 ; 5.2] p=0.83

Values are means ± standard deviation. β: regression coefficient.

M1: model 1 adjusted for gender and the parameter at baseline
M2: model 2 adjusted for gender, the parameter at baseline and the quantity of ethanol consumed during the second week of the program. *** p<0.001, ** p<0.01, * p<0.05 paired T-test or Wilcoxon test : intra-group comparison. AUD, alcohol use disorder; Vit, Vitamin

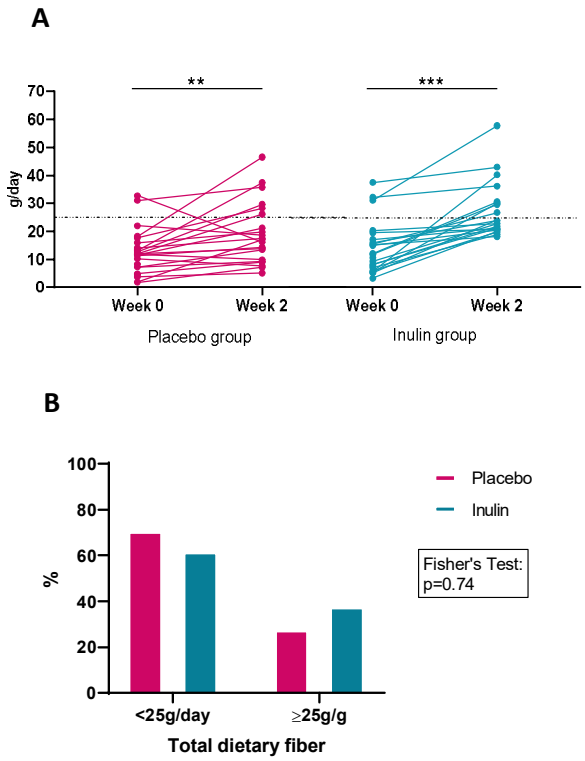

Figure S2: Evolution of total dietary fiber intake in AUD patients

A. Wilcoxon paired tests were performed to compare the evolution from baseline to week 2 (at home) in each groups **B.** Fisher's exact test was performed to compare the proportion of patients who reach 25 grams of total dietary fiber per day (Dietary guidelines- Belgian Superior health council) in each groups during the second week of the program. Eight grams of inulin have been added to the dietary fiber intake (from food) for the inulin group to take into account the supplementation during the second week of the detoxification program.

3.7 Reference

1. Baj J, Flieger W, Teresiński G, Buszewicz G, Sitarz E, Forma A, Karakuła K, Maciejewski R. Magnesium, Calcium, Potassium, Sodium, Phosphorus, Selenium, Zinc, and Chromium Levels in Alcohol Use Disorder: A Review. J Clin Med 2020; 9.
2. Carvalho AF, Heilig M, Perez A, Probst C, Rehm J. Alcohol use disorders. Lancet 2019; 394:781–92.
3. Addolorato G, Leggio L, Abenavoli L, Gasbarrini G, Alcoholism Treatment Study Group. Neurobiochemical and clinical aspects of craving in alcohol addiction: a review. Addict Behav 2005; 30:1209–24.
4. Yeomans MR, Caton S, Hetherington MM. Alcohol and food intake. Curr Opin Clin Nutr Metab Care 2003; 6:639–44.
5. Bach P, Koopmann A, Kiefer F. The Impact of Appetite-Regulating Neuropeptide Leptin on Alcohol Use, Alcohol Craving and Addictive Behavior: A Systematic Review of Preclinical and Clinical Data. Alcohol Alcohol 2020;
6. Temko JE, Bouhlal S, Farokhnia M, Lee MR, Cryan JF, Leggio L. The Microbiota, the Gut and the Brain in Eating and Alcohol Use Disorders: A 'Ménage à Trois'? Alcohol Alcohol 2017; 52:403–13.
7. Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. Biol Psychiatry 2014; 76:725–33.
8. Bajaj JS. Alcohol, liver disease and the gut microbiota. Nat Rev Gastroenterol Hepatol 2019; 16:235–46.
9. Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, Leclercq I, Fouts D, Schnabl B, Stärkel P. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. Gut Microbes 2020; 12:1782157.
10. Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L. Characterization of fecal microbial communities in patients with liver cirrhosis. Hepatology 2011; 54:562–72.
11. Bjørkhaug ST, Aanes H, Neupane SP, Bramness JG, Malvik S, Henriksen C, Skar V, Medhus AW, Valeur J. Characterization of gut microbiota composition and functions in patients with chronic alcohol overconsumption. Gut Microbes 2019; 10:663–75.
12. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, Tito RY, Schiweck C, Kurilshikov A, Joossens M, Wijmenga C, et al. The neuroactive potential of the human gut microbiota in quality of life and depression. Nature Microbiology 2019; :1.
13. Sharon G, Cruz NJ, Kang D-W, Gandal MJ, Wang B, Kim Y-M, Zink EM, Casey CP, Taylor BC, Lane CJ, et al. Human Gut Microbiota from Autism Spectrum Disorder Promote Behavioral Symptoms in Mice. Cell 2019; 177:1600-1618.e17.

14. Cryan JF, O'Riordan KJ, Sandhu K, Peterson V, Dinan TG. The gut microbiome in neurological disorders. The Lancet Neurology 2020; 19:179–94.
15. Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. Microbiota and the social brain. Science 2019; 366:eaar2016.
16. Leclercq S, Le Roy T, Furgiuele S, Coste V, Bindels LB, Leyrolle Q, Neyrinck AM, Quoilin C, Amadieu C, Petit G, et al. Gut Microbiota-Induced Changes in β-Hydroxybutyrate Metabolism Are Linked to Altered Sociability and Depression in Alcohol Use Disorder. Cell Rep 2020; 33:108238.
17. Rothschild D, Weissbrod O, Barkan E, Kurilshikov A, Korem T, Zeevi D, Costea PI, Godneva A, Kalka IN, Bar N, et al. Environment dominates over host genetics in shaping human gut microbiota. Nature 2018; 555:210–5.
18. Amadieu C, Leclercq S, Coste V, Thijssen V, Neyrinck AM, Bindels LB, Cani PD, Piessevaux H, Stärkel P, de Timary P, et al. Dietary fiber deficiency as a component of malnutrition associated with psychological alterations in alcohol use disorder. Clinical Nutrition 2021; 40:2673–82.
19. Stephen AM, Champ MM-J, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, Burley VJ. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev 2017; 30:149–90.
20. Delzenne NM, Olivares M, Neyrinck AM, Beaumont M, Kjølbæk L, Larsen TM, Benítez-Páez A, Romaní-Pérez M, Garcia-Campayo V, Bosscher D, et al. Nutritional interest of dietary fiber and prebiotics in obesity: Lessons from the MyNewGut consortium. Clinical Nutrition [Internet] 2019 [cited 2019 Aug 14]; Available from: http://www.sciencedirect.com/science/article/pii/S0261561419301153
21. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017; 14:491–502.
22. Roberfroid MB. Introducing inulin-type fructans. Br J Nutr 2005; 93:S13–25.
23. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PGB, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen J-P, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 2013; 62:1112–21.
24. Le Bastard Q, Chapelet G, Javaudin F, Lepelletier D, Batard E, Montassier E. The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. Eur J Clin Microbiol Infect Dis 2020; 39:403–13.
25. Chambers ES, Byrne CS, Morrison DJ, Murphy KG, Preston T, Tedford C, Garcia-Perez I, Fountana S, Serrano-Contreras JI, Holmes E, et al. Dietary supplementation with inulin-propionate ester or inulin improves insulin sensitivity in adults with overweight and

obesity with distinct effects on the gut microbiota, plasma metabolome and systemic inflammatory responses: a randomised cross-over trial. Gut 2019; 68:1430–8.

26. Guarner F. Studies with Inulin-Type Fructans on Intestinal Infections, Permeability, and Inflammation. The Journal of Nutrition 2007; 137:2568S-2571S.

27. Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal Tolerance of Chicory Inulin Products. Journal of the American Dietetic Association 2010; 110:865–8.

28. Hiel S, Bindels LB, Pachikian BD, Kalala G, Broers V, Zamariola G, Chang BPI, Kambashi B, Rodriguez J, Cani PD, et al. Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. The American Journal of Clinical Nutrition 2019; 109:1683–95.

29. Cain KC, Jarrett ME, Burr RL, Rosen S, Hertig VL, Heitkemper MM. Gender Differences in Gastrointestinal, Psychological, and Somatic Symptoms in Irritable Bowel Syndrome. Dig Dis Sci 2009; 54:1542–9.

30. Wang L, Yang H, Huang H, Zhang C, Zuo H-X, Xu P, Niu Y-M, Wu S-S. Inulin-type fructans supplementation improves glycemic control for the prediabetes and type 2 diabetes populations: results from a GRADE-assessed systematic review and dose–response meta-analysis of 33 randomized controlled trials. J Transl Med 2019; 17:410.

31. Ojo O, Ojo OO, Zand N, Wang X. The Effect of Dietary Fibre on Gut Microbiota, Lipid Profile, and Inflammatory Markers in Patients with Type 2 Diabetes: A Systematic Review and Meta-Analysis of Randomised Controlled Trials. Nutrients 2021; 13:1805.

32. Delzenne NM, Cani PD, Daubioul C, Neyrinck AM. Impact of inulin and oligofructose on gastrointestinal peptides. Br J Nutr 2005; 93:S157–61.

33. Morris LS, Voon V, Leggio L. Stress, Motivation, and the Gut-Brain Axis: A Focus on the Ghrelin System and Alcohol Use Disorder. Alcohol Clin Exp Res 2018; 42:1378–89.

34. Farokhnia M, Faulkner ML, Piacentino D, Lee MR, Leggio L. Ghrelin: From a gut hormone to a potential therapeutic target for alcohol use disorder. Physiology & Behavior 2019; 204:49–57.

35. de Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre P-F, Badaoui A, Leclercq S, Delzenne NM, Stärkel P. The Loss of Metabolic Control on Alcohol Drinking in Heavy Drinking Alcohol-Dependent Subjects. PLoS One [Internet] 2012 [cited 2020 Jun 25]; 7. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3392266/

36. Autry AE, Monteggia LM. Brain-Derived Neurotrophic Factor and Neuropsychiatric Disorders. Pharmacol Rev 2012; 64:238–58.

37. Micka A, Siepelmeyer A, Holz A, Theis S, Schön C. Effect of consumption of chicory inulin on bowel function in healthy subjects with constipation: a randomized, double-blind, placebo-controlled trial. International Journal of Food Sciences and Nutrition 2017; 68:82–9.

38. Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2016; 44:693–703.
39. Menni C, Jackson MA, Pallister T, Steves CJ, Spector TD, Valdes AM. Gut microbiome diversity and high-fibre intake are related to lower long-term weight gain. Int J Obes (Lond) 2017; 41:1099–105.
40. Lin D, Peters BA, Friedlander C, Freiman HJ, Goedert JJ, Sinha R, Miller G, Bernstein MA, Hayes RB, Ahn J. Association of dietary fibre intake and gut microbiota in adults. Br J Nutr 2018; 120:1014–22.
41. So D, Whelan K, Rossi M, Morrison M, Holtmann G, Kelly JT, Shanahan ER, Staudacher HM, Campbell KL. Dietary fiber intervention on gut microbiota composition in healthy adults: a systematic review and meta-analysis. Am J Clin Nutr 2018; 107:965–83.
42. Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, Verbeke K, Raes J. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017; 66:1968–74.
43. Reimer RA, Willis HJ, Tunnicliffe JM, Park H, Madsen KL, Soto-Vaca A. Inulin-type fructans and whey protein both modulate appetite but only fructans alter gut microbiota in adults with overweight/obesity: A randomized controlled trial. Mol Nutr Food Res 2017; 61:1700484.
44. Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, Harris HMB, Coakley M, Lakshminarayanan B, O'Sullivan O, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012; 488:178–84.
45. Hiel S, Gianfrancesco MA, Rodriguez J, Portheault D, Leyrolle Q, Bindels LB, Gomes da Silveira Cauduro C, Mulders MDGH, Zamariola G, Azzi A-S, et al. Link between gut microbiota and health outcomes in inulin -treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. Clinical Nutrition 2020; :S0261561420301606.
46. Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. Br J Nutr 2018; 119:176–89.
47. first 1000 cultured species of the human gastrointestinal microbiota | FEMS Microbiology Reviews | Oxford Academic [Internet]. [cited 2021 Jun 28]; Available from: https://academic.oup.com/femsre/article/38/5/996/498300?login=true
48. de Timary P, Leclercq S, Stärkel P, Delzenne N. A dysbiotic subpopulation of alcohol-dependent subjects. Gut Microbes 2015; 6:388–91.
49. Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, Windey K, Tremaroli V, Bäckhed F, Verbeke K, et al. Intestinal permeability, gut-bacterial dysbiosis,

and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci USA 2014; 111:E4485-4493.

50. Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of Bifidobacterium adolescentis and Faecalibacterium prausnitzii. Br J Nutr 2009; 101:541–50.

51. Alcock J, Maley CC, Aktipis CA. Is eating behavior manipulated by the gastrointestinal microbiota? Evolutionary pressures and potential mechanisms. BioEssays 2014; 36:940–9.

52. Daud NM, Ismail NA, Thomas EL, Fitzpatrick JA, Bell JD, Swann JR, Costabile A, Childs CE, Pedersen C, Goldstone AP, et al. The impact of oligofructose on stimulation of gut hormones, appetite regulation and adiposity. Obesity (Silver Spring) 2014; 22:1430–8.

53. Alarcon R, Tiberghien M, Trouillet R, Pelletier S, Luquiens A, Ahmed SH, Nalpas B, Alaux-Cantin S, Naassila M, Perney P. Sugar intake and craving during alcohol withdrawal in alcohol use disorder inpatients. Addiction Biology [Internet] 2020 [cited 2020 Jun 26]; Available from: https://onlinelibrary.wiley.com/doi/abs/10.1111/adb.12907

54. Stickel A, Rohdemann M, Landes T, Engel K, Banas R, Heinz A, Müller CA. Changes in Nutrition-Related Behaviors in Alcohol-Dependent Patients After Outpatient Detoxification: The Role of Chocolate. Subst Use Misuse 2016; 51:545–52.

55. Wiss DA, Avena N, Rada P. Sugar Addiction: From Evolution to Revolution. Front Psychiatry 2018; 9:545.

56. Koob GF. Negative reinforcement in drug addiction: the darkness within. Current Opinion in Neurobiology 2013; 23:559–63.

57. Smith AP, Sutherland D, Hewlett P. An Investigation of the Acute Effects of Oligofructose-Enriched Inulin on Subjective Wellbeing, Mood and Cognitive Performance. Nutrients 2015; 7:8887–96.

58. Leyrolle Q, Cserjesi R, D.G.H. Mulders M, Zamariola G, Hiel S, Gianfrancesco MA, Portheault D, Amadieu C, Bindels LB, Leclercq S, et al. Prebiotic effect on mood in obese patients is determined by the initial gut microbiota composition: A randomized, controlled trial. Brain, Behavior, and Immunity 2021; 94:289–98.

59. Moludi J, Khedmatgozar H, Nachvak SM, Abdollahzad H, Moradinazar M, Sadeghpour tabaei A. The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical trial. Nutritional Neuroscience 2021; :1–10.

60. Burokas A, Arboleya S, Moloney RD, Peterson VL, Murphy K, Clarke G, Stanton C, Dinan TG, Cryan JF. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry 2017; 82:472–87.

61. Matt SM, Allen JM, Lawson MA, Mailing LJ, Woods JA, Johnson RW. Butyrate and Dietary Soluble Fiber Improve Neuroinflammation Associated With Aging in Mice. Front Immunol 2018; 9:1832.

62. Deltheil T, Guiard BP, Cerdan J, David DJ, Tanaka KF, Repérant C, Guilloux J-P, Coudoré F, Hen R, Gardier AM. Behavioral and serotonergic consequences of decreasing or increasing hippocampus brain-derived neurotrophic factor protein levels in mice. Neuropharmacology 2008; 55:1006–14.

63. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, Deng Y, Blennerhassett P, Macri J, McCoy KD, et al. The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. Gastroenterology 2011; 141:599-609.e3.

64. Schéle E, Grahnemo L, Anesten F, Hallén A, Bäckhed F, Jansson J-O. The Gut Microbiota Reduces Leptin Sensitivity and the Expression of the Obesity-Suppressing Neuropeptides Proglucagon (Gcg) and Brain-Derived Neurotrophic Factor (Bdnf) in the Central Nervous System. Endocrinology 2013; 154:3643–51.

65. Klein AB, Williamson R, Santini MA, Clemmensen C, Ettrup A, Rios M, Knudsen GM, Aznar S. Blood BDNF concentrations reflect brain-tissue BDNF levels across species. Int J Neuropsychopharm 2011; 14:347–53.

66. Pan W, Banks WA, Fasold MB, Bluth J, Kastin AJ. Transport of brain-derived neurotrophic factor across the blood–brain barrier. Neuropharmacology 1998; 37:1553–61.

67. Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, Leigh Gibson E, Nielsen DS, Costabile A. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome 2018; 6:133.

68. Petit G, Luminet O, Cordovil de Sousa Uva M, Monhonval P, Leclercq S, Spilliaert Q, Zammit F, Maurage P, de Timary P. Gender Differences in Affects and Craving in Alcohol-Dependence: A Study During Alcohol Detoxification. Alcohol Clin Exp Res 2017; 41:421–31.

69. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen M, Hercog R, Jung F-E, et al. Towards standards for human fecal sample processing in metagenomic studies. Nat Biotechnol 2017; 35:1069–76.

70. Escudié F, Auer L, Bernard M, Mariadassou M, Cauquil L, Vidal K, Maman S, Hernandez-Raquet G, Combes S, Pascal G. FROGS: Find, Rapidly, OTUs with Galaxy Solution. Bioinformatics 2018; 34:1287–94.

71. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods 2013; 10:57–9.

72. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research 2012; 41:D590–6.

73. Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997; 11:395–402.
74. Neyrinck AM, Nazare J-A, Rodriguez J, Jottard R, Dib S, Sothier M, Berghe LVD, Alligier M, Alexiou H, Maquet V, et al. Development of a Repertoire and a Food Frequency Questionnaire for Estimating Dietary Fiber Intake Considering Prebiotics: Input from the FiberTAG Project. Nutrients 2020; 12:2824.
75. Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, Mikolajczak M, Delzenne NM, de Timary P. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. Brain Behav Immun 2012; 26:911–8.
76. Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995; 39:315–25.
77. Whelan DC, Zelenski JM. Experimental Evidence That Positive Moods Cause Sociability. Social Psychological and Personality Science 2012; 3:430–7.
78. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. Int J Food Sci Nutr 2014; 65:117–23.

B. Inulin supplementation during alcohol withdrawal in alcohol use disorder patients does not improve liver alterations: a randomized, double-blind, placebo-controlled study

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3.1 Abstract

Background & Aims: Emerging evidence highlights that targeting the gut microbiota could be an interesting approach to improve alcohol liver disease (ALD) due to its important plasticity. This study aimed to evaluate the effects of inulin supplementation on liver parameters in alcohol use disorder (AUD) patients (whole sample) and in a subpopulation with progressive ALD.

Methods: Fifty AUD patients, hospitalized for a 3-week detoxification program, were enrolled in a randomized, double-blind, placebo-controlled study and assigned to inulin *versus* placebo for 17 days. Liver damage, microbial translocation, inflammatory markers and 16S rDNA sequencing were measured at the beginning (T1) and at the end of the study (T2). The results in AUD patients were compared to 14 healthy subjects (HS).

Results: Compared to HS, AUD patients had significantly higher level of AST, ALT, CK18-M65 at T1. After alcohol cessation, transaminases but not CK18-M65 decreased but remained significantly higher at T2. sCD14 significantly decreased without improving inflammatory status. Compared to placebo, AST, ALT and IL-18 remained significantly higher in the inulin group in the whole sample at T2. In the progressive ALD subgroup, inulin supplementation lead to specific changes in the gut microbiota, including an increase in *Bifidobacterium* and a decrease of *Bacteroides*. Despite those changes, AST and ALT at T2 were higher in the inulin group compared to placebo.

Conclusions: This pilot study shows that 17 days of inulin supplementation versus placebo, even though it induces specific changes in the gut microbiota, did not alleviate liver damage in AUD patients.

Keywords: Alcohol use disorder, prebiotics, inulin, alcoholic liver disease, gut microbiota, inflammation

3.2 Introduction

Alcoholic liver disease (ALD) is one of the leading causes of chronic liver disease worldwide. Although the majority of patients with alcohol use disorder (AUD) present with steatosis, only 10-20 % develop progressive forms of liver disease and its related complications. Currently, the pathophysiological mechanisms implicated in liver disease progression are not completely understood and there is no drug approved for treatment of ALD [1].

Recent reports have highlighted the potential role of the gut-liver axis in ALD progression. Gut barrier dysfunction together with alterations in the composition of the intestinal microbiota as well as elevated systemic microbial translocation have been associated with ALD progression [2].

Murine models of chronic ethanol exposure have been used to discover new potential therapeutic targets at the frontier of the gut-liver axis in ALD[3]. Manipulations designed to restore gut barrier function, thus preventing microbial translocation, or alleviating dysbiosis all improved liver disease in animals [4–8]. However, these data cannot necessarily be extrapolated to human pathology for several reasons. Animals have a natural aversion to alcohol [9,10], a 5 times faster ethanol metabolism [11], and profound differences in their immune system [12] and their microbiota [13] compared to humans. Animals do only develop mild forms of ALD upon chronic alcohol feeding and do not resume the liver-damage pattern observed in humans [14]. Clinical studies targeting the gut microbiota in AUD patients are scarce and generally focused on patients with severe alcoholic hepatitis and decompensated cirrhosis [15]. Little is known about the impact of a gut microbiota modifying strategy in AUD patients on earlier non-cirrhotic disease stages of ALD. One potential way of modulating the gut microbiota in those patients might be the use of prebiotics. Prebiotics are defined as 'substrates that are selectively used by host microorganisms conferring a health benefit' [16] meaning that they promote the growth of some specific bacteria. Of particular interest, inulin-type fructan (ITF) is known to favor *Bifidobacterium* and *F prautznizi* two well-recognized beneficial bacteria which are decreased in AUD patients[17–19]. It has been shown that Bifidobacteria negatively correlate with pro-inflammatory cytokines and improve intestinal health in humans [20,21]. *F prausnitzii,* a butyrate producer, exhibits anti-inflammatory properties both in vitro and in vivo studies [22,23]. ITF supplementation and subsequent restoration of an optimal microbial balance, could exert beneficial effects on gut barrier function, reduce microbial translocation and thus attenuate systemic inflammation [15,18,24,25]. We therefore designed a

randomized, double-blind, placebo-controlled study to modulate the gut microbiota of AUD patients using a 3-weeks inulin supplementation. Since the liver is closely connected to the gut via the portal vein, we hypothesized that restoring the microbial balance with subsequent improvement of the gut barrier function could exert a beneficial effect on ALD development.

Therefore, one of the outcomes of this Gut2Brain study was to test the efficacy of this intervention on liver disease. The principal aim of this sub-study was to investigate the effect of inulin supplementation on liver parameters and systemic inflammation in AUD subjects. The secondary objective was to study the effect of supplementation in a subgroup of patients with progressive ALD.

3.3 Materials and Methods

Study population

Fifty AUD patients undergoing elective alcohol withdrawal were recruited between October 2018 and December 2019. They followed a 3 weeks highly standardized alcohol-detoxification and rehabilitation program (Supplementary figure 1) in an academic hospital. This program includes 1 week in the hospital, 1 week at home and another week in the hospital (Supplementary figure 1).

AUD patients were diagnosed by a psychiatrist according to the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition.

Eighteen to 65 years old male or female subjects who were actively drinking until at least 48 hours prior to admission were included in the study. The following exclusion criteria applied: presence of another addiction (except tobacco), inflammatory bowel disease, chronic inflammatory diseases (such as rheumatoid arthritis), cancer, obesity (BMI ≥ 30 kg/m2), diabetes, bariatric surgery, or severe cognitive impairment (Mini Mental State Examination (MMSE) < 24). Patients with known cirrhosis or significant hepatic fibrosis (≥ F2) detected by Fibroscan (> 7.6 kPa) immediately after admission were also excluded from the study. Patients who have been taking antibiotics, probiotics or prebiotics during the 3 months prior to enrolment or who had regularly used non-steroidal anti-inflammatory drugs or glucocorticoids during the month prior to enrolment were excluded.

Fourteen healthy subjects (HS) matched for age, gender and BMI who consumed less than 20g of alcohol per day (social drinkers) were also recruited using flyers posted in the public setting of the city.

Study design

The Gut2Brain study was a randomized, double blind, placebo-controlled trial. Each subject was randomly assigned to daily intake of inulin (Fibruline®; Inulin group) or maltodextrin (Placebo group).The randomization was performed using the method of randomly permuted blocks (50 subjects randomized into 5 blocks) via the website www.randomization.com, by a person not clinically involved in order to ensure the double blind. Neither the patient nor the investigator knew which boxes contained inulin or placebo until unblinding at the end of the study. In order to reduce potential

gastrointestinal side effects, the dose of inulin or maltodextrin increased gradually from 4 to 16 gram per day during the 17 days of treatment (4g from day 3 to day 4; 8g from day 5 to day 14 and 16g from day 15 to day 19 of the detoxification program; Supplementary figure 1).

The trial protocol was published on protocols.io (dx.doi.org/10.17504/protocols.io.bvs2n6ge). This study was approved by the ethics committee of the hospital (Nb: 190616V1). All participants signed informed consent prior to inclusion and the trial was registered in the clinicaltrials.gov registry (ClinicalTrials.gov identifier: NCT03803709).

Examinations and sample collections

A Fibroscan® (Echosense, Paris, France) combined with the controlled attenuation parameter (CAP) was performed at admission and repeated after 19 days. Fasting blood samples were drawn and stool samples collected twice on day 2 (T1) and 19 (T2) after the admission. Blood samples were centrifuged at 1000g for 15 min at 4°C. Plasma, Serum and stool samples were stored at -80°C until use.

Inflammatory markers

Plasma concentrations of inflammatory markers (IL-18, MCP-1, IFN-γ, IL-8, IL-10, TNF-a, IL-6) and Fibroblast growth factor 21 (FGF-21) were determined using the Meso Scale Discovery (MSD) U-PLEX assay (Rockville, MD, USA) following the manufacturer's instructions.

Microbial translocation and serum biomarkers of liver cell damage

Microbial translocation was determined using Lipopolysaccharide Binding Protein (LBP), soluble CD14 (sCD14) (Human LBP duoset ELISA and Human CD14 Quantikine ELISA kit sCD14 , Biotechne Ltd, Abingdon, United Kingdom) and Peptidoglycan Recognition Proteins (Human PGRPs ELISA kit, Thermofisher, Merelbeke, Belgium). Liver damage was assessed by measuring serum cytokeratin 18 (CK18) (CK18-M65 ELISA kit; TECOmedical AG, Sissach, Switzerland). All assays were performed in duplicate following the

manufacturer's instructions. Routine biochemical analyses were performed by the clinical biochemistry laboratory of the hospital.

Classification of patients according to severity of liver disease

AUD patient were classified according to the severity of liver disease using clinical biomarkers as previously described by our group[2]. Briefly, patients with a normal or increased CAP values but normal transaminases and CK18-M65 < 270 U/L were considered as having non-progressive liver disease (minimal liver involvement or simple steatosis) whereas those with CAP values > 250 dB/m AND CK18-M65 >270 U/L and/or AST/ALT>40 U/L were classified into the progressive ALD group (essentially alcoholic steato-hepatitis). According to the predefined inclusion/exclusion criteria, patients with liver stiffness values > 7.6 kPa on Fibroscan at admission (significant fibrosis) were excluded from the study.

16S rRNA sequencing and data analysis

Stool samples were collected at Day 2 (T1) and at the end of the intervention (Day 19 – T2). They were collected and stored immediately at -20°C and then transferred to -80°C within 5 to 10 hours. Genomic DNA was extracted from the feces using a QIAamp DNA Stool Mini Kit (Qiagen, Germany), including a bead - beating step and following the protocol Q towards standards for human fecal sample processing in metagenomic studies). The composition of the gut microbiota was analysed by Illumina sequencing of the 16S rRNA gene and qPCR of 16S rDNA was used to quantify the abundance of total bacteria and Bifidobacterium spp. (see supplementary Material).

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM) or mean ± standard error (SD). Normality was assessed by the Shapiro–Wilk test. According to data distribution, Wilcoxon or paired t-tests were used to analyse the evolution of the different parameters between T1 and T2. Linear regression models adjusted for gender, the quantity of ethanol consumed during the second week of the program and the baseline measurement of the outcome were performed to analyse the difference between placebo and inulin groups at T2. For the comparison between AUD and HS, we used ANOVA or

Kruskal Wallis test followed by a Holm-Sidak's or Dunn's test. A p value <.05 was considered as statistically significant. Statistical analyses were performed using SAS version 9.4 and Graphpad Prism 8.0.

For the gut microbiota analysis only the phyla, families and genera with an average relative abundance superior to 0.1% were analyzed. We used a Mann–Whitney U test in R to compare the relative abundance between groups and the within group analyses were evaluated using a Wilcoxon paired test.

Spearman partial correlations (adjusted for gender and the quantity of ethanol consumed during the second week of the program) were performed to assess the relationships between biological outcomes and microbial data.

Sample size was estimated using G*Power based on the bifidogenic effect of inulin [18]. Therefore, we estimated that a total sample size of 50 participants, with a 20% drop out during the study and 20 patients in each group completing the study provides 80% power to observed an effect size of 0.34 for the relative abundance of *Bifidobacterium* genus using a power calculation test with a 0.05 two-sided significance level.

3.4 Results

Study population

To reach the final sample, 150 patients were screened, 77 did not meet the inclusion criteria and 23 refused to participate. Finally, 50 AUD patients were randomized. Among those, 43 patients completed the study (Supplementary Figure 2). Compliance with the nutritional supplementation was 96% in the placebo group and 98% in the inulin group. The two groups of treatment where comparable at baseline in terms of age, BMI, marital status and alcohol history. However, there was a women predominance in the inulin group (20% in placebo vs 48% in the inulin group, p=0.04) and they had one additional criteria in the DSM-5 classification compared to the placebo group (7.8 ± 1.8 in placebo vs 9.1 ± 1.5 in inulin group, p=0.01). During the second week of the detoxification program, 8 patients relapsed in the placebo group vs 12 in the inulin group (32% vs 48% respectively, p=0.25). Patients who relapsed in placebo group consumed 79 g/d alcohol on average vs 76 g/d in inulin group (p=0.96).

Table 1 depicts demographics and various baseline characteristics of AUD patients and HS. Both groups were similar in terms of age, sex, BMI and marital status. AUD patients were less educated and had a lower cognitive function (MMSE score) than HS. There were more smokers in the AUD group than in the HS group. On average AUD patients consumed 132 ± 73 g of ethanol/day compared to 7.6 ± 10.4 g/day in healthy subjects.

In order to study the effect of 19 days of abstinence, we first studied the evolution between T1 and T2 of the hepatic, inflammatory and microbial translocation parameters in the whole sample (n=50) without taking into account the supplementation. This sample was called “global population”.

Then, we studied the effect of inulin supplementation compared to the placebo group.

Finally, we stratified the population according to the severity of the liver disease (progressive vs non-progressive ALD) to study the effect of inulin supplementation in patients with more severe liver disease.

Table 1: Baseline characteristics of AUD patients compared to healthy subjects

	HS n=14	AUD n=50	p[1]
Sociodemographic characteristics			
Age (y)	47.28 ± 11.46	48.44± 9.23	0.81
Female, n(%)	6 (42.86)	18(36.00)	0.64
Marital status			0.23
Couple/ married	8 (57.14)	20 (40.00)	
Single	3 (21.43)	22 (44.00)	
Separated/divorced	2 (14.28)	8 (16.00)	
Educational level			0.006
Primary	0 (0.00)	5 (10.00)	
Secondary	0 (0.00)	17 (34.00)	
Superior	14 (100.00)	28 (56.00)	
Clinical examination			
Weight	71.43 ± 10.72	72.34 ± 12.36	0.74
BMI	23.70 ± 3.16	23.97 ± 3.25	0.522
MMSE score	29.36 ± 0.63	27.96 ± 2.30	0.01
Tobacco	2 (14.28)	40 (80.0)	<0.001
Alcohol history			
DSM-V score	0.00 ± 0.00	8.46 ± 1.78	1.00
Age of loss of control (y)	-	32.31 ± 11.02	-
Numbers of withdrawal	-	2.20 ± 2.20	-
Duration of drinking habit	-	15.81 ± 10.54	-
Alcohol consumption (g/d)	7,48	135.44±73.66	<0.001
AUDIT score	3.11 ± 2.30	-	-

Values are means ± standard deviation or n (%)

[1] p values were calculated using a T-test or Mann Whitney Wilcoxon's test and Chi2 test or Fisher's test for categorical variables.

AUD, Alcohol use disorders; Alcohol Use Disorders Test; BMI, Body mass index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; HS, Healthy subjects MMSE, Mini Mental State Examination

Inulin intake, alcohol abstinence, liver damage and inflammatory markers in AUD patients

Evolution of liver damage markers, microbial translocation and inflammatory markers after 19 days of withdrawal in the global population

AST, ALT as well as CK18-M65, reflecting liver cell damage, were significantly higher in AUD patients compared to HS at admission (T1). At the end of the detoxication program (T2) AST and ALT values declined but remained significantly higher compared to HS while no reduction in CK18-M65 was observed. In addition, CAP, an estimate for liver steatosis, decreased significantly between T1 and T2 in AUD patients (Figure 1A). Interestingly, FGF-21 was significantly increased in AUD patients compared to HS at T1. Although a reduction was observed, after the 19 days of abstinence, levels remained significantly higher than in HS (T2) (Figure 1A).

Regarding microbial translocation markers, sCD14 and PGRP were significantly increased in AUD patients at T1 compared to HS (Figure 1B) whereas LBP levels did not change. sCD14 decreased significantly between T1 and T2 (Figure 1B) but neither PGRPs nor LBP were modified by alcohol withdrawal.

Since bacterial endotoxin translocation could favor systemic inflammation, we measured pro and anti-inflammatory cytokines. Except for IFNγ, the systemic inflammatory markers TNF-α, IL-6, IL-18, IL-8, MCP-1 and IL-10 were significantly upregulated in AUD patients compared to HS at T1 (Figure 1C). IL-8 and MCP1 significantly dropped with alcohol withdrawal while the decrease in IL-18 between T1 and T2 did not reach the significance level ($p=0.07$). However, IL-18, IL-8 and MCP-1 remained higher than HS at T2. TNF-α, IL-6 and IL-10 remained on a similar high level at the end of the program (Figure 1C). Overall and despite some changes, alcohol cessation during a period of 3 weeks did not restore the inflammatory status of AUD patients nor completely alleviate liver damage.

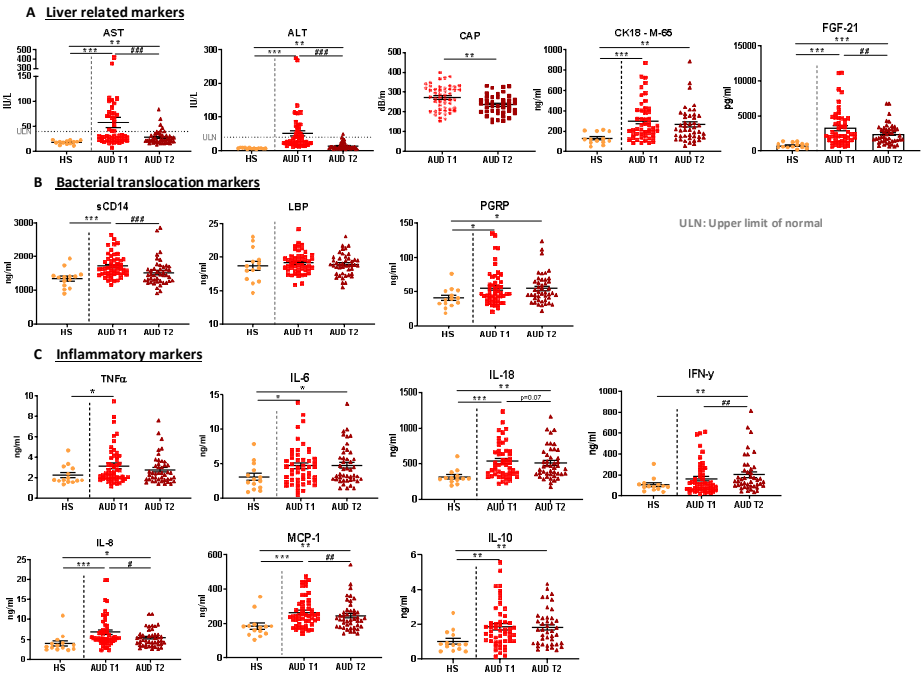

Figure 1: Evolution of liver, microbial translocation and inflammatory markers between T1 and T2 in AUD patients

HS n=14, AUD T1 n=50 and AUD T2 n=44. T test or Mann Whitney test were performed to compare HS with AUD at T1 and T2 (*p<0.05, **p<0.01,***p<0.001). Paired T-test or Wilcoxon paired test were performed to compare the evolution of markers between T1 and T2 in AUD population (#p<0.05, ##p<0.01, ###p<0.001).
Grey line corresponds to the upper limit of normal value.

Inulin supplementation did not improve liver damage, microbial translocation and inflammatory markers over abstinence alone

Since we observed significant changes for several markers in the overall population at the end of the withdrawal program, we wanted to know whether inulin supplementation procures an additional benefit over placebo in AUD patients.

AST and ALT decreased significantly after 17 days regardless of the treatment group (Figure 2A). AST returned below the upper limit of normal (ULN, 40 IU/L) in 100% of the patients of the placebo group and even reached values of the HS in the vast majority. By contrast, AST in the inulin group remained significantly higher than the values observed in HS (Inulin vs HS, p=0.01; Supplementary Table 1) and only 76% of the patients decreased to a level below the ULN (Figure 2A). ALT dropped below the ULN in both groups (Figure 2A) but remained significantly higher than in HS (Supplementary Table 1).In addition, linear regression analysis, adjusted for gender, the baseline value of the outcome and the quantity of ethanol consumed during the second week of the program, revealed that the levels of AST and ALT at T2 were higher in the inulin group than in the placebo group (Table 2). CAP values decreased significantly only in the inulin group (Figure 2A). However, no difference was observed between the inulin group and the placebo group at T2 after adjustment for gender, the baseline value of the outcome and the quantity of ethanol consumed during the second week of the program (Table 2).

Serum CK18-M65 and FGF-21 did not significantly change in the placebo group nor in the inulin group (Figure 2A) and remained higher than HS in both groups at T2 (Supplementary Table 1).

The observed changes of microbial translocation and inflammatory markers in the global population between T1 and T2 were homogenously distributed between both groups. We observed a significant reduction of sCD14 in the placebo group and of MCP-1 in the inulin group (figure 2B and C) but the adjusted linear regression models revealed no difference between inulin and placebo at T2 (Table 2). IL-18 specifically decreased in the placebo group whereas almost no variations were found in the inulin supplemented patients (Figure 2C). In addition, the adjusted linear regression analysis confirmed a significantly higher level of IL-18 in inulin group compared to placebo group at T2 (Table 2).

Taken together, our results suggest that 17 days of inulin supplementation has no beneficial effect on liver, bacterial translocation and inflammatory markers during a period of 3 weeks of abstinence in AUD patients.

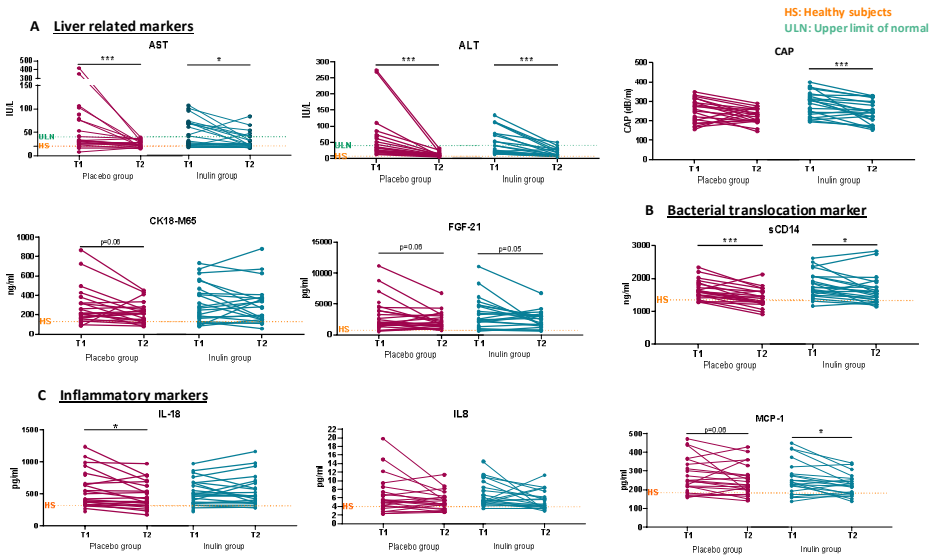

Figure 2: Evolution of liver, microbial translocation and inflammatory markers in AUD patients receiving inulin or placebo for 3 weeks

n=25 at T1 in both groups; n=21 at T2 in placebo and n=22 in inulin group at T2. Paired T-test or Wilcoxon paired test were performed to compare the evolution between T1 and T2.

Orange line corresponds to the mean for healthy subjects and the green line correspond to the upper limit of normal value. *p<0.05, **p<0.01,***p<0.001.

Table 2: Effect of inulin supplementation on liver function, bacterial translocation and inflammatory markers*

	AUD patients n=50		Progressive ALD patients n=22	
	Adjusted difference at T2 (inulin vs placebo) β [95% CI]	p	Adjusted difference at T2 (inulin vs placebo) β [95% CI]	p
Liver parameters				
AST	8.09 [1.77 ; 14.41]	0.01	16.67 [2.84 ; 30.51]	0.02
ALT	5.18 [1.30 ; 9.06]	0.01	9.09 [0.65 ; 17.53]	0.04
CAP	8.36 [-21.24 ; 37.96]	0.57	1.52 [-45.24 ; 48.29]	0.95
CK18-M-65	76.70 [-4.13 ; 157.54]	0.06	127.52 [-38.83 ; 293.88]	0.12
Bacterial translocation				
sCD14	80.70 [-100.21 ; 261.69]	0.37	209.79 [-104.84 ; 524.41]	0.18
Inflammation				
FGF-21	-164.70 [-867.38 ; 537.97]	0.64	-258.20 [-1428.38 ; 911.98]	0.65
IL-18	113.86 [23.02 ; 204.71]	0.02	103.90 [-19.78 ; 227.58]	0.09
IL-8	0.22 [-1.09 ; 1.53]	0.73	-0.55 [-2.60 ; 1.50]	0.58
MCP-1	-2.08 [-49.00 ; 44.83]	0.93	-49.24 [-112.37 ; 13.88]	0.12

*Linear regression model adjusted for gender, the baseline measurement of the outcome and the quantity of ethanol consumed during the second week of the program

AST, Aspartate transaminase; ALD, Alcohol liver disease; ALT, Alanine transaminase; CAP, controlled attenuation parameter; CK18-M65, Serum cytokeratin 18 ; sCD14, soluble CD14.

The impact of inulin supplementation in AUD patients with progressive alcohol liver disease

We have previously shown that the gut microbiota of patients with progressive ALD was particularly impaired [2]. Therefore, we wanted to study the effect of inulin supplementation in a subgroup of patients with progressive ALD. For this exploratory analysis, we stratified the study population into patients with non-progressive and progressive liver disease according to clinical parameters. As by definition, patients with progressive ALD patients were characterized by elevated AST, ALT, and serum CK18-M65 compared to non-progressive ALD and HS. In addition, we found that among the upregulated markers, two of them, sCD14 and FGF-21, also distinguished non-progressive from progressive ALD with significantly higher levels in the latter one (Supplementary Figure 3).

Inulin supplementation induced different changes in gut microbiota composition at the phylum, family and genus level in progressive and non-progressive ALD groups

Microbiota analysis at baseline revealed that the progressive ALD group displayed a decreased richness since the number of observed species and the Chao-1 index were significantly decreased compared to HS (Figure 3A). Total bacteria measured by qPCR, was not different between the groups (Figure 3B). The LEfSE analysis showed that Firmicutes and especially Clostridia were higher in HS while Proteobacteria were significantly higher in progressive ALD patients (Figure 3C and D). At the genus level, the relative abundance of *NK4A214 group* from *Oscillospiraceae* family and *Dialister* were significantly higher in HS while *Flavonifractor and Lachnoclostridium* were significantly increased in progressive ALD (Figure 3D). These findings are in accordance with our previous data of more severe dysbiosis in patients with progressive ALD[2].

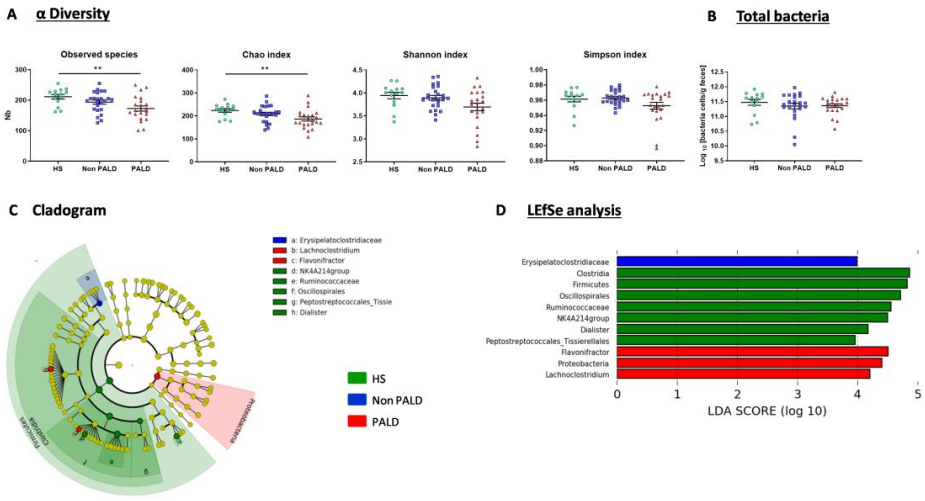

Figure 3: Gut microbiota composition and microbial diversity according to the severity of alcoholic liver disease at baseline (T1)

HS: n=14, Non progressive ALD (Non PALD): n=24, Progressive ALD (PALD) : n=22. Kruskal Wallis tests were performed to compare the three groups followed by a Dunn's test. For each panel, data are expressed as mean ± SEM.

(A) Measure of alpha-diversity indexes: Number of observed species, chao-1, Shannon, Simpson. (**B**) Total bacteria measured by qPCR. (**C**) Cladogram using LEfSe method indicating the phylogenetic distribution of gut microbiota of HS and AUD patient according to progressive liver disease. Each successive circle represents a phylogenetic level. (**D**) Histogram of the LDA scores reveals the most differentially abundant taxa among different groups. Taxa enriched in the HS group are highlighted in green, in blue for Non-PALD group and in red for the PALD group in the linear discriminant analysis (LDA). Graphical representation was performed using Galaxy/Hutlab tool (huttenhower.sph.harvard.edu/galaxy).

The effects of inulin supplementation on the gut microbiota composition have been already published elsewhere, showing namely an increase in *Bifidobacterium* as a "signature" of inulin intake (Amadieu C et al, in revision). We compared the change from baseline of *Bifidobacterium* between placebo and the inulin group. *Bifidobacterium* increased significantly in the inulin group compared to placebo in progressive ALD as well as non-progressive ALD (Figure 4A and B). The bifidogenic effect of inulin was therefore confirmed in both groups.

Inulin supplementation induced a significant decrease of all α diversity indexes compared to placebo in the progressive ALD group (Figure 4C) while only the number of observed species was decreased in the non-progressive ALD group (Figure 4D). In the progressive

ALD group, analysis of phylum and family levels of bacteria revealed minor changes in those who received placebo while an increase of *Bifidobacteriaceae* (Actinobacteriota) and a decrease of *Bacteroidaceae* (Bacteroidota) was found in patients supplemented with inulin (Figure 4E). In the non-progressive ALD group, inulin supplementation has no effect at phylum level but similar changes were observed at family level with an additional increase of *Veillonellaceae* family (Figure 4F).

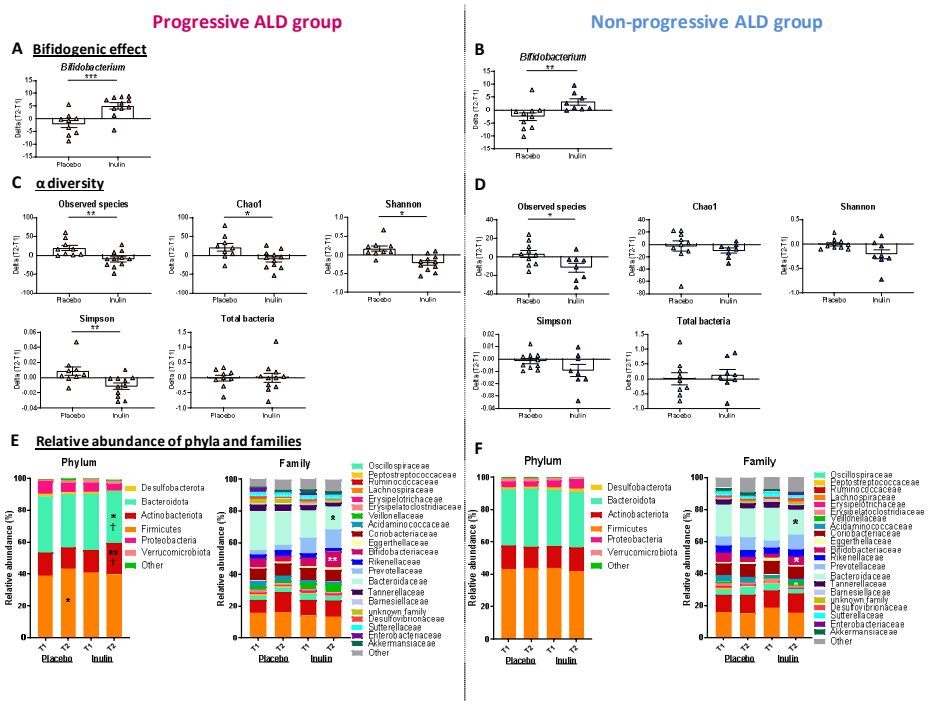

Figure 4: Effect of inulin supplementation on gut microbiota composition in progressive and non progressive ALD patients.

Progressive ALD: n=9 and n=11 in placebo and inulin groups respectively. Non progressive ALD: n=10 and n=8 in placebo and inulin groups respectively (**A-B**) Change in relative abundance of Bifidobacterium between T1 and T2. (**C-D**) Changes in measure of alpha-diversity indexes: Number of observed species, Chao-1, Shannon, Simpson and total bacteria measured by qPCR. (**E-F**) Relative abundances of bacterial taxa accounting for more than 1%, at the phylum and family level, assessed using Illumina 16S rRNA gene sequencings in AUD patients supplemented with placebo (n=19) or inulin (n=19) according to the severity of liver disease. Wilcoxon paired tests were performed to compare the evolution from baseline in each groups. *p<0.05, **p<0.01,*** p<0.001.

At the genus level, prebiotic treatment also increased *Dialister* whereas *Bacteroides*, *Ruminococcus torques* and *Dorea* decreased in the progressive ALD group (Table 3). In the placebo group, *Fusicatenibacter*, *Oscillospiraceae UCG-002*, *Monoglobus*, *Alistipes* and *Lachnospiraceae ND3007* increased significantly between T1 and T2 while *Oscillibacter*, *Flavonifractor*, *Colidextribacter* and *Sutterella* decreased (Table 3).

The genera modified between T1 and T2 in non-progressive ALD group are presented in Supplementary Table 2. *Bifidobacterium* and *Veillonella* increased significantly after 17 days of inulin supplementation. In contrast, *Lachnoclostridium*, *Ruminococcus torques group*, *Dorea*, *Tizzerella*, *Oscillibacter*, *Colidextribacter*, *Erysipelotrichaceae UCG-003* and *Bacteroides* decreased significantly (Supplementary Table 2). In the placebo group, *Lachnospiraceae NK4A136 group* increased while *Blautia*, *Eubacterium eligens group* and *Acidaminococcus* decreased after 17 days (Supplementary Table 2).

Globally, inulin supplementation exacerbated the decrease in α-diversity but did not impact the bacteria shown to be already altered at baseline in progressive ALD patients.

Table 3: Significant changes in relative abundance of gut bacteria at the genus level in AUD patient with progressive liver disease receiving inulin or placebo for 3 weeks*

	Progressive ALD group		
	T1	**T2**	**p**
Significant changes in inulin group			
Bifidobacterium	9.91 ± 0.70	10.42 ± 0.46	0.01
Dialister	1.30 ± 1.83	3.73 ± 3.15	0.03
Bacteroides	19.00 ± 10.61	14.34 ± 10.33	0.04
Ruminococcus torques group	0.23 ± 0.32	0.04 ± 0.05	0.03
Dorea	0.27 ± 0.21	0.09 ± 0.06	0.004
Eubacterium ruminantium group	0.07 ± 0.17	0.004 ± 0.008	0.03
Significant changes in placebo group			
Fusicatenibacter	0.27 ± 0.20	0.55 ± 0.37	0.04
Oscillospiraceae UCG 002	0.83 ± 0.90	1.72 ± 1.22	0.004
Monoglobus	0.13 ± 0.23	0.33 ± 0.26	0.01
Alistipes	2.19 ± 1.40	3.41 ± 1.96	0.03
Lachnospiraceae ND3007 group	0.11 ± 0.13	0.22 ± 0.27	0.04
Eubacterium ventriosum group	0.04 ± 0.05	0.15 ± 0.13	0.04
Oscillibacter	0.46 ± 0.40	0.18 ± 0.15	0.01
Flavonifractor	0.40 ± 0.38	0.20 ± 0.30	0.009
Colidextribacter	0.26 ± 0.14	0.13 ± 0.13	0.002
Sutterella	2.17 ± 1.52	1.72 ± 1.38	0.04

*Genus significantly modified after 17 days of treatment were identified using Wilcoxon paired test.

Inulin supplementation did not improve liver damage, microbial translocation and inflammatory markers in the subgroup of patient suffering from progressive alcohol liver disease

Regarding the evolution of liver and microbial translocation markers between T1 and T2 in patients with progressive ALD, AST, ALT and CAP decreased significantly in both placebo and inulin groups while no significant changes were observed for CK18-M65 and sCD14. By contrast, FGF-21 and IL-18 were specifically reduced in the placebo group while IL-8 and MCP-1 decreased significantly between T1 and T2 in patients supplemented with inulin (Figure 5). The adjusted linear regression models revealed that AST and ALT at T2 remained higher in the inulin group compared to placebo ($p=0.02$ and $p=0.04$; Table 2). No significant differences were observed between placebo and inulin for the other markers after adjustment.

Overall, our data do not support beneficial effect of inulin supplementation compared with placebo, even in the sub-group of patients with more severe liver disease at baseline.

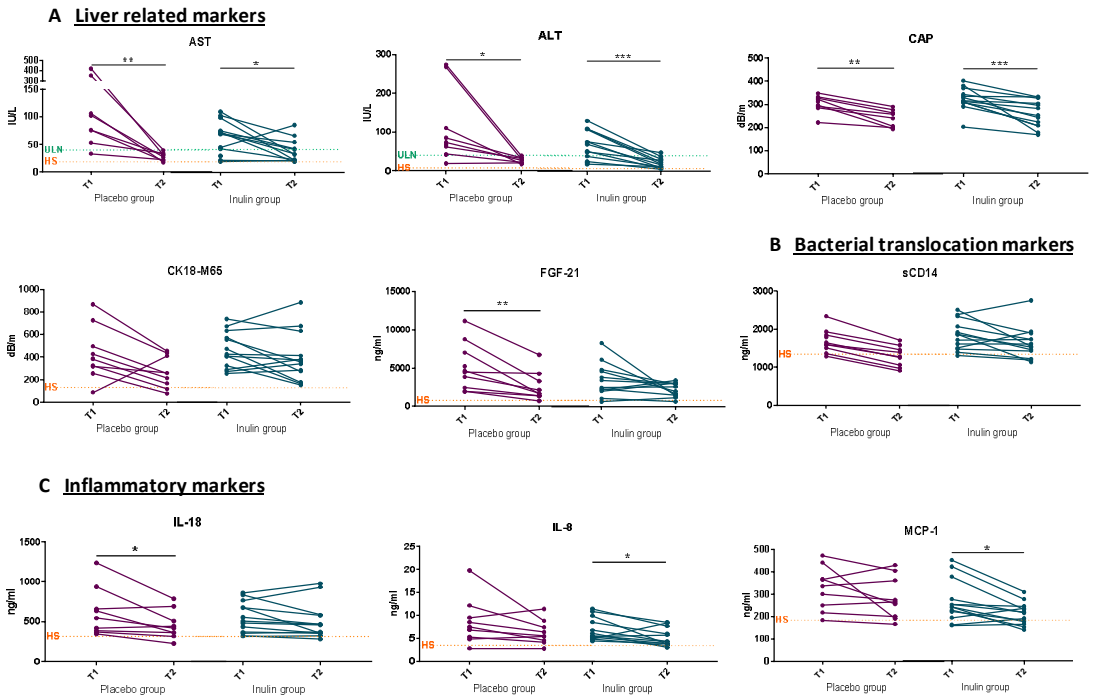

Figure 6: Evolution between T1 and T2 of liver, microbial translocation and inflammatory markers in patient with progressive alcohol liver disease according to inulin or placebo supplementation

Wilcoxon paired test were performed to compare the evolution between T1 and T2.Orange line corresponds to the mean for healthy subjects and the green line correspond to the upper limit of normal value. *p<0.05, **p<0.01,***p<0.001.

Link between liver alterations, inflammatory markers and microbiota in the subgroup of patient with progressive ALD

In order to investigate whether changes in liver or inflammatory markers after treatment could be related to certain bacterial genera, we performed partial correlations adjusted for gender and the amount of alcohol consumed during the second week in the progressive ALD group. The variations of Shannon and Simpson indexes as well as *Fusicatenibacter* were negatively correlated with the variation of AST level (r=-0.49, p=0.03; r=-0.47, p=0.04 and r=-0.46, p=0.048 respectively; Supplementary Figure 4). The variation of Shannon and Simpson indexes, *Oscillospiraceae UCG-002*, and *Alistipes* were negatively associated with the variation of the translocation marker sCD14 level (r=-0.49, p=0.03; r=-0.50, p=0.027 ; r=-0.55, p=0.01 and r=-0.59, p=0.007 respectively) while *Sutterella* was positively associated (r=0.46, p=0.048). *Oscillibacter* and *Colidextribacter* were positively correlated with IL-10 (r=0.60, p=0.007; r=0.48, p=0.04 respectively). No correlation between alpha diversity indexes or bacterial genera and IL-18 levels was observed (supplementary Figure 4).

3.5 Discussion

This work aimed at studying the effect of inulin supplementation on the gut-liver axis in AUD patients during alcohol withdrawal. Inulin is an interesting prebiotic since several studies highlight its beneficial effect on gut microbiota composition and metabolism[26,27]. We hypothesized that inulin, through amelioration of gut dysbiosis, could improve liver damage in AUD patients especially in a subgroup of patients with progressive ALD defined by clinical parameters. Alcohol withdrawal, regardless of the nutritional intervention, had a beneficial effect on AST, ALT, sCD14, IL8, MCP-1 and IL-18. However, most of the markers remained higher than HS levels after 19 days of abstinence. We further demonstrate that 17 days of inulin supplementation did not elicited additional benefit over abstinence alone on liver disease and inflammatory parameters in AUD patients. Surprisingly, we observed even a less pronounced reduction of AST, ALT and IL-18 in patients supplemented with inulin compared to placebo. By stratifying the patients according to the severity of liver disease, we did not find a benefit of inulin in the subgroup of patient suffering from more severe ALD at baseline. AST and ALT remained significantly higher in inulin group than in placebo group at the end of the intervention.

Studies in mice reported a positive effect of inulin on inflammatory markers [28,29], decreased blood lipopolysaccharide (LPS) levels and increased short chain fatty acids (SCFA) concentrations [30,31] after alcohol feeding. In humans, several studies have shown a decrease in AST and ALT after inulin supplementation in obese or diabetic patients[32,33] as well as a decrease in circulating LPS, IL-6 and TNFα [34] in the latter. In a pilot study, supplementation with oligofructose, a short chain ITF, during 8 weeks in patients with nonalcoholic steatohepatitis significantly decreased serum AST and ALT [35]. However, our findings revealed that changes were observed in placebo and as such more related to abstinence. Inulin does not improve liver function or inflammatory markers in the specific context of AUD.

IL-18 was found to be higher in inulin supplemented subjects than in placebo subjects at T2. The blunted decrease in IL-18 observed in inulin-treated patients, that mirrors the less pronounced decrease of ALT and AST, is intriguing as we did not observe the same for others cytokines. Several reports have proposed that IL-18 could play a specific role in liver injury [36,37]. As the main signature of inulin is to modulate the gut microbiota composition, one might postulate that some specific inulin-related changes could be responsible for this intriguing result. However, we did not find any correlation between the changes in IL-18 levels and changes in genera abundance. It would be interesting to study whether changes

in metabolites from the gut microbiota like SCFA or bile acids could explain the changes observed in IL-18 levels. In the specific context of AUD, it would also be of interest to ensure that inulin does not promote the growth of ethanol-producing bacteria, which could explain the smaller decrease not only in liver enzymes but also in IL-18. Indeed, some studies have revealed that *Bifidobacterium* can produce ethanol through the fermentation of fibers, which is susceptible to partially counteract the beneficial effect of inulin[38,39]. This effect might be especially relevant in an AUD population.

In this work, we studied the effect of inulin in patients with progressive ALD as we observed, in a previous study, that these patients had a particularly altered microbiota[2]. We observed that patients with progressive ALD had reduced bacterial richness and that 17 days of inulin supplementation further reduced this microbial richness compared to placebo. Few studies have shown a decrease in microbial diversity with dietary fiber supplementation [40,41]. In our study, this decrease in diversity was negatively correlated with the decrease AST and CD14 levels in progressive ALD patients and as such might rather be an unfavourable outcome. Decreased microbial diversity has often been associated with a pathological context in the literature [42,43] and it has also been shown that patients with ALD have a lower microbial diversity compared to HS [2,44].

Progressive ALD patients are characterized by an overrepresentation of the phylum Proteobacteria as well as the genera *Lachnoclostridium* and *Flavonifractor* compared to HS that were not modulated by inulin supplementation. We solely observed an increase in Actinobacteriota coupled with a decrease in Bacteroidota in the progressive ALD group. Among those bacteria, the phylum Proteobacteria*,* is of particular interest. It is composed of gram-negative bacteria and has been shown to be increased in patients with liver cirrhosis[45,46] and in non-alcoholic steatohepatitis patients with advanced fibrosis and cirrhosis [47]. Alcohol use has also been associated with an increase in Proteobacteria considered to be responsible for endotoxemia and hepatic inflammation[48–50]. At least in our context, inulin was not able to counteract this potentially negative impact. At the genus level, inulin increased the abundance of *Bifidobacterium* and *Dialister* while it decreased *Bacteroides*, *Ruminococcus torques group*, *Dorea* and *Eubacterium ruminantium group*. Most of these changes have already been observed in the literature in a different context[19,51] but their significance in AUD and ALD is not clear. Addolorato *et al* showed that *Bacteroides* was enriched in AUD patients and that this is associated with circulating LPS [44]. Bifidobacteria, a core genus in human gut microbiota, are generally associated with good health outcome [52]. They seem to have a beneficial effect on gut

barrier integrity[30,31] and improved gut barrier function was associated with alleviated liver damage in animals [53,54]. Our group previously reported that AUD patients had a decreased abundance of bifidobacteria compared to healthy individuals [17] but another study showed an increased fecal abundance of *Bifidobacterium* in AUD patient with severe hepatitis and cirrhosis [55]. Promoting Bifidobacteria, e.g. through inulin supplementation, might not be beneficial in some circumstances such as in AUD patients. A negative impact could eventually result from the ability of *Bifidobacterium* to produce ethanol or to increase butyrate levels which has been described in several studies [38,39]. Indeed, it has been shown that in TLR5-KO mice presenting gut dysbiosis, inulin supplementation, via the increase of butyrate, induced hepatic alterations [56]. It is possible that potential beneficial effects of inulin only applies to particular pathological contexts[57,58]. It is also possible that the effects of inulin are dependent on the diet, the metabolic status or the initial microbial composition.

Multiple alterations of the intestinal barrier have been associated with chronic alcohol consumption and eventually liver disease severity[2,7,8]. Modulating the microbiota might not improve immunity and bacterial translocation, important contributors in the gut-liver communication in ALD pathogenesis [59]. Therefore, a mono therapeutic approach targeting only the microbiota with prebiotic fibers could be insufficient in a pathology where multiple factors contribute to disease progression.

The principal strength of our pilot study is the blinded-randomized-placebo controlled design. However, some limitations do apply. Since gender is known to influence the biological parameters including liver damage [60,61], a higher proportion of female in the inulin group could have impacted the results. Although relapse rates were similar between both groups of treatment we cannot exclude that this parameter could have influenced liver recovery. In order to limit the consequences of these potential biases, we adjusted the linear regression models for both variables. It would nevertheless be of interest to stratify randomization of the population according to these two parameters for future studies. Finally, the sample size for the subgroup analyses was small which could have impacted the results.

Overall, our pilot study shows that modulation of the microbiota can be obtained via inulin in AUD patients. However, 17 days of inulin supplementation versus placebo did not have a beneficial effect on liver recovery after short-term alcohol withdrawal even in subjects with more pronounced ALD. In addition, further studies testing other gut

microbiota targeting strategies, especially those eventually leading to a decrease in bacterial diversity, should be planned and performed carefully since potential adverse effects of gut microbiota modulations on liver alterations in AUD subjects could be a concern.

Acknowledgements

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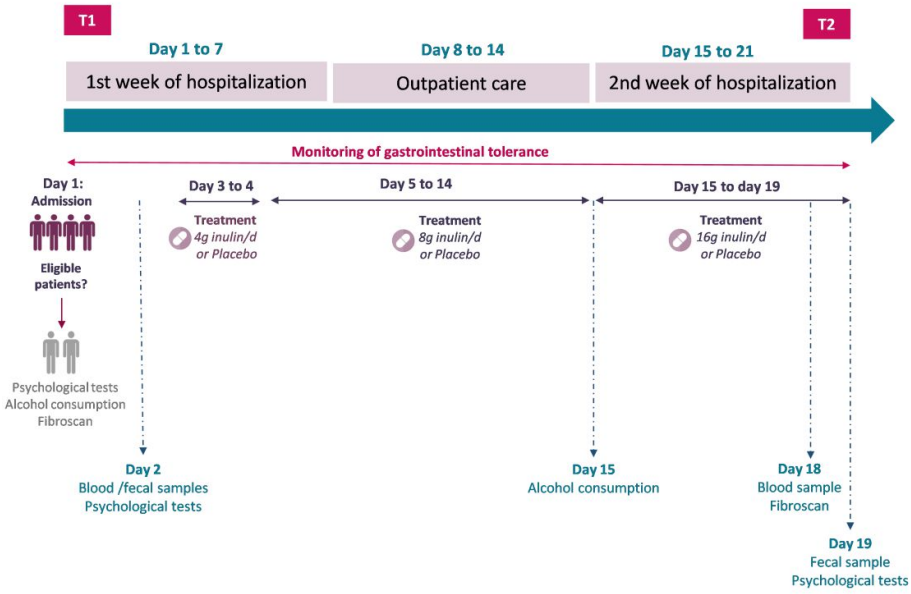
Data availability statement

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request. The accession number for the raw data generated with the 16S rRNA gene sequencing reported in this paper is BioProject PRJNA745947 (SRA) and data are available through the following link https://www.ncbi.nlm.nih.gov/bioproject/PRJNA745947/ .

3.6 Supplemental information



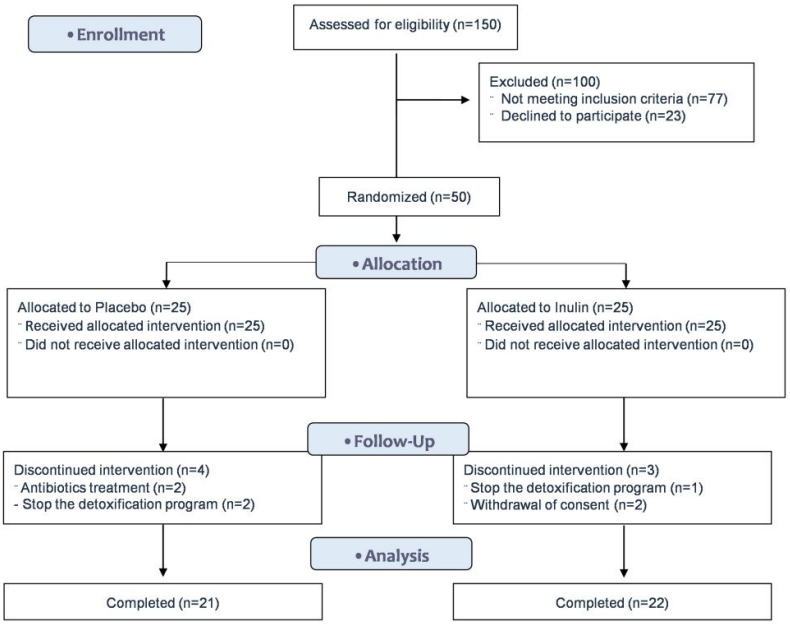
Supplementary figure 1: Design of the Gut2Brain study in the alcohol withdrawal unit.

Supplementary methods

Microbiota analysis

The composition of the gut microbiota was analysed by Illumina sequencing of the 16S rRNA gene (see supplementary Material). The V3-V4 region of the 16S rRNA gene was PCR-enriched using the primer pairs V3F_Nextera (CCTACGGGAGGCAGCAG) and Meta_V4_806R (GGACTACHVGGGTWTCTAAT). The amplicons were purified, quantified and sequenced using an Illumina Miseq to produce 2x300-bp sequencing products at the University of Minnesota Genomics Center. 16S rDNA amplicon sequences were analysed using FROGS pipeline 26. Amplicons were filtered according to their size then clustered into OTUs using Swarm (aggregation parameter d = 1 + d = 3). Chimera were removed using VSEARCH combined with an innovative chimera cross-validation and OTUs were kept when representing more than 0.005% of the total number of sequences 27. OTUs were classified using the reference database Silva138 16S with a pintail quality of 100 28. Relative abundance of each OTU was calculated after data normalization using a threshold of 33133 reads per sample.

qPCR of 16S rDNA was used to quantify the abundance of total bacteria (Forward: ACT-CCT-ACG-GGA-GGC-AGC-AG, Reverse: ATT-ACC-GCG-GCT-GCT-GG) and Bifidobacterium spp. (Forward: GAT-TCT-GGC-TCA-GGA-TGA-ACG-C, Reverse:CTG-ATA-GGA-CGC-GAC-CCC-AT). PCR amplification was carried out as follows: 10 min at 95 °C, followed by 45 cycles of 3 s at 95 °C, 26 s at 58 °C or 60 °C, and 10 s at 72 °C. Detection was achieved with the QuantStudio3instrument and software (Applied Biosystems) using the GoTaqqPCR MasterMix Plus for SYBR Assay (Promega). BSA was added to samples. Each assay was performed in duplicate in the same run. For construction of standard curves, fivefold dilution series from target species genomic DNA preparations (DSMZ) were applied to the PCR.

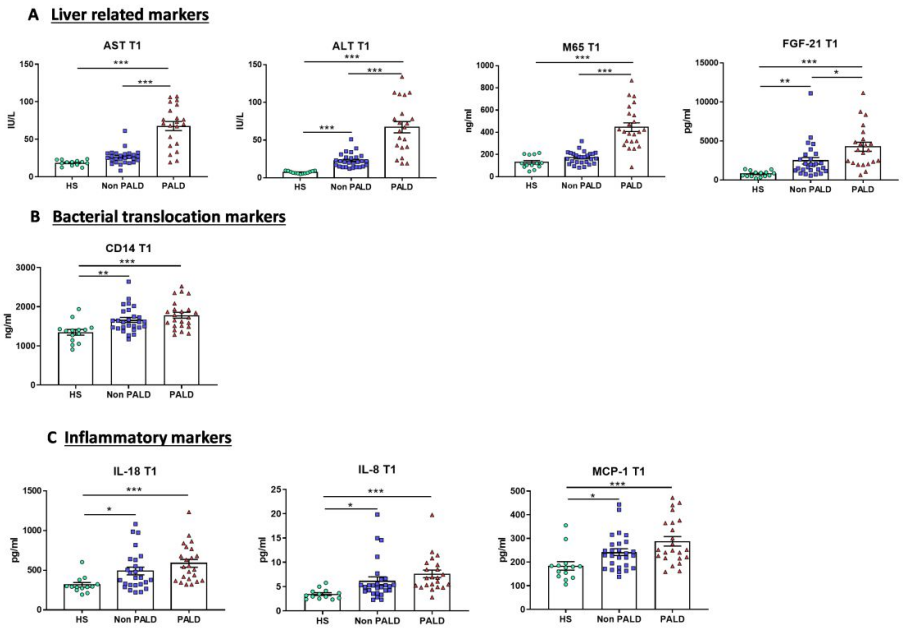


Supplementary Figure 2: CONSORT flow diagram of the gut2Brain study

Supplementary table 1 : Comparison between placebo, inulin and healthy subjects at T2*

	HS	Placebo T2	Inulin T2	p KW	p Placebo vs HS	p Inulin vs HS
Liver parameters						
AST	20.1 ± 7.3	24.9 ± 7.4	31.6 ± 17.7	0.03	0.11	0.01
ALT	9.6 ± 8.7	12.2 ± 6.8	16.6 ± 12.8	0.01	0.02	0.02
CK18-M-65	129.9 ± 57.0	221.1 ± 109.7	306.9 ± 204.9	0.003	0.04	0.002
Bacterial translocation						
CD14s	1349.0± 273.8	1406.8±282.2	1647.8 ± 448.7	0.05	-	-
Inflammation						
FGF-21	778.8 ± 378.6	2240.2±1409.9	2590.3± 515.7	<.001	<.001	<.001
IL-18	452.4 ± 518.1	474.4 ± 208.4	545.3 ± 239.5	0.002	0.02	0.001
IFN-y	103.9 ± 66.8	191.7 ± 137.5	212.5 ± 203.2	0.03	0.03	0.06
IL-8	4.0 ± 2.2	5.3 ± 2.2	5.5 ± 2.2	0.02	0.03	0.01
MCP-1	183.9 ± 66.7	249.1 ± 81.8	240.0 ± 87.5	0.02	0.01	0.04

*ANOVA or Kruskal Wallis test were performed to compare the three groups followed by a Holm-Sidak's or Dunn's test comparing AUD patients to healthy subjects.
AST, Aspartate transaminase; ALT, Alanine transaminase; CAP, controlled attenuation parameter; CK18-M65, Serum cytokeratin 18 ; KW, Kruskal wallis test; sCD14, soluble CD14



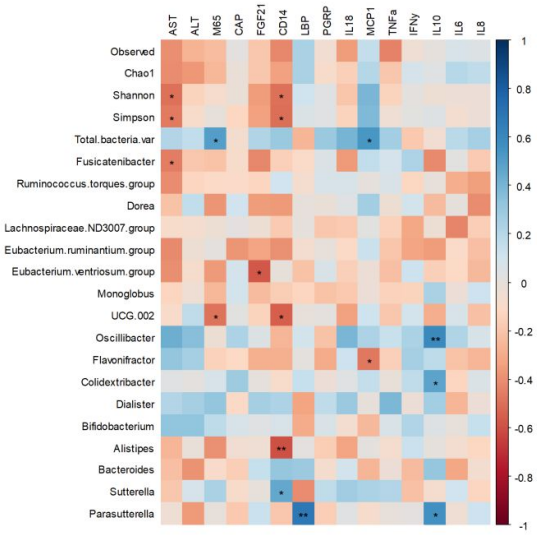
Supplementary figure 3: Baseline clinical characteristics of AUD patients according to clinical evaluation of alcohol liver disease.

Kruskal Wallis tests were performed to compare the three groups followed by a Dunn's test. *p<0.05, **p<0.01,***p<0.001

Supplementary Table 2: Significant changes in relative abundance of gut bacteria at the genus level in AUD patient with non progressive liver disease receiving inulin or placebo for 3 weeks*

	Non-progressive ALD group		
	T1	**T2**	**p**
Significant changes in inulin group			
Bifidobacterium	9.96 ± 0.73	10.20 ± 0.83	0.02
Veillonella	0.29 ± 0.87	0.64 ± 1.44	0.03
Lachnoclostridium	0.97 ± 1.63	0.62 ± 1.15	0.008
Ruminococcus torques group	0.29 ± 0.41	0.09 ± 0.13	0.008
Dorea	0.31 ± 0.36	0.08 ± 0.05	0.02
Tyzzerella	0.30 ± 0.42	0.24 ± 0.39	0.036
Oscillibacter	0.32 ± 0.35	0.20 ± 0.20	0.008
Colidextribacter	0.32 ± 0.34	0.15 ± 0.08	0.016
Erysipelotrichaceae UCG 003	1.03 ± 1.20	0.21 ± 0.27	0.008
Bacteroides	20.88 ± 8.36	15.51 ± 10.99	0.04
Significant changes in placebo group			
Lachnospiraceae NK4A136 group	0.07 ± 0.14	0.23 ± 0.40	0.03
Blautia	2.10 ± 1.12	1.65 ± 1.04	0.01
Acidaminococcus	0.94 ± 1.54	0.41 ± 0.86	0.01
Eubacterium eligens group	0.26 ± 0.67	0.21 ± 0.53	0.04

*Genus significantly modified after 17 days of treatment were identified using Wilcoxon paired test.



Supplemental figure 4: Partial correlations between biological and microbial data
Spearman partial correlations between the variations (T2-T1) of liver, microbial translocation, inflammatory markers and α diversity and microbial genera in progressive ALD subjects. Correlations were adjusted for gender and the quantity of ethanol consumed during the second week of the program. *p<0.05, **p<0.01,***p<0.001. n=20.

3.7 References

1. WHO | Global status report on alcohol and health 2018. *WHO*. http://www.who.int/substance_abuse/publications/global_alcohol_report/en/. Accessed January 11, 2019
2. Maccioni L, Gao B, Leclercq S, et al. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. *Gut Microbes*. 2020;12:1782157.
3. Starkel P, Leclercq S, de Timary P, Schnabl B. Intestinal dysbiosis and permeability: the yin and yang in alcohol dependence and alcoholic liver disease. *Clinical Science*. 2018;132:199.
4. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor I and mediates alcoholic liver disease in mice. *Hepatology*. 2015;61:883–894.
5. Hendrikx T, Duan Y, Wang Y, et al. Bacteria engineered to produce IL-22 in intestine induce expression of REG3G to reduce ethanol-induced liver disease in mice. *Gut*. 2019;68:1504–1515.
6. Chen P, Torralba M, Tan J, et al. Supplementation of saturated long-chain fatty acids maintains intestinal eubiosis and reduces ethanol-induced liver injury in mice. *Gastroenterology*. 2015;148:203-214.e16.
7. Hartmann P, Chen P, Wang HJ, et al. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. *Hepatology*. 2013;58:108–119.
8. Wang L, Fouts DE, Stärkel P, et al. Intestinal REG3 Lectins Protect against Alcoholic Steatohepatitis by Reducing Mucosa-Associated Microbiota and Preventing Bacterial Translocation. *Cell Host Microbe*. 2016;19:227–239.
9. Wilkin RJW, Lalor PF, Parker R, Newsome PN. Murine Models of Acute Alcoholic Hepatitis and Their Relevance to Human Disease. *The American Journal of Pathology*. 2016;186:748–760.
10. Tabakoff B, Hoffman PL. Animal models in alcohol research. *Alcohol Res Health*. 2000;24:77–84.
11. Cederbaum AI. Alcohol Metabolism. *Clinics in Liver Disease*. 2012;16:667–685.
12. J M, Cc H. Of mice and not men: differences between mouse and human immunology. *Journal of immunology (Baltimore, Md : 1950)*. 2004;172.
13. Nguyen TLA, Vieira-Silva S, Liston A, Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech*. 2015;8:1–16.
14. Gao B, Xu M-J, Bertola A, Wang H, Zhou Z, Liangpunsakul S. Animal Models of Alcoholic Liver Disease: Pathogenesis and Clinical Relevance. *Gene Expr*. 2017;17:173–186.
15. Leclercq S, de Timary P, Stärkel P. Targeting the gut microbiota to treat alcoholic liver diseases: evidence and promises. *Acta Gastroenterol Belg*. 2020;83:616–621.
16. Gibson GR, Hutkins R, Sanders ME, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol*. 2017;14:491–502.

17. Leclercq S, Matamoros S, Cani PD, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci USA*. 2014;111:E4485-4493.
18. Dewulf EM, Cani PD, Claus SP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut*. 2013;62:1112–1121.
19. Le Bastard Q, Chapelet G, Javaudin F, Lepelletier D, Batard E, Montassier E. The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. *Eur J Clin Microbiol Infect Dis*. 2020;39:403–413.
20. Ouwehand AC, Bergsma N, Parhiala R, et al. Bifidobacterium microbiota and parameters of immune function in elderly subjects. *FEMS Immunology & Medical Microbiology*. 2008;53:18–25.
21. Whorwell PJ, Altringer L, Morel J, et al. Efficacy of an encapsulated probiotic Bifidobacterium infantis 35624 in women with irritable bowel syndrome. *Am J Gastroenterol*. 2006;101:1581–1590.
22. Sokol H, Pigneur B, Watterlot L, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci U S A*. 2008;105:16731–16736.
23. Miquel S, Martín R, Rossi O, et al. Faecalibacterium prausnitzii and human intestinal health. *Curr Opin Microbiol*. 2013;16:255–261.
24. Kellow NJ, Coughlan MT, Reid CM. Metabolic benefits of dietary prebiotics in human subjects: a systematic review of randomised controlled trials. *Br J Nutr*. 2014;111:1147–1161.
25. Stachowska E, Portincasa P, Jamioł-Milc D, Maciejewska-Markiewicz D, Skonieczna-Żydecka K. The Relationship between Prebiotic Supplementation and Anthropometric and Biochemical Parameters in Patients with NAFLD-A Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Nutrients*. 2020;12:E3460.
26. Meyer D, Stasse-Wolthuis M. The bifidogenic effect of inulin and oligofructose and its consequences for gut health. *Eur J Clin Nutr*. 2009;63:1277–1289.
27. Kolida S, Tuohy K, Gibson GR. Prebiotic effects of inulin and oligofructose. *Br J Nutr*. 2002;87 Suppl 2:S193-197.
28. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut*. 2009;58:1091–1103.
29. Yasuda K, Dawson HD, Wasmuth EV, et al. Supplemental Dietary Inulin Influences Expression of Iron and Inflammation Related Genes in Young Pigs. *The Journal of Nutrition*. 2009;139:2018–2023.
30. Wang Z, Zhang X, Zhu L, et al. Inulin alleviates inflammation of alcoholic liver disease via SCFAs-inducing suppression of M1 and facilitation of M2 macrophages in mice. *Int Immunopharmacol*. 2020;78:106062.
31. Yang X, He F, Zhang Y, et al. Inulin Ameliorates Alcoholic Liver Disease via Suppressing LPS-TLR4-MΨ Axis and Modulating Gut Microbiota in Mice. *Alcohol Clin Exp Res*. 2019;43:411–424.

32. Hiel S, Gianfrancesco MA, Rodriguez J, et al. Link between gut microbiota and health outcomes in inulin -treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. *Clinical Nutrition*. 2020;S0261561420301606. doi:10.1016/j.clnu.2020.04.005
33. Farhangi MA, Javid AZ, Dehghan P. The effect of enriched chicory inulin on liver enzymes, calcium homeostasis and hematological parameters in patients with type 2 diabetes mellitus: A randomized placebo-controlled trial. *Prim Care Diabetes*. 2016;10:265–271.
34. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutr*. 2014;65:117–123.
35. Daubioul CA, Horsmans Y, Lambert P, Danse E, Delzenne NM. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. *Eur J Clin Nutr*. 2005;59:723–726.
36. Flisiak-Jackiewicz M, Bobrus-Chociej A, Tarasów E, Wojtkowska M, Białokoz-Kalinowska I, Lebensztejn DM. Predictive Role of Interleukin-18 in Liver Steatosis in Obese Children. *Can J Gastroenterol Hepatol*. 2018;2018:3870454.
37. Bachmann M, Pfeilschifter J, Mühl H. A Prominent Role of Interleukin-18 in Acetaminophen-Induced Liver Injury Advocates Its Blockage for Therapy of Hepatic Necroinflammation. *Front Immunol*. 2018;9:161.
38. Van der Meulen R, Makras L, Verbrugghe K, Adriany T, De Vuyst L. In Vitro Kinetic Analysis of Oligofructose Consumption by *Bacteroides* and *Bifidobacterium* spp. Indicates Different Degradation Mechanisms. *Appl Environ Microbiol*. 2006;72:1006–1012.
39. Van der Meulen R, Adriany T, Verbrugghe K, De Vuyst L. Kinetic Analysis of Bifidobacterial Metabolism Reveals a Minor Role for Succinic Acid in the Regeneration of NAD $^{+}$ through Its Growth-Associated Production. *Appl Environ Microbiol*. 2006;72:5204–5210.
40. Reimer RA, Willis HJ, Tunnicliffe JM, Park H, Madsen KL, Soto-Vaca A. Inulin-type fructans and whey protein both modulate appetite but only fructans alter gut microbiota in adults with overweight/obesity: A randomized controlled trial. *Mol Nutr Food Res*. 2017;61:1700484.
41. Vandeputte D, Falony G, Vieira-Silva S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. *Gut*. 2017;66:1968–1974.
42. Deng F, Li Y, Zhao J. The gut microbiome of healthy long-living people. *Aging*. 2019;11:289–290.
43. Mosca A, Leclerc M, Hugot JP. Gut Microbiota Diversity and Human Diseases: Should We Reintroduce Key Predators in Our Ecosystem? *Front Microbiol*. 2016;7.
44. Addolorato G, Ponziani FR, Dionisi T, et al. Gut microbiota compositional and functional fingerprint in patients with alcohol use disorder and alcohol-associated liver disease. *Liver Int*. 2020;40:878–888.
45. Qin N, Yang F, Li A, et al. Alterations of the human gut microbiome in liver cirrhosis. *Nature*. 2014;513:59–64.
46. Chen Y, Yang F, Lu H, et al. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology*. 2011;54:562–572.

47. de Faria Ghetti F, Oliveira DG, de Oliveira JM, de Castro Ferreira LEVV, Cesar DE, Moreira APB. Influence of gut microbiota on the development and progression of nonalcoholic steatohepatitis. *Eur J Nutr*. 2018;57:861–876.
48. Fukui H. Role of Gut Dysbiosis in Liver Diseases: What Have We Learned So Far? *Diseases*. 2019;7:58.
49. Philips CA, Augustine P, Yerol PK, et al. Modulating the Intestinal Microbiota: Therapeutic Opportunities in Liver Disease. *J Clin Transl Hepatol*. 2020;8:87–99.
50. Mutlu EA, Gillevet PM, Rangwala H, et al. Colonic microbiome is altered in alcoholism. *American Journal of Physiology-Gastrointestinal and Liver Physiology*. 2012;302:G966–G978.
51. Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. *Br J Nutr*. 2018;119:176–189.
52. O'Callaghan A, van Sinderen D. Bifidobacteria and Their Role as Members of the Human Gut Microbiota. *Front Microbiol*. 2016;7.
53. Keshavarzian A, Choudhary S, Holmes EW, et al. Preventing gut leakiness by oats supplementation ameliorates alcohol-induced liver damage in rats. *J Pharmacol Exp Ther*. 2001;299:442–448.
54. Forsyth CB, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. Lactobacillus GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol*. 2009;43:163–172.
55. Dubinkina VB, Tyakht AV, Odintsova VY, et al. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome*. 2017;5:141.
56. Singh V, Yeoh BS, Chassaing B, et al. Dysregulated Microbial Fermentation of Soluble Fiber Induces Cholestatic Liver Cancer. *Cell*. 2018;175:679-694.e22.
57. Weitkunat K, Schumann S, Petzke KJ, Blaut M, Loh G, Klaus S. Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice. *The Journal of Nutritional Biochemistry*. 2015;26:929–937.
58. Li L-L, Wang Y-T, Zhu L-M, Liu Z-Y, Ye C-Q, Qin S. Inulin with different degrees of polymerization protects against diet-induced endotoxemia and inflammation in association with gut microbiota regulation in mice. *Sci Rep*. 2020;10:978.
59. Stärkel P, Schnabl B. Bidirectional Communication between Liver and Gut during Alcoholic Liver Disease. *Semin Liver Dis*. 2016;36:331–339.
60. Guy J, Peters MG. Liver Disease in Women: The Influence of Gender on Epidemiology, Natural History, and Patient Outcomes. *Gastroenterology & Hepatology*. 2013;9:633.
61. Buzzetti E, Parikh PM, Gerussi A, Tsochatzis E. Gender differences in liver disease and the drug-dose gender gap. *Pharmacological Research*. 2017;120:97–108.

GENERAL DISCUSSION AND FUTURE PERSPECTIVES

1 Summary of the main outcomes

AUD is a major public health problem affecting 5 to 10% of the population in developed countries. AUD patients are prone to develop emotional and cognitive symptoms that contribute to the persistence of addictive behaviour [302] and to the risk of relapse [465]. Pharmacological agents have been developed in order to reduce the frequency of heavy drinking and alcohol craving but clinical studies reported a small efficacy of theses drugs [153], and a high proportion of relapse (70%) among the recently detoxified AUD subjects [138]. Although AUD has traditionally been considered a brain disease, other organs are damaged, notably the gut and the liver. In this thesis work we focused on the role of the gut microbiota in the biological (metabolism, liver damages and inflammation) and psychological alterations induced by chronic alcohol consumption.

The gut microbiota is now considered a potential new therapeutic target due to its key role on host physiology and behaviour [293,301,466,467]. Altered composition of the gut microbiota has been reported in several diseases such as obesity, diabetes or Crohn's disease and, more recently, in diseases with neurological components such as autism and AUD [468–470]. Gut dysbiosis has been linked with intestinal permeability that promotes the translocation of gut-derived bacterial components in bloodstream which can in turn activate the inflammatory response. The latter is known to trigger psychological and liver alterations in several disorders including in AUD [293,298,301].

ITF are interesting DF that respond to the definition of prebiotic: they are used as substrates by selective gut microorganisms and confer health benefit to the host [348]. The effects of ITF on gut health, inflammation and metabolism have been widely studied particularly in the context of obesity and metabolic disorders. More recently, ITF treatment in mice has been shown to exert antidepressant and anxiolytic effects, improve brain function and reduces stress-induced corticosterone release and pro-inflammatory cytokines [402,403].

In this PhD work, we hypothesized that malnutrition in AUD patients can contribute to alterations of the gut microbiota and psychological disorders, and that modulating the gut microbiota by supplementation with inulin in the period of alcohol withdrawal can positively impact host health and behaviour.

In the first chapter, we demonstrated that AUD patients have disorganized dietary habits and that alcohol account for almost 40% of their total energy intake. We also showed that AUD patients consumed less fiber, both soluble and insoluble, and that the

latter were associated with higher sociability scores. Fructans intake was negatively associated with anxiety scores meaning that patients that consumed more fructans were less anxious.

Social functioning has been shown to be impaired in various psychiatric disorders including AUD. Recently, a link between sociability and the gut microbiota has been suggested in both clinical and preclinical studies. We therefore wanted to better understand the relationship between the gut microbiota and social functioning in actively drinking AUD patients (chapter 2).

In our sample, 16 out of 46 patients were considered dysbiotic. Non-dysbiotic patients had a microbial composition comparable to healthy subjects whereas dysbiotic subjects had more *Parabacteroides*, *Lachnoclostridium*, *Erysipelatoclostridium* and *Flavonifractor* and less *Ruminococcus*, *Christensenellaceae R7 group*, *Oscillospiraceae NK4A214 group* and *Oscillospiraceae UCG 003* compared to healthy subjects. We found that dysbiotic subjects were younger and thinner than non-dysbiotic subjects and that their craving score was significantly higher. Interestingly, we observed that dysbiotic patients had a smaller and less connected social network than non-dysbiotic subjects. Patients with a more altered gut microbiota also tended to give more emphasis to their perspective than to the other's. No differences were observed in terms of nutritional profile or medication.

In the last chapter we demonstrated that inulin supplementation during 17 days was well tolerated by AUD patients and induced significant changes in gut microbiota composition (8 genera including *Bifidobacterium*). Inulin supplementation had no effect on biological parameters except BDNF which increased in inulin group compared to placebo. No significant differences were observed for the change in depression, anxiety or alcohol craving between inulin and placebo groups during alcohol withdrawal. However patients supplemented with inulin displayed an increased sociability score after 17 days of supplementation compare to placebo.

Regarding liver and inflammatory parameters, patients supplemented with inulin had higher levels of AST and ALT at the end of the detoxification program compared to placebo. They also displayed a higher IL-18 level. The liver enzymes were also higher in inulin group compare to placebo when we stratified the population according to the severity of liver disease. Those data suggest that when administrated during the

withdrawal procedure, inulin does not improve, and even slightly worsens, the hepatic alterations.

We propose to put those conclusions in a more general context, and to discuss some points requiring attention. First, we will discuss the advantages and disadvantages of our study design. Next, we will focus on the link between nutrition, and in particular dietary fiber, and psychological symptoms. We will also address the particularly interesting link between gut microbiota and sociability. Finally, we will discuss the impact of inulin on the composition of the gut microbiota and on inflammation and liver parameters.

2 General discussion

Advantages and limitations of the study design

The detoxification program instaured at St Luc Hospital provided highly standardised conditions to unravel the role of the gut microbiota and nutrition in AUD patients. Indeed, the patients received the same hospitalisation program, including for all a similar period "at home" in the middle of the withdrawal procedure and the same psychological support. The patients were tested before and after inulin/placebo supplementation using the same standardised tests, methods and conditions including the experimenter which limits bias. Furthermore, all the samples were taken and stored in an adequate manner to ensure appropriate analysis. Another strength of this study is that factors that could influence psychological symptoms or biological data (and notably the gut microbiota) such as nutrition or medication were also carefully collected in this study.

We conducted a randomised, double-blind, placebo-controlled study. This design avoids bias and ensures comparability of groups on all factors except treatment. The double-blind design was maintained until the end of the study to avoid the influence of the judgement criteria and to maintain the comparability between the groups throughout the trial. In our case a gender difference was observed between the placebo and inulin groups at baseline. In the case of small sample sizes, it is possible that an imbalance between groups occurs [471]. In order to minimise the risk of bias we therefore adjusted all analyses for gender.

The design of the detoxification program included a week at home. During this week some patients relapsed. This has the advantage of studying the effect of inulin under representative conditions, as AUD is a disease with a high rate of relapse [472]. However, this may also introduce a bias if the number of relapses is not well distributed between the two intervention groups. In our study 8 patients relapsed during the second week of the program (at home) in placebo group vs 12 in inulin group (32% vs 48% respectively $p=0.25$). Even if the difference was not statistically significant we adjusted the analyses on this parameter because alcohol consumption influences microbial changes but also the recovery of psychological and biological parameters [119,293].

The definition of relapse is complex and requires to distinguish simple lapse (one drink or brief episode) from heavy relapse (4 -5 or more drinks in a day during several consecutive days) [473]. We therefore adjusted the analyses on the quantity of alcohol consumed, which is more informative than a qualitative variable (relapse: yes/no).

As mentioned, patients were recruited in the context of a detoxification programme. This had the advantage to be highly standardised conditions, however the population of AUD patients recruited is not representative of the general population of AUD patients. Indeed, in this work, the patients selected are patients who were hospitalised on a voluntary basis, and are therefore patients who are motivated to undertake withdrawal.

Malnutrition in AUD patients: focus on the relationship between dietary fiber intake and psychological symptoms

We studied the intake of different types of soluble, insoluble fibers and prebiotic (oligo)saccharides (FOS and GOS) in AUD patients. To our knowledge these intakes have never been studied in patients diagnosed with severe AUD. For this purpose we used a tool developed in the FiberTag study [474]. This tool allowed us to take into account the different types of fibers including the prebiotics fructans and galactans, that are missing in most official nutritional database such as the Belgian nutritional database Nubel.

Dietary fibers (DF) can be classified according to their structure or their physiological and physicochemical properties (fermentability, solubility in water, viscosity). In our study DF are classified according to water solubility which depends on the bonds between the monomeric units, the nature of the monomeric units and the degree of polymerisation [474]. The classification according to the solubility of fibers is highly debated because there are exceptions, notably resistant starch which is insoluble in water and highly fermentable. It has been proposed to classify fibers according to 1) their solubility and viscosity, 2) their fermentability in the colon and 3) bulking effect in the colon. However, data on all these characteristics are not yet available for many dietary fiber types and sources [446].

Soluble fibers are often considered highly fermentable fibers (e.g. β-glucan, pectin, psyllium, inulin) whereas insoluble fibers are partially fermented or not fermented by gut bacteria (e.g. cellulose, hemicellulose, chitosan, lignin) [475,476]. Insoluble fibers have a high water-retaining capacity and have been shown to decrease intestinal transit speed

[477]. In contrast, the majority of soluble fibers do not contribute to stool bulk, while they increase transit time and are fermented by gut bacteria and thus produce SCFAs [476]. The type of fiber, according to their origin, chemical composition and physicochemical properties, can therefore affect differently host physiology (**Figure 16**). It is therefore interesting to study each fiber types in relation to gastrointestinal symptoms, biological parameters and mood [478].

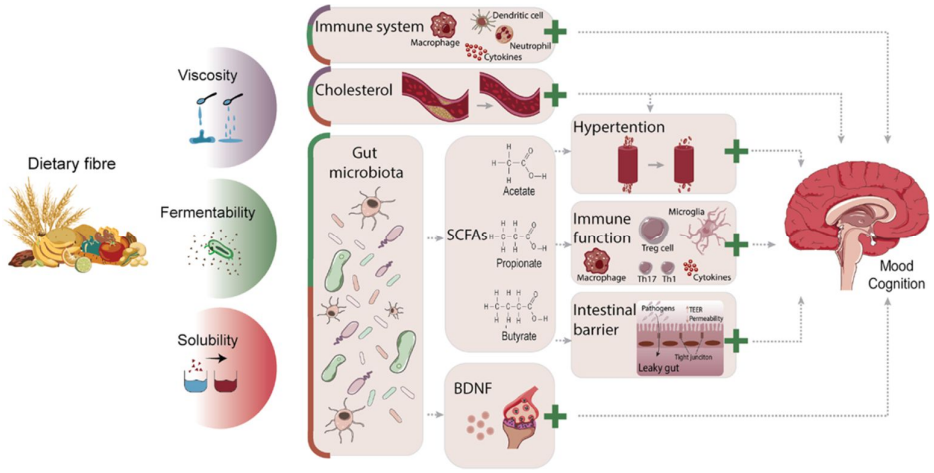


Figure 16: Potential biological mechanisms underlying the effects of dietary fiber on mood and cognition based on its physicochemical properties [478]

We found a positive correlation between satisfaction with gastrointestinal symptoms and soluble fibers intake. Soluble fiber consumption was also associated with a lower gastrointestinal discomfort score, whereas no association was found with insoluble fibers (Chapter 1). These results are consistent with an interventionnal study conducted in patients with IBD over a 12-week period. It was demonstrated that patients supplemented with psyllium, a soluble fiber, had an improvement in the severity of IBD symptoms. In contrast, patients supplemented with bran, an insoluble fiber, showed no improvement of those symptoms [479].

Concerning the link with behaviour and psychological alterations, the dietary source of fiber has been differentially associated with the prevalence of depression in a cross-sectional study of nearly 3000 Korean adults [480]. In the chapter 1, we observed an association between the severity of psychological symptoms and fiber intake at baseline.

A negative association was found between fructans and anxiety, with patients consuming more fructans having lower anxiety scores. We also observed a positive association between sociability score and soluble and insoluble fiber intake. Knowing this, we expected to observe an effect of inulin supplementation on anxiety. However, we observed an effect of inulin supplementation on sociability, but not on anxiety. It is possible that in our intervention study, the duration of supplementation was too short to observe an effect on anxiety. Indeed studies that have shown an effect of dietary prebiotic fibers on anxiety scores used a higher duration of supplementation. For example in IBD 4 weeks of short chain FOS or 12 weeks of trans-galactooligosaccharide improved anxiety score [395,481].

To avoid side effects, the dose of inulin was gradually increased from 4 to 16g during the study. Patients were supplemented with 4g for 2 days, 8g for 10 days and 16g only for 5 days. It is possible that significant changes in the gut microbiota took some time to occur, thus limiting the influence on biological and psychological parameters. Although Bouhnik *et al* showed that a 5g inulin supplementation for 7 days had a bifidogenic effect in healthy subjects, it is possible that the latency is longer in subjects with an altered microbiota [331]. A stool sample at several time points would have allowed us to determine whether or not the microbial changes occurred quickly after the inulin intake.

Furthermore, since alcohol withdrawal itself has a very important effect of improving anxiety, craving and depression at 3 weeks, it is possible that, if any, the more moderate effect of inulin supplementation has been masked.

Finally, sociability, unlike the others, did not improve during withdrawal. Maybe this is why we were able to observe a moderate positive effect of inulin.

The link between the gut microbiota and sociability in AUD patients

We have highlighted a link between sociability and the gut microbiota in chapters 2 and 3. In the second chapter, we demonstrated that subjects with altered gut microbiota had a smaller and less connected social network and tended to have difficulty taking the perspective of others. Although this study is exploratory and limited in terms of subjects, it reveals a novel aspect namely the link between social network and gut microbiota composition, which had never been established in AUD patients. This is a cross-sectional study, and therefore, it does not allow to prove causality. We do not know if it is the reduced social network that leads to dysbiosis, or if dysbiosis will lead to a lower openness to the outside world. In the light of the literature, it seems that the relationship between intestinal microbiota and sociability is bidirectional. Indeed, studies suggest that social behaviour favours a horizontal transmission of microbes between individuals. Observational studies in humans and animals have shown an impact of social group and social network on microbial composition and diversity [181,482,483].

The causal role of the microbiome on sociability was revealed first using GF mice. Indeed these mice exhibit social impairment that was normalised following GF bacterial colonisation [484]. Then, by transplanting fecal microbiota from offspring of high-fat fed dams presenting social deficit to GF mice, it has been shown that recipient mice developed social behavioural disorders and showed impaired long-term potentiation in the ventral tegmental area, as well as reduced oxytocin-expressing neurons [447]. Several interventional studies have also shown a positive impact of pro- or prebiotics on social behaviour in children with autism [451,485,486]. In the context of AUD it has been shown that transferring the gut microbiota of AUD patients to antibiotic-treated mice induced alterations in social behaviour [251]. In chapter 3, we also showed that 17 days of inulin supplementation increased the sociability score.

The mechanisms by which the gut microbiota influences sociability are still poorly understood, however, many pathways have been proposed in the literature (**Figure 17**). Preclinical studies have demonstrated the involvement of the vagus nerve, the immune system and bacterial metabolites. Through these pathways, the gut microbiota could affect neuroinflammation, myelination, oxytocin secretion and therefore influence social behaviour [487]. It has also recently been shown that intestinal bacteria can influence the stress response by limiting the activation of the HPA axis and thus restore the social deficit in mice [488].

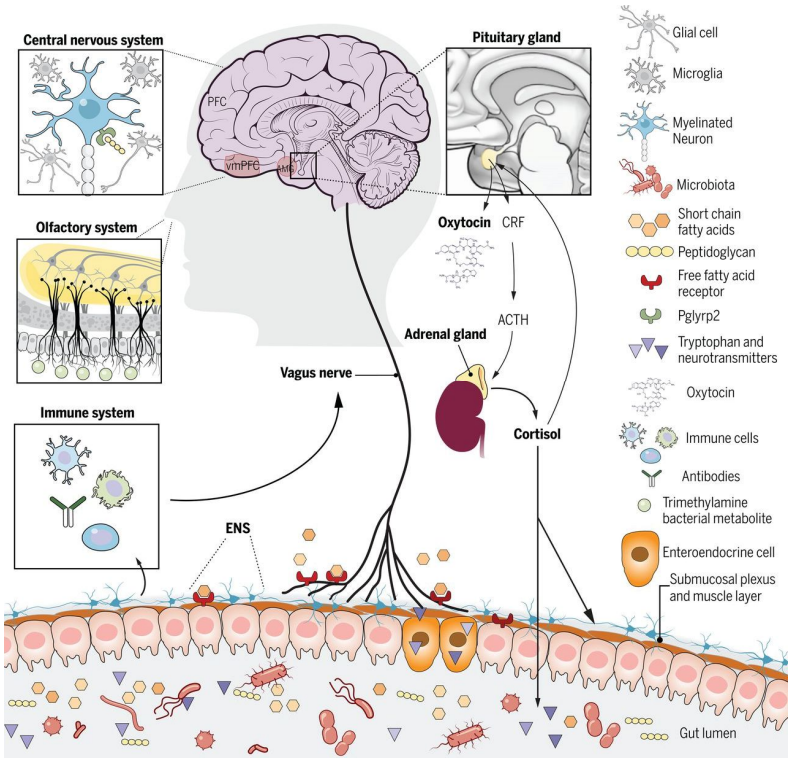


Figure 17 : Biological pathways through which the gut microbiota may influence the social behaviour [487]

Numerous studies have demonstrated alexithymia in AUD patients that was maintained after 3 weeks of withdrawal [489–491]. Recognizing emotions and ability to communicate them to others, are two skills that underlie social interactions and thus this can have a significant impact on the interpersonal relationships of alcohol-dependent individuals. AUD patients also exhibit impaired emotional intelligence which has been associated with problematic drinking behaviours [492–494]. Impaired abilities for taking the perspective of others have also been observed in AUD patients [495].

Social difficulties could be a factor in sustaining alcoholism as well as a risk factor for relapse. In our study, social activity scores were negatively correlated with anxiety and alcohol craving at baseline (**Figure 18**). The ego bias was associated with higher depression and anxiety score while alter bias was negatively associated with alcohol craving in actively drinking AUD patients (**Figure 18**). Furthermore, social difficulties as well as poor emotion recognition performance have been shown to be predictors of relapse [94,496]. Knowing this, understanding the role that social cognition plays in AUD could lead to the development of prevention and intervention strategies to promote abstinence and support long-term recovery from addiction. Since microbiota is a modifiable factor, it could be an interesting target to influence social behaviour and thus the risk of relapse.

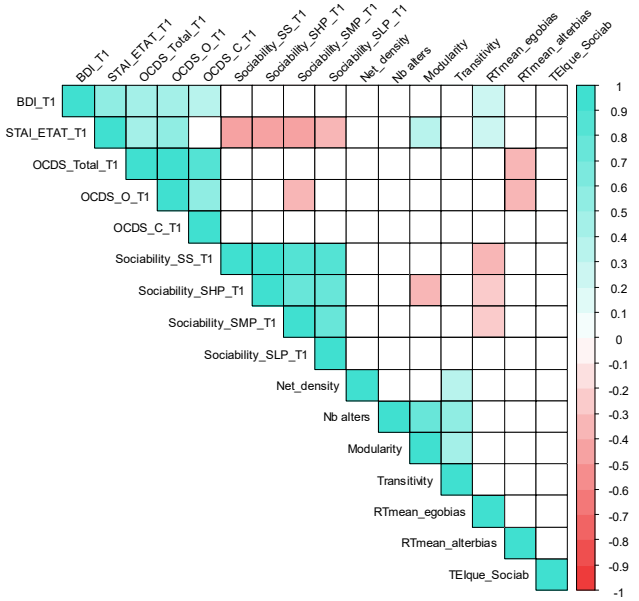


Figure 18: Correlation matrix between mood, alcohol craving and sociability scores in AUD patients at baseline (T1). Colored boxes represent significant correlations ($p<0.05$). Non-significant correlations are represented by white boxes. OCDS: Obsessive Compulsive Drinking Scale; OCDS C: Obsessive Compulsive Drinking Scale, compulsive score; OCDS O: Obsessive Compulsive Drinking Scale, obsessive score. SHP: social high pleasant activity; SMP: social medium pleasant activity; SLP: social low pleasant activity; TEI: trait emotional intelligence questionnaire.

Despite some limitations, our work allows us to highlight the role of the gut microbiota in sociability and its potential consequences in AUD. We took into account two important confounding factors the medications and the diet [175,497]. Furthermore, even after adjustment for the familial status and the number of children the results remained

significant (data not shown) meaning that the differences observed for the social network structure were not due to these factors. However the use of the term “dysbiosis” is widely debated in the literature [498,499]. We choose this term to define AUD patients who had an altered gut microbiota compare to HS. However, the number of HS recruited in the study is limited and therefore not representative of the healthy Belgian population. This group of patients could have been called “altered gut microbiota”. Moreover there is no consensus regarding the “normal” gut microbiota definition, it would therefore be important to increase the sample size of HS. This study is exploratory and further research would allow to confirm the reproducibility of these results.

Inulin supplementation induces specific changes in gut microbiota composition of AUD patients

Inulin has been described as the first non digestible carbohydrate able to modulate the gut microbiota in favour of *Bifidobacterium*, leading to the concept of prebiotics in the ninetee’s. The relevance of gut microbiota modification by inulin in AUD patients was questioned, since it was considered as the rationale to conduct an intervention study. Our pilot study allowed us to demonstrate for the first time that 17 days of inulin supplementation in AUD patients induces significant changes in the composition of the gut microbiota. We observed an increase in the relative abundance of *Bifidobacterium* and a decrease in *Bacteroides*, *Dorea* and *Ruminococcus torques*. These results are consistent with the systematic review conducted by Le Bastard and the work of Healey *et al* [354,358]. The Bifidogenic effect is considered as the signature of inulin intake. Among the 19 patients for whom we had sequencing data of the intestinal microbiota in the inulin group, 17 had an increase in the relative abundance of *Bifidobacterium*, which confirms the good compliance with the intervention (98%). However, a large variability in the response has been observed. As it has been shown that the response to inulin may depend on dietary fiber intake [277] or on the baseline microbiota [278], it would be interesting to investigate these aspects in future larger studies. In our study, we observed a negative correlation between the amount of bifidobacteria at baseline and the variation between T1 and T2 (**Figure 19**). The increase in bifidobacteria was more pronounced in patients with lower baseline bifidobacteria concentration than in those with higher baseline bifidobacteria concentration (**Figure 19**). This is consistent with many studies

[360,361,500,501]. No correlation was observed between the amount of dietary fiber consumed and the change in *Bifidobacterium* between T1 and T2 (**Figure 19**).

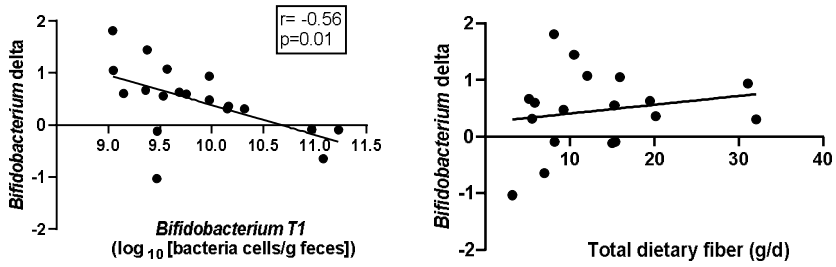


Figure 19: Associations between the change of *Bifidobacterium* between T1 and T2 and the baseline level of *Bifidobacterium* or the dietary fiber intake in AUD patients supplemented with inulin

In parallel, we observed a decrease in microbial diversity after inulin supplementation. This result is not surprising since it has been observed in other interventionnal studies conducted in different pathological contexts that ITF supplementation decreased or had no effect on microbial diversity [354–357].

This effect can be explained by different parameters. First, we showed that supplemented patients had a significant increase of *Bifidobacterium* (Percentil of Bifidobacterium delta (T1-T2): Q1=0.89%; Q2=4.5%; Q3=7.4%). No other bacteria increased significantly after supplementation. It is possible that *Bifidobacterium* has taken the place of other bacteria in the ecosystem which could explain the decrease in diversity. Furthermore, we supplemented the patients with a single type of fiber known for its bifidogenic effect. The healthy food diversity index has been shown to be positively associated with α-diversity indices in the literature, meaning that a healthy diverse diet promotes a more diverse gut microbiota [502]. We have seen in chapter 1 that AUD patients had an unbalanced diet rich in ultra-processed food known to be associated with a decrease in microbial diversity [503]. Thus, it is not surprising that a nutritional approach with one single prebiotic fiber did not increase diversity. Moreover, it has been shown in a study from Zhao and colleagues that a diet rich in prebiotic fibers can decrease diversity [504].

Effect of inulin supplementation on BDNF

In chapter 3, besides the effect of inulin supplementation on sociability we observed an effect on plasma BDNF levels in AUD patients. This is particularly interesting knowing the various effect of this neurotrophin on brain function and behaviour. BDNF is expressed

in many brain structures but the highest levels were found in the hippocampus [505]. It is also expressed at the peripheral level (blood, immune cells, liver) and has been shown to be able to cross the BBB [505]. BDNF has a major role during development via the regulation of dendritic spine maturation and pruning and plays an important role in neuronal growth, differentiation and survival [506,507]. In adults, BDNF is also critical as it is involved in synaptic plasticity, learning and memory [507]. It has been shown that serum and cerebral BDNF levels are decreased in depressed, bipolar and schizophrenic patients [508–510]. In actively drinking AUD patients, serum BDNF levels are decreased compared to healthy subjects and increase again after 1 to 7 days of withdrawal [511–513].

In our study, plasma BDNF levels increased significantly in subjects supplemented with inulin compared to placebo. This result is consistent with preclinical studies showing an increase in brain BDNF levels with prebiotic fiber supplementation in rodents [403,514,515]. Indeed, it has been shown that fibers can increase the level of circulating BDNF via the increase of *Bifidobacterium* and *Lactobacillus* [516,517]. Regarding the possible mechanisms, the SCFA, known to be promoted by some prebiotics intervention, have been demonstrated in mice to increase brain BDNF levels [518]. In humans, colonic administration of SCFA has not shown any effect on BDNF levels [338]. Further studies are needed to understand the mechanisms underlying the effect of prebiotic fibers on BDNF levels.

In humans, the measurement of central BDNF levels, via the cerebrospinal fluid, is too invasive, therefore we measured it in the plasma. As mentioned previously, BDNF is able to cross the BBB in a bidirectional manner and preclinical studies have shown that peripheral levels are correlated with central levels. Thus peripheral BDNF levels are generally considered to reflect those of the CNS [511]. The methodology for measuring BDNF in the periphery is highly variable in the literature and much discussed [519,520]. BDNF is stored in platelets and it has been shown that plasma BDNF (free BDNF) is a better reflect of BDNF present in the brain than serum BDNF [519].

Effect of inulin on inflammatory and hepatic parameters

It is now well recognized that gut microbiota largely influence inflammatory parameters and hepatic function [298,521,522]. Beside its effect on brain function and behaviour, inflammation is also tightly related to liver function [521,523]. In the chapter 3 we have explored the effect of inulin on both hepatic enzymes and inflammatory markers.

We have seen that AST and ALT return to normal values after 17 days of abstinence in the placebo group. However it was not the case for all the patients in inulin group. The level of CK18-M65 decreased significantly between T1 and T2 but remained higher than HS at T2. This confirms previous studies showing that 3 weeks of abtinence are sufficient to normalize or partially normalize liver damages. Conversely, parameters such as systemic inflammation did not improve with abstinence. This is consistent with previous observations [119,301]. It thus seems that systemic inflammation is not the main cause of hepatic alterations. This confirms the major impact of ethanol consumption compared to other parameters (gut microbiota, inflammation) on the liver alterations in patients without severe liver disease [524–526]. Of note, it has been shown that recovery depends on the degree of liver damage [524].

It has been shown that three weeks to 1 month of abstinence produces only incomplete recovery of the gut microbiota composition [293,294] indicating that alcohol consumption has a more lasting effect on gut dysbiosis. In the present work, we also observe that regardless of the treatment group the bacterial profile of AUD patients does not cluster with the control group (CT) at T2 (**Figure 20**). This could explain the maintenance of systemic inflammation in AUD patients after 3 weeks. In this case, it is possible that the way the 3 weeks-inulin supplementation affect the gut microbiota (mainly a bifidogenic effect) is not sufficient to act on inflammatory parameters. Thus, other gut microbiota targeting approaches could be more suitable to restore these parameters.

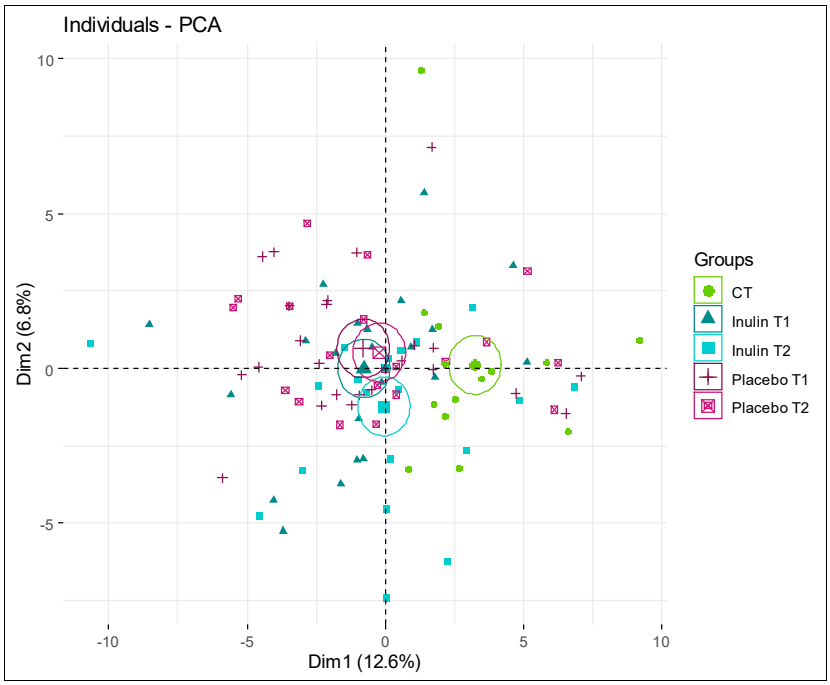


Figure 20: Principal component analysis with all microbial genera of healthy subjects and AUD patients according to the intervention group

3 Future perspectives

Improvement of study design

- Stratified randomization and increased sample size

As mentioned in the previous part we observed a dysbalance in gender proportion between groups. In the future it would be interesting to use gender-stratified randomization. This kind of stratification consists of generating an independent randomization list for each combination of strata of the factor to be considered. This ensures that the numbers in the strata combinations are homogeneously distributed between the treatment groups [471]. Indeed, gender is an important confounding factor, especially in the context of AUD. The recovery trajectory in terms of psychological and biological parameters is different between men and women [527–529].

In our study we have seen that different factors could modify the effect of inulin on the outcomes studied. This is the case of gender and relapse. We adjusted our analyses on these factors to avoid bias but it would be interesting to perform stratified analyses to see if inulin has a different effect according to these factors. Unfortunately our sample size did not allow us to perform such analyses but in future studies these factors should be taken into account. In order to distribute them equally between the groups of treatment, these factors could be taken into account during randomization.

We have also seen that initial bifidobacteria levels can potentially influence the response to inulin. Increasing the sample size would also allow us to see if the effects of inulin on biological and psychological outcomes depend on the basal composition of the gut microbiota. It is likely that inulin has a greater effect on those with with a greater increase in the amount of fecal *Bifidobacterium.*

Globally, the sample size is the biggest limitation of this study. Given the inter-individual variability (in terms of microbial composition, liver alterations, psychological parameters and response to supplementation) and the different potential modification factors (gender, relapse, baseline microbiota composition), subgroup analysis might have helped to highlight a treatment effect in a specific subgroup and thus target a specific patient population to propose personalized strategies.

- Study population

In our study, we excluded patients with hepatic fibrosis at the admission and we did not observe marked effects of inulin supplementation. It would be interesting to study the role of gut microbiota in more advanced stages of liver disease were the recovery will be slower after alcohol cessation.

- Duration of supplementation

Our study revealed that inulin supplementation for 17 days had limited effects on biological and psychological parameters. In the context of withdrawal, it might be relevant to supplement patients for a longer period of time in order to study the possible additional effect of a prebiotic. It is known that antidepressants need to be administered for at least 3 weeks to have a significant effect on mood [530]. Although the neurobiological mechanisms underlying the effect of prebiotics on behaviour are still poorly understood, it is possible that more time is needed to observe an effect on mood. To avoid the acute effect of withdrawal on the judgement criteria, it would also have been interesting to supplement patients after this period even if the follow-up would be more complicated to implement and compliance more difficult to reach.

Even if this pilot study does not allow us to conclude on the effect of inulin on anxiety, depression or craving, it could allow us to design a study aimed specifically at studying the effect of prebiotic supplementation on psychological symptoms. Indeed, this study demonstrated the good tolerance of AUD patients to inulin as well as the feasibility of the study design. It could also help to calculate the sample size needed to design a new study where the primary outcome would be psychological symptoms.

Investigation of the link between sociability and the gut microbiota

An interesting perspective of this work would be to study whether patients with lower sociability scores and a less dense social network have a higher risk of relapse. In a future study, it would also be interesting to know if individuals in the social network suffer from AUD. Indeed, the composition and not only the size of the network is important to take into account in order to study the factors influencing relapse. Indeed, it has been shown, with a different methodology, that patients with a network rich in "pro-abstainers" increased the number of days of abstinence while a network rich in "pro-drinkers" decreased it [531].

In order to study the causal role of gut microbiota on social behaviour it would also be interesting to transplant mice with the gut microbiota of dysbiotic or non-dysbiotic patients and look at the impact on behaviour including sociability. This study would also allow to unravel the neurobiological mechanisms involved.

Alternatives to inulin-prebiotic to modulate the gut microbiota composition

The gut microbiome is a modifiable factor that can be modulated through a number of strategies: diet, probiotics, prebiotics, synbiotics or even fecal microbiota transplantation. We have shown that inulin supplementation has few effects on biological and psychological parameters in AUD patients.

As mentioned earlier, different types of fiber may have different effects on metabolism and behaviour. For example, soluble fibers may have an effect on the brain via the SCFA which impact the gut barrier, systemic and central inflammation and BDNF production [478]. FOS or inulin improved alcohol-induced liver injury in mice [390,391,532]. Pectin, another soluble fiber, restored *Bacteroides* levels in mice and prevented alcohol-induced liver injury [533]. Viscous and non-fermentable fibers are still poorly studied and could also have an effect on cognition and/or behaviour even without stimulating SCFA production [478]. Combining different prebiotic fibers known to promote different beneficial bacterial strains can be an interesting approach. Namely, combining pectins, shown to improve liver function and inflammation in mice model of AUD, and inulin could be of interest [533]. If pectin is also a soluble fiber it did not elicited the same changes in the gut microbiota composition compared to inulin with a lower bifidogenic effect and modulation of other genera (*Bacteroides, Prevotella, Faecalibacterium, Roseburia*…) [534–536].

Another possible approach would be to use probiotics like *Lactobacillus* or *Bifidobacterium* which have been shown to improve alcohol-induced liver inflammation and intestinal permeability in preclinical studies [290,537]. In clinical settings, *Bifidobacterium* is known to have a beneficial effect on gut barrier function, immunity and to have positive effects on psychological symptoms in irritable bowel syndrome (IBS) patients [538]. Despite the fact that we observe an increase in Bifidobacteria in our study, it is possible that the stimulated strain is not the right one or that fiber fermentation in AUD patients is not that beneficial.

More and more studies are looking at the use of synbiotics (association of pre and probiotics), complete dietary interventions or combined approaches, and show encouraging results [539–541]. In the case of a complex pathology such as AUD, combining metabolic and psychological disorders, these approaches could be an interesting strategy.

FMT is another innovative approach used firstly to fight resistant infection (*C. Difficile*) [340,341]. A recent phase 1 randomized placebo control study conducted in 20 Cirrhotic patients demonstrated that FMT improved duodenal mucosal diversity, dysbiosis and anti-microbial peptid expression. FMT also reduced lipopolysaccharide-binding protein and improved cognitive performance [345]. As gut barrier disruption, dysbiosis and circulating inflammation are known to promote psychological symptoms, it would be of interest to test this FMT in AUD to tackle concomitantly peripheral and central alterations.

Interest of studying the metabolomic profile of AUD patients

Another future perspective of this work would be to perform a metabolomic analysis on fecal and plasma samples. Despite the fact that PCoA shows no difference in bacterial profile between the placebo and inulin group, some bacterial genera are significantly modulated. Therefore, we cannot exclude that specific bacterial metabolites can be produced and act as physiological modulators via the different pathways described in parts 2.1.2 and 2.2.2. Gut-derived metabolites that could influence host metabolism and/or behaviour include SCFAs, secondary bile acids, indole derivatives, phenols, neurotransmitters (serotonin, GABA) or trimethylamine-N-oxide [542–545]. Several studies have found altered metabolomic profiles in blood, urine, or stool related to alcohol consumption [546,547]. However, few studies have linked alterations in the metabolome with alterations in the gut microbiota, especially in patients without severe liver disease. Dysbiosis can influence the quantity, the absorption and the transfer of metabolites. Patients with increased gut permeability displayed higher fecal levels of phenol and lower levels of indole compared to AUD patients with low gut permeability [293]. Alcohol-induced dysbiosis could also affect the BA profile. Studies demonstrated higher secondary BA profile in the stool and higher serum BA profile of conjugated secondary BAs in actively drinking non-cirrhotic and cirrhotic patients [548–550]. Furthermore, actively drinking cirrhotic patients had decreased levels of AA like threonine and serine compared to controls [550]. Finally, dysbiosis induced by chronic alcohol

consumption has been shown to impair SCFA production and induce a reduction in fecal SCFA content in patients with alcoholic cirrhosis [551]. It has also been shown that patients with alcoholic hepatitis had a quantitative reduction of SCFA in stool compared to high drinking controls [552].

To date, few studies have performed a metabolomic analysis after ITF supplementation. In healthy volunteers, inulin supplementation enriched with oligofructose induced a decrease in urine ammonia and p-cresol excretion in parallel with an increase in bifidobacteria [360]. The effect of ITF on the BA profile was investigated in several studies [356,553–556]. Only one showed that inulin and FOS supplementation in young men significantly altered the bile acid profile, notably by decreasing fecal deoxycholic acid [556]. The authors argued that this decrease in secondary bile acids could be protective and decrease the risk of developing colon cancer [556]. SCFA are produced following ITF fermentation in the colon. In humans, the effect of ITF supplementation on SCFA has shown heterogeneous results [554,557–560]. Studies in healthy or overweight/obese subjects have shown an increase in SCFA with ITF supplementation [558–560] while another showed a decrease in obese women [557]. Thus, it appears that the changes elicited by the same prebiotic can vary a lot depending on the targeted population.

In our studies, characterization of the entire metabolome using non-targeted techniques would be of great interest to link microbial activity to biological and behavioural outcomes. It would be interesting to study the effect of inulin on the metabolome and to see if certain metabolites are correlated with the parameters affected by our intervention (sociability, inflammatory and liver parameters). For example, it has been shown that SCFAs can modulate BNDF levels which is elevated by inulin supplementation in our study [274]. AA derivatives (p-cresol, 4-ethylphenylsulfate) have been associated with alterations in social behaviour in mice [561,562] while indole lactate, a tryptophan derivative, was decreased in autistic children [563]. It has recently been shown that this metabolite is produced by bifidobacteria [564] which is particularly interesting regarding the bifidogenic effect we observed in our patients treated with inulin.

Concerning the hepatic parameters, indole derivatives such as indole-3-acetic acid have been associated with an improvement of the hepatic damages associated with alcohol [300] and indole reduces liver inflammation in mice [299].

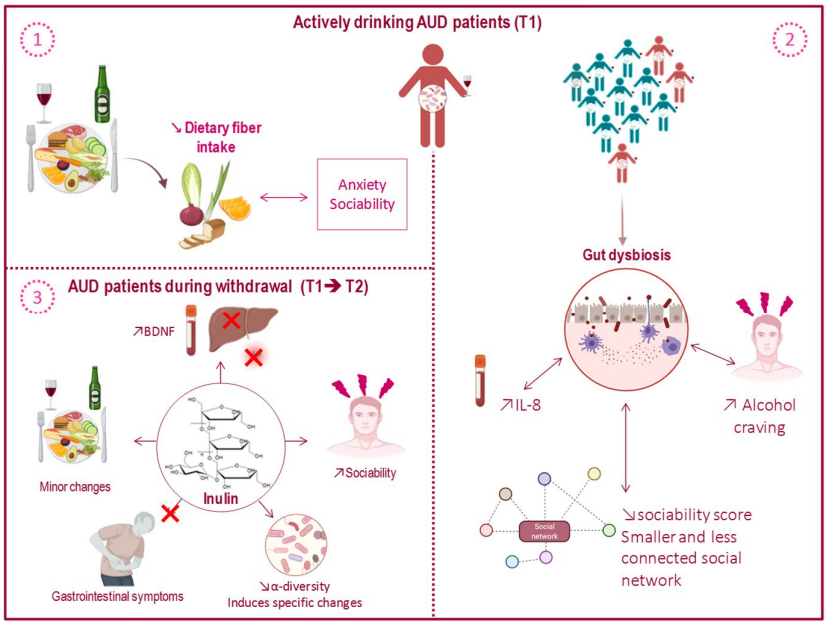
Overall, the study of the fecal and blood metabolome would improve the characterization of the effect of inulin in AUD and to a better understanding of the

relationship between gut microbiota composition and the clinical parameters of these patients.

In conclusion, throughout this thesis, we have been able to highlight that 90% of AUD patients have an insufficient intake of DF that is associated with anxiety and sociability. This work demonstrated for the first time that inulin supplementation is feasible, well tolerated and induces specific changes of gut microbiota composition in AUD patients upon withdrawal. This is of particular interest since we observed that the withdrawal alone (i.e in placebo group) was not sufficient to restore the gut microbiota composition after 3 weeks. This raises questions about the enduring effect of alcohol on the gut microbiota: is it the alcohol *per se* that induces microbial changes? Or are 3 weeks of abstinence not enough to restore these stigmas?

Although this prebiotic fiber did not affect mood or the biological parameters studied, we observed a beneficial effect on the sociability score. Our work shows a link between gut microbiota and sociability as we also demonstrated that AUD patients with dysbiosis had impaired sociability and a smaller and less connected social network. Social cognition is an important parameter in alcoholic pathology that can influence relapse. The present work highlights the need to include the study of the gut microbiota when investigating this relationship in the context of AUD.

Main conclusions:



- Ninety percent of AUD patients had a DF intake below the recommended value of 25g/d. Fructan intake was negatively associated with anxiety while total DF, insoluble, soluble DF and galacto-oligosaccharide intakes were associated with higher sociability score.

- Only a subset of AUD patients presents a gut dysbiosis. Compared to non dysbiotics these patients had a smaller and less connected social network et tend to had difficulties to take into account the perspective of others. Patients that displayed an altered gut microbiota had a higher alcohol craving but no differences were observed for nutritional intake nor medication.

- Inulin induces specific changes of gut microbiota composition without major gastrointestinal symptoms. Seventeen days of inulin supplementation had minor effect on behavioural parameters, but improved some features of sociability score and increased plasma BDNF level compared to placebo.

- In AUD patients, inulin supplementation had no beneficial effect on liver or inflammatory parameters even in patients with more severe liver disease.

Main future perspectives

- To study the impact of inulin supplementation on the host metabolome (stool and blood) using untargeted techniques.

- To test association between blood metabolites, biological parameters and psychological symptoms.

- Large-scale studies in humans suffering from AUD with less restricted exclusion criteria (especially for liver disease) and higher duration of supplementation.

- To test the neurobiological mechanisms by which inulin could improve social behaviour in a mice model of FMT.

- To test the causal role of gut dysbiosis in social behaviour using FMT from dysbiotic and non dysbiotic patients to mice.

- To study the association between social cognition and the risk of relapse.

REFERENCES

[1] WHO | Global status report on alcohol and health 2018. WHO n.d. http://www.who.int/substance_abuse/publications/global_alcohol_report/en/ (accessed January 11, 2019).

[2] Risques liés à la consommation d'alcool. Bruxelles: CSS; Avis N° 9438: Conseil Supérieur de la Santé; 2018.

[3] Carvalho AF, Heilig M, Perez A, Probst C, Rehm J. Alcohol use disorders. Lancet 2019;394:781–92. https://doi.org/10.1016/S0140-6736(19)31775-1.

[4] Hoffmann NG, Kopak AM. How Well Do the DSM-5 Alcohol Use Disorder Designations Map to the ICD-10 Disorders? Alcohol Clin Exp Res 2015;39:697–701. https://doi.org/10.1111/acer.12685.

[5] Saha TD, Chou SP, Grant BF. Toward an alcohol use disorder continuum using item response theory: results from the National Epidemiologic Survey on Alcohol and Related Conditions. Psychol Med 2006;36:931. https://doi.org/10.1017/S003329170600746X.

[6] Proudfoot H, Baillie AJ, Teesson M. The structure of alcohol dependence in the community. Drug and Alcohol Dependence 2006;81:21–6. https://doi.org/10.1016/j.drugalcdep.2005.05.014.

[7] Harford TC, Yi H, Faden VB, Chen CM. The Dimensionality of DSM-IV Alcohol Use Disorders Among Adolescent and Adult Drinkers and Symptom Patterns by Age, Gender, and Race/Ethnicity. Alcoholism: Clinical and Experimental Research 2009;33:868–78. https://doi.org/10.1111/j.1530-0277.2009.00910.x.

[8] Alcohol Use Disorder: A Comparison Between DSM–IV and DSM–5 | National Institute on Alcohol Abuse and Alcoholism (NIAAA) n.d. https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-use-disorder-comparison-between-dsm (accessed October 24, 2021).

[9] Bratberg GH, C Wilsnack S, Wilsnack R, Håvås Haugland S, Krokstad S, Sund ER, et al. Gender differences and gender convergence in alcohol use over the past three decades (1984–2008), The HUNT Study, Norway. BMC Public Health 2016;16:723. https://doi.org/10.1186/s12889-016-3384-3.

[10] Keyes KM, Li G, Hasin DS. Birth Cohort Effects and Gender Differences in Alcohol Epidemiology: A Review and Synthesis: GENDER DIFFERENCES IN ALCOHOL EPIDEMIOLOGY. Alcoholism: Clinical and Experimental Research 2011;35:2101–12. https://doi.org/10.1111/j.1530-0277.2011.01562.x.

[11] Grittner U, Kuntsche S, Gmel G, Bloomfield K. Alcohol consumption and social inequality at the individual and country levels—results from an international study. European Journal of Public Health 2013;23:332–9. https://doi.org/10.1093/eurpub/cks044.

[12] Kendler KS, Gardner C, Dick DM. Predicting alcohol consumption in adolescence from alcohol-specific and general externalizing genetic risk factors, key

environmental exposures and their interaction. Psychol Med 2011;41:1507–16. https://doi.org/10.1017/S003329171000190X.
[13] Castaldelli-Maia JM, Bhugra D. Investigating the interlinkages of alcohol use and misuse, spirituality and culture - Insights from a systematic review. Int Rev Psychiatry 2014;26:352–67. https://doi.org/10.3109/09540261.2014.899999.
[14] Morgenstern M, Sargent JD, Sweeting H, Faggiano F, Mathis F, Hanewinkel R. Favourite alcohol advertisements and binge drinking among adolescents: a cross-cultural cohort study. Addiction 2014;109:2005–15. https://doi.org/10.1111/add.12667.
[15] Yang P, Tao R, He C, Liu S, Wang Y, Zhang X. The Risk Factors of the Alcohol Use Disorders—Through Review of Its Comorbidities. Front Neurosci 2018;12:303. https://doi.org/10.3389/fnins.2018.00303.
[16] Schneier FR, Foose TE, Hasin DS, Heimberg RG, Liu S-M, Grant BF, et al. Social Anxiety Disorder and Alcohol Use Disorder Comorbidity in the National Epidemiologic Survey on Alcohol and Related Conditions. Psychological Medicine 2010;40:977. https://doi.org/10.1017/S0033291709991231.
[17] Ralevski E, Olivera-Figueroa LA, Petrakis I. PTSD and comorbid AUD: a review of pharmacological and alternative treatment options. Subst Abuse Rehabil 2014;5:25–36. https://doi.org/10.2147/SAR.S37399.
[18] Dworkin ER, Wanklyn S, Stasiewicz PR, Coffey SF. PTSD symptom presentation among people with alcohol and drug use disorders: Comparisons by substance of abuse. Addict Behav 2018;76:188–94. https://doi.org/10.1016/j.addbeh.2017.08.019.
[19] Head M, Goodwin L, Debell F, Greenberg N, Wessely S, Fear NT. Post-traumatic stress disorder and alcohol misuse: comorbidity in UK military personnel. Soc Psychiatry Psychiatr Epidemiol 2016;51:1171–80. https://doi.org/10.1007/s00127-016-1177-8.
[20] Bramming M, Becker U, Jørgensen MB, Neermark S, Bisgaard T, Tolstrup JS. Bariatric surgery and risk of alcohol use disorder: a register-based cohort study. Int J Epidemiol 2021;49:1826–35. https://doi.org/10.1093/ije/dyaa147.
[21] Connor JP, Haber PS, Hall WD. Alcohol use disorders. The Lancet 2016;387:988–98. https://doi.org/10.1016/S0140-6736(15)00122-1.
[22] Cruz MT, Bajo M, Schweitzer P, Roberto M. Shared mechanisms of alcohol and other drugs. Alcohol Res Health 2008;31:137–47.
[23] Littleton J. Neurochemical mechanisms underlying alcohol withdrawal. Alcohol Health Res World 1998;22:13–24.
[24] Valenzuela CF. Alcohol and neurotransmitter interactions. Alcohol Health Res World 1997;21:144–8.
[25] Kumar S, Porcu P, Werner DF, Matthews DB, Diaz-Granados JL, Helfand RS, et al. The role of GABAA receptors in the acute and chronic effects of ethanol: a decade of progress. Psychopharmacology (Berl) 2009;205:529. https://doi.org/10.1007/s00213-009-1562-z.
[26] Gonzales RA, Job MO, Doyon WM. The role of mesolimbic dopamine in the development and maintenance of ethanol reinforcement. Pharmacology &

Therapeutics 2004;103:121–46. https://doi.org/10.1016/j.pharmthera.2004.06.002.
[27] Koob GF, Le Moal M. Plasticity of reward neurocircuitry and the “dark side” of drug addiction. Nat Neurosci 2005;8:1442–4. https://doi.org/10.1038/nn1105-1442.
[28] MA H, ZHU G. The dopamine system and alcohol dependence. Shanghai Arch Psychiatry 2014;26:61–8. https://doi.org/10.3969/j.issn.1002-0829.2014.02.002.
[29] Gilpin NW, Koob GF. Neurobiology of alcohol dependence: focus on motivational mechanisms. Alcohol Res Health 2008;31:185–95.
[30] Nutt DJ. The role of the opioid system in alcohol dependence. J Psychopharmacol 2014;28:8–22. https://doi.org/10.1177/0269881113504017.
[31] Cowen MS, Lawrence AJ. The role of opioid-dopamine interactions in the induction and maintenance of ethanol consumption. Progress in Neuro-Psychopharmacology and Biological Psychiatry 1999;23:1171–212. https://doi.org/10.1016/S0278-5846(99)00060-3.
[32] Jesse S, Bråthen G, Ferrara M, Keindl M, Ben-Menachem E, Tanasescu R, et al. Alcohol withdrawal syndrome: mechanisms, manifestations, and management. Acta Neurol Scand 2017;135:4–16. https://doi.org/10.1111/ane.12671.
[33] Pinto E. [Neurobiological mechanisms of alcohol dependence]. Rev Med Liege 2019;74:274–80.
[34] Mukherjee S, Das SK, Vaidyanathan K, Vasudevan DM. Consequences of alcohol consumption on neurotransmitters -an overview. Curr Neurovasc Res 2008;5:266–72. https://doi.org/10.2174/156720208786413415.
[35] Lovinger DM. Serotonin’s Role in Alcohol’s Effects on the Brain. Alcohol Health Res World 1997;21:114–20.
[36] LeMarquand D, Pihl RO, Benkelfat C. Serotonin and alcohol intake, abuse, and dependence: Clinical evidence. Biological Psychiatry 1994;36:326–37. https://doi.org/10.1016/0006-3223(94)90630-0.
[37] Sinha R. The role of stress in addiction relapse. Curr Psychiatry Rep 2007;9:388–95. https://doi.org/10.1007/s11920-007-0050-6.
[38] Lu Y-L, Richardson HN. Alcohol, stress hormones, and the prefrontal cortex: A proposed pathway to the dark side of addiction. Neuroscience 2014;277:139–51. https://doi.org/10.1016/j.neuroscience.2014.06.053.
[39] Rivier C, Lee S. Acute alcohol administration stimulates the activity of hypothalamic neurons that express corticotropin-releasing factor and vasopressin. Brain Research 1996;726:1–10. https://doi.org/10.1016/0006-8993(96)00301-0.
[40] Willey AR, Anderson RI, Morales M, Ramirez RL, Spear LP. Effects of ethanol administration on corticosterone levels in adolescent and adult rats. Alcohol 2012;46:29–36. https://doi.org/10.1016/j.alcohol.2011.08.005.
[41] Koob GF. Neurobiology of Addiction. FOC 2011;9:55–65. https://doi.org/10.1176/foc.9.1.foc55.
[42] Robinson G, Most D, Ferguson LB, Mayfield J, Harris RA, Blednov YA. Neuroimmune Pathways in Alcohol Consumption: Evidence from Behavioral and Genetic Studies in Rodents and Humans. Int Rev Neurobiol 2014;118:13–39. https://doi.org/10.1016/B978-0-12-801284-0.00002-6.

[43] He J, Crews FT. Increased MCP-1 and microglia in various regions of the human alcoholic brain. Experimental Neurology 2008;210:349–58. https://doi.org/10.1016/j.expneurol.2007.11.017.
[44] Enache D, Pariante CM, Mondelli V. Markers of central inflammation in major depressive disorder: A systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. Brain Behav Immun 2019;81:24–40. https://doi.org/10.1016/j.bbi.2019.06.015.
[45] de Timary P, Stärkel P, Delzenne NM, Leclercq S. A role for the peripheral immune system in the development of alcohol use disorders? Neuropharmacology 2017;122:148–60. https://doi.org/10.1016/j.neuropharm.2017.04.013.
[46] Dantzer R, O'Connor JC, Freund GG, Johnson RW, Kelley KW. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat Rev Neurosci 2008;9:46–56. https://doi.org/10.1038/nrn2297.
[47] Konsman JP, Parnet P, Dantzer R. Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 2002;25:154–9. https://doi.org/10.1016/s0166-2236(00)02088-9.
[48] Mayfield J, Ferguson L, Harris RA. Neuroimmune signaling: a key component of alcohol abuse. Current Opinion in Neurobiology 2013;23:513–20. https://doi.org/10.1016/j.conb.2013.01.024.
[49] Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. Gene expression in human alcoholism: microarray analysis of frontal cortex. Alcohol Clin Exp Res 2000;24:1873–82.
[50] Pascual M, Baliño P, Aragón CMG, Guerri C. Cytokines and chemokines as biomarkers of ethanol-induced neuroinflammation and anxiety-related behavior: role of TLR4 and TLR2. Neuropharmacology 2015;89:352–9. https://doi.org/10.1016/j.neuropharm.2014.10.014.
[51] Streit WJ, Mrak RE, Griffin WST. Microglia and neuroinflammation: a pathological perspective. Journal of Neuroinflammation 2004;1:14. https://doi.org/10.1186/1742-2094-1-14.
[52] Jurga AM, Paleczna M, Kuter KZ. Overview of General and Discriminating Markers of Differential Microglia Phenotypes. Frontiers in Cellular Neuroscience 2020;14:198. https://doi.org/10.3389/fncel.2020.00198.
[53] Mondelli V, Vernon AC, Turkheimer F, Dazzan P, Pariante CM. Brain microglia in psychiatric disorders. Lancet Psychiatry 2017;4:563–72. https://doi.org/10.1016/S2215-0366(17)30101-3.
[54] Chastain LG, Sarkar DK. Chapter Four - Role of Microglia in Regulation of Ethanol Neurotoxic Action. In: Cui C, Shurtleff D, Harris RA, editors. International Review of Neurobiology, vol. 118, Academic Press; 2014, p. 81–103. https://doi.org/10.1016/B978-0-12-801284-0.00004-X.
[55] Kim SU, de Vellis J. Microglia in health and disease. Journal of Neuroscience Research 2005;81:302–13. https://doi.org/10.1002/jnr.20562.
[56] McClain JA, Morris SA, Deeny MA, Marshall SA, Hayes DM, Kiser ZM, et al. Adolescent binge alcohol exposure induces long-lasting partial activation of microglia. Brain Behav Immun 2011;25 Suppl 1:S120-128. https://doi.org/10.1016/j.bbi.2011.01.006.

[57] Marshall SA, McClain JA, Kelso ML, Hopkins DM, Pauly JR, Nixon K. Microglial activation is not equivalent to neuroinflammation in alcohol-induced neurodegeneration: The importance of microglia phenotype. Neurobiol Dis 2013;54:239–51. https://doi.org/10.1016/j.nbd.2012.12.016.

[58] Marshall SA, McClain JA, Wooden JI, Nixon K. Microglia Dystrophy Following Binge-Like Alcohol Exposure in Adolescent and Adult Male Rats. Frontiers in Neuroanatomy 2020;14:52. https://doi.org/10.3389/fnana.2020.00052.

[59] Osterndorff-Kahanek EA, Becker HC, Lopez MF, Farris SP, Tiwari GR, Nunez YO, et al. Chronic Ethanol Exposure Produces Time- and Brain Region-Dependent Changes in Gene Coexpression Networks. PLoS One 2015;10:e0121522. https://doi.org/10.1371/journal.pone.0121522.

[60] Lippai D, Bala S, Petrasek J, Csak T, Levin I, Kurt-Jones EA, et al. Alcohol-induced IL-1β in the brain is mediated by NLRP3/ASC inflammasome activation that amplifies neuroinflammation. J Leukoc Biol 2013;94:171–82. https://doi.org/10.1189/jlb.1212659.

[61] Marshall SA, Casachahua JD, Rinker JA, Blose AK, Lysle DT, Thiele TE. IL-1 receptor signaling in the basolateral amygdala modulates binge-like ethanol consumption in male C57BL/6J mice. Brain Behav Immun 2016;51:258–67. https://doi.org/10.1016/j.bbi.2015.09.006.

[62] Wu Y, Lousberg EL, Moldenhauer LM, Hayball JD, Robertson SA, Coller JK, et al. Attenuation of microglial and IL-1 signaling protects mice from acute alcohol-induced sedation and/or motor impairment. Brain Behav Immun 2011;25 Suppl 1:S155-164. https://doi.org/10.1016/j.bbi.2011.01.012.

[63] Valenta JP, Gonzales RA. Chronic Intracerebroventricular Infusion of Monocyte Chemoattractant Protein-1 Leads to a Persistent Increase in Sweetened Ethanol Consumption During Operant Self-Administration But Does Not Influence Sucrose Consumption in Long-Evans Rats. Alcohol Clin Exp Res 2016;40:187–95. https://doi.org/10.1111/acer.12928.

[64] Pearse DD, Hughes ZA. PDE4B as a microglia target to reduce neuroinflammation. Glia 2016;64:1698–709. https://doi.org/10.1002/glia.22986.

[65] Wen R-T, Zhang F-F, Zhang H-T. Cyclic nucleotide phosphodiesterases: potential therapeutic targets for alcohol use disorder. Psychopharmacology (Berl) 2018;235:1793–805. https://doi.org/10.1007/s00213-018-4895-7.

[66] Logrip ML. Phosphodiesterase regulation of alcohol drinking in rodents. Alcohol 2015;49:795–802. https://doi.org/10.1016/j.alcohol.2015.03.007.

[67] Gong M-F, Wen R-T, Xu Y, Pan J-C, Fei N, Zhou Y-M, et al. Attenuation of ethanol abstinence-induced anxiety- and depressive-like behavior by the phosphodiesterase-4 inhibitor rolipram in rodents. Psychopharmacology 2017;234:3143–51. https://doi.org/10.1007/s00213-017-4697-3.

[68] Ray LA, Bujarski S, Shoptaw S, Roche DJ, Heinzerling K, Miotto K. Development of the Neuroimmune Modulator Ibudilast for the Treatment of Alcoholism: A Randomized, Placebo-Controlled, Human Laboratory Trial. Neuropsychopharmacology 2017;42:1776–88. https://doi.org/10.1038/npp.2017.10.

[69] Grodin EN, Bujarski S, Towns B, Burnette E, Nieto S, Lim A, et al. Ibudilast, a neuroimmune modulator, reduces heavy drinking and alcohol cue-elicited neural activation: a randomized trial. Transl Psychiatry 2021;11:355. https://doi.org/10.1038/s41398-021-01478-5.

[70] Kessler RC, Crum RM, Warner LA, Nelson CB, Schulenberg J, Anthony JC. Lifetime co-occurrence of DSM-III-R alcohol abuse and dependence with other psychiatric disorders in the National Comorbidity Survey. Arch Gen Psychiatry 1997;54:313–21. https://doi.org/10.1001/archpsyc.1997.01830160031005.

[71] Pignon B, Sescousse G, Amad A, Benradia I, Vaiva G, Thomas P, et al. Alcohol Use Disorder Is Differently Associated With Psychotic Symptoms According To Underlying Psychiatric Disorders: A General Population Study. Alcohol and Alcoholism 2020;55:112–20. https://doi.org/10.1093/alcalc/agz077.

[72] Le Berre A-P, Fama R, Sullivan EV. Executive Functions, Memory, and Social Cognitive Deficits and Recovery in Chronic Alcoholism: A Critical Review to Inform Future Research. Alcohol Clin Exp Res 2017;41:1432–43. https://doi.org/10.1111/acer.13431.

[73] Carbia C, Lannoy S, Maurage P, López-Caneda E, O'Riordan KJ, Dinan TG, et al. A biological framework for emotional dysregulation in alcohol misuse: from gut to brain. Mol Psychiatry 2021;26:1098–118. https://doi.org/10.1038/s41380-020-00970-6.

[74] Le Berre A-P. Emotional processing and social cognition in alcohol use disorder. Neuropsychology 2019;33:808–21. https://doi.org/10.1037/neu0000572.

[75] Gross JJ, Jazaieri H. Emotion, Emotion Regulation, and Psychopathology: An Affective Science Perspective. Clinical Psychological Science 2014;2:387–401. https://doi.org/10.1177/2167702614536164.

[76] Herman AM, Duka T. Facets of impulsivity and alcohol use: What role do emotions play? Neurosci Biobehav Rev 2019;106:202–16. https://doi.org/10.1016/j.neubiorev.2018.08.011.

[77] Mowbray O, Quinn A, Cranford JA. Social networks and alcohol use disorders: findings from a nationally representative sample. Am J Drug Alcohol Abuse 2014;40:181–6. https://doi.org/10.3109/00952990.2013.860984.

[78] Han M, Jiang G, Luo H, Shao Y. Neurobiological Bases of Social Networks. Frontiers in Psychology 2021;12.

[79] Brooks AT, Lòpez MM, Ranucci A, Krumlauf M, Wallen GR. A Qualitative Exploration of Social Support during Treatment for Severe Alcohol Use Disorder and Recovery. Addict Behav Rep 2017;6:76–82. https://doi.org/10.1016/j.abrep.2017.08.002.

[80] Moon T-J, Mathias CW, Mullen J, Karns-Wright TE, Hill-Kapturczak N, Roache JD, et al. The role of social support in motivating reductions in alcohol use: A test of three models of social support in alcohol-impaired drivers. Alcohol Clin Exp Res 2019;43:123–34. https://doi.org/10.1111/acer.13911.

[81] Dahal P, Koirala P. Perceived Social Support and its Influence on Motivation to Change Drinking Behavior: An Observation from Kathmandu, Nepal. Alcoholism Treatment Quarterly 2021;39:383–92. https://doi.org/10.1080/07347324.2020.1866475.

[82] Franko DL, Dorer DJ, Keel PK, Jackson S, Manzo MP, Herzog DB. How do eating disorders and alcohol use disorder influence each other? International Journal of Eating Disorders 2005;38:200–7. https://doi.org/10.1002/eat.20178.
[83] Temko JE, Bouhlal S, Farokhnia M, Lee MR, Cryan JF, Leggio L. The Microbiota, the Gut and the Brain in Eating and Alcohol Use Disorders: A 'Ménage à Trois'? Alcohol Alcohol 2017;52:403–13. https://doi.org/10.1093/alcalc/agx024.
[84] Galmiche M, Déchelotte P, Lambert G, Tavolacci MP. Prevalence of eating disorders over the 2000-2018 period: a systematic literature review. Am J Clin Nutr 2019;109:1402–13. https://doi.org/10.1093/ajcn/nqy342.
[85] Mellentin AI, Skøt L, Guala MM, Støving RK, Ascone L, Stenager E, et al. Does receiving an eating disorder diagnosis increase the risk of a subsequent alcohol use disorder? A Danish nationwide register-based cohort study. Addiction 2022;117:354–67. https://doi.org/10.1111/add.15639.
[86] Bahji A, Mazhar MN, Hudson CC, Nadkarni P, MacNeil BA, Hawken E. Prevalence of substance use disorder comorbidity among individuals with eating disorders: A systematic review and meta-analysis. Psychiatry Res 2019;273:58–66. https://doi.org/10.1016/j.psychres.2019.01.007.
[87] Bogusz K, Kopera M, Jakubczyk A, Trucco EM, Kucharska K, Walenda A, et al. Prevalence of alcohol use disorder among individuals who binge eat: a systematic review and meta-analysis. Addiction 2021;116:18–31. https://doi.org/10.1111/add.15155.
[88] Grilo CM, Sinha R, O'Malley SS. Eating Disorders and Alcohol Use Disorders. Alcohol Res Health 2002;26:151–60.
[89] Gregorowski C, Seedat S, Jordaan GP. A clinical approach to the assessment and management of co-morbid eating disorders and substance use disorders. BMC Psychiatry 2013;13:289. https://doi.org/10.1186/1471-244X-13-289.
[90] Bénard M, Bellisle F, Kesse-Guyot E, Julia C, Andreeva VA, Etilé F, et al. Impulsivity is associated with food intake, snacking, and eating disorders in a general population. Am J Clin Nutr 2019;109:117–26. https://doi.org/10.1093/ajcn/nqy255.
[91] Smith DG, Robbins TW. The neurobiological underpinnings of obesity and binge eating: a rationale for adopting the food addiction model. Biol Psychiatry 2013;73:804–10. https://doi.org/10.1016/j.biopsych.2012.08.026.
[92] Schreiber LRN, Odlaug BL, Grant JE. The overlap between binge eating disorder and substance use disorders: Diagnosis and neurobiology. J Behav Addict 2013;2:191–8. https://doi.org/10.1556/JBA.2.2013.015.
[93] Uher R, Murphy T, Brammer MJ, Dalgleish T, Phillips ML, Ng VW, et al. Medial prefrontal cortex activity associated with symptom provocation in eating disorders. Am J Psychiatry 2004;161:1238–46. https://doi.org/10.1176/appi.ajp.161.7.1238.
[94] Zywiak WH, Westerberg VS, Connors GJ, Maisto SA. Exploratory findings from the Reasons for Drinking Questionnaire. Journal of Substance Abuse Treatment 2003;25:287–92. https://doi.org/10.1016/S0740-5472(03)00118-1.

[95] Beck AT, Steer RA, Ball R, Ranieri W. Comparison of Beck Depression Inventories -IA and -II in psychiatric outpatients. J Pers Assess 1996;67:588–97. https://doi.org/10.1207/s15327752jpa6703_13.
[96] Spielberger CD, Gorsuch RC, Lushene RE. Manual for the State-Trait Anxiety Inventory (Form Y):("self-evaluation questionnaire") Consulting Psychologists Press. Palo Alto, CA 1983.
[97] Anton RF, Moak DH, Latham P. The Obsessive Compulsive Drinking Scale: a self-rated instrument for the quantification of thoughts about alcohol and drinking behavior. Alcohol Clin Exp Res 1995;19:92–9. https://doi.org/10.1111/j.1530-0277.1995.tb01475.x.
[98] Mikolajczak M, Luminet O, Leroy C, Roy E. Psychometric properties of the Trait Emotional Intelligence Questionnaire: factor structure, reliability, construct, and incremental validity in a French-speaking population. J Pers Assess 2007;88:338–53. https://doi.org/10.1080/00223890701333431.
[99] Whelan DC, Zelenski JM. Experimental Evidence That Positive Moods Cause Sociability. Social Psychological and Personality Science 2012;3:430–7. https://doi.org/10.1177/1948550611425194.
[100] Samson D, Apperly IA, Braithwaite JJ, Andrews BJ, Bodley Scott SE. Seeing it their way: evidence for rapid and involuntary computation of what other people see. J Exp Psychol Hum Percept Perform 2010;36:1255–66. https://doi.org/10.1037/a0018729.
[101] Wyngaerden F, Tempels M, Feys J-L, Dubois V, Lorant V. The personal social network of psychiatric service users. Int J Soc Psychiatry 2020;66:682–92. https://doi.org/10.1177/0020764020927447.
[102] Lieber CS. Relationships between nutrition, alcohol use, and liver disease. Alcohol Res Health 2003;27:220–31.
[103] Breslow RA, Guenther PM, Smothers BA. Alcohol Drinking Patterns and Diet Quality: The 1999–2000 National Health and Nutrition Examination Survey. American Journal of Epidemiology 2006;163:359–66. https://doi.org/10.1093/aje/kwj050.
[104] Goldsmith RH, Iber FL, Miller PA. Nutritional status of alcoholics of different socioeconomic class. Journal of the American College of Nutrition 1983;2:215–20. https://doi.org/10.1080/07315724.1983.10719925.
[105] Santolaria F, González-Reimers E. Alcohol and Nutrition: An Overview. In: Watson RR, Preedy VR, Zibadi S, editors. Alcohol, Nutrition, and Health Consequences, Totowa, NJ: Humana Press; 2013, p. 3–14. https://doi.org/10.1007/978-1-62703-047-2_1.
[106] de Timary P, Cani PD, Duchemin J, Neyrinck AM, Gihousse D, Laterre P-F, et al. The loss of metabolic control on alcohol drinking in heavy drinking alcohol-dependent subjects. PLoS ONE 2012;7:e38682. https://doi.org/10.1371/journal.pone.0038682.
[107] Kwok A, Dordevic AL, Paton G, Page MJ, Truby H. Effect of alcohol consumption on food energy intake: a systematic review and meta-analysis. Br J Nutr 2019;121:481–95. https://doi.org/10.1017/S0007114518003677.

[108] Santolaria F, Pérez-Manzano JL, Milena A, González-Reimers E, Gómez-Rodríguez MA, Martínez-Riera A, et al. Nutritional assessment in alcoholic patients. Its relationship with alcoholic intake, feeding habits, organic complications and social problems. Drug Alcohol Depend 2000;59:295–304. https://doi.org/10.1016/s0376-8716(99)00129-5.
[109] Lands WE, Zakhari S. The case of the missing calories. Am J Clin Nutr 1991;54:47–8. https://doi.org/10.1093/ajcn/54.1.47.
[110] Lieber CS. Relationships between nutrition, alcohol use, and liver disease. Alcohol Res Health 2003;27:220–31.
[111] Baj J, Flieger W, Teresiński G, Buszewicz G, Sitarz E, Forma A, et al. Magnesium, Calcium, Potassium, Sodium, Phosphorus, Selenium, Zinc, and Chromium Levels in Alcohol Use Disorder: A Review. J Clin Med 2020;9. https://doi.org/10.3390/jcm9061901.
[112] O'Shea RS, Dasarathy S, McCullough AJ, Practice Guideline Committee of the American Association for the Study of Liver Diseases, Practice Parameters Committee of the American College of Gastroenterology. Alcoholic liver disease. Hepatology 2010;51:307–28. https://doi.org/10.1002/hep.23258.
[113] Seitz HK, Gärtner U, Egerer G, Simanowski UA. Ethanol metabolism in the gastrointestinal tract and its possible consequences. Alcohol Alcohol Suppl 1994;2:157–62.
[114] Elamin EE, Masclee AA, Dekker J, Jonkers DM. Ethanol metabolism and its effects on the intestinal epithelial barrier. Nutrition Reviews 2013;71:483–99. https://doi.org/10.1111/nure.12027.
[115] Bujanda L. The effects of alcohol consumption upon the gastrointestinal tract. Am J Gastroenterol 2000;95:3374–82. https://doi.org/10.1111/j.1572-0241.2000.03347.x.
[116] Paone P, Cani PD. Mucus barrier, mucins and gut microbiota: the expected slimy partners? Gut 2020;69:2232–43. https://doi.org/10.1136/gutjnl-2020-322260.
[117] Duerkop BA, Vaishnava S, Hooper LV. Immune Responses to the Microbiota at the Intestinal Mucosal Surface. Immunity 2009;31:368–76. https://doi.org/10.1016/j.immuni.2009.08.009.
[118] Bishehsari F, Magno E, Swanson G, Desai V, Voigt RM, Forsyth CB, et al. Alcohol and Gut-Derived Inflammation. Alcohol Res 2017;38:163–71.
[119] Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, Mikolajczak M, et al. Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. Brain Behav Immun 2012;26:911–8. https://doi.org/10.1016/j.bbi.2012.04.001.
[120] Meroni M, Longo M, Dongiovanni P. Alcohol or Gut Microbiota: Who Is the Guilty? IJMS 2019;20:4568. https://doi.org/10.3390/ijms20184568.
[121] Hartmann P, Chen P, Wang HJ, Wang L, McCole DF, Brandl K, et al. Deficiency of intestinal mucin-2 ameliorates experimental alcoholic liver disease in mice. Hepatology 2013;58:108–19. https://doi.org/10.1002/hep.26321.
[122] Bergstrom KSB, Kissoon-Singh V, Gibson DL, Ma C, Montero M, Sham HP, et al. Muc2 Protects against Lethal Infectious Colitis by Disassociating Pathogenic and

Commensal Bacteria from the Colonic Mucosa. PLOS Pathogens 2010;6:e1000902. https://doi.org/10.1371/journal.ppat.1000902.
[123] Grewal RK, Mahmood A. Ethanol induced changes in glycosylation of mucins in rat intestine. Annals of Gastroenterology 2009:178–83.
[124] Leclercq S, de Timary P, Stärkel P. Targeting the gut microbiota to treat alcoholic liver diseases: evidence and promises. Acta Gastroenterol Belg 2020;83:616–21.
[125] Lieber CS. Alcohol and the liver: metabolism of alcohol and its role in hepatic and extrahepatic diseases. Mt Sinai J Med 2000;67:84–94.
[126] Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 2004;34:9–19. https://doi.org/10.1016/j.alcohol.2004.07.008.
[127] Osna NA, Donohue TM, Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. Alcohol Res 2017;38:147–61.
[128] Degré D, Stauber RE, Englebert G, Sarocchi F, Verset L, Rainer F, et al. Long-term outcomes in patients with decompensated alcohol-related liver disease, steatohepatitis and Maddrey's discriminant function <32. J Hepatol 2020;72:636–42. https://doi.org/10.1016/j.jhep.2019.12.023.
[129] Avila MA, Dufour J-F, Gerbes AL, Zoulim F, Bataller R, Burra P, et al. Recent advances in alcohol-related liver disease (ALD): summary of a Gut round table meeting. Gut 2020;69:764–80. https://doi.org/10.1136/gutjnl-2019-319720.
[130] Fuster D, Samet JH. Alcohol Use in Patients with Chronic Liver Disease. N Engl J Med 2018;379:1251–61. https://doi.org/10.1056/NEJMra1715733.
[131] Louvet A, Mathurin P. Alcoholic liver disease: mechanisms of injury and targeted treatment. Nat Rev Gastroenterol Hepatol 2015;12:231–42. https://doi.org/10.1038/nrgastro.2015.35.
[132] Jiang L, Stärkel P, Fan J-G, Fouts DE, Bacher P, Schnabl B. The gut mycobiome: a novel player in chronic liver diseases. J Gastroenterol 2021;56:1–11. https://doi.org/10.1007/s00535-020-01740-5.
[133] Hall W, Zador D. The alcohol withdrawal syndrome. The Lancet 1997;349:1897–900. https://doi.org/10.1016/S0140-6736(97)04572-8.
[134] Saitz R. Clinical practice. Unhealthy alcohol use. N Engl J Med 2005;352:596–607. https://doi.org/10.1056/NEJMcp042262.
[135] Mirijello A, D'Angelo C, Ferrulli A, Vassallo G, Antonelli M, Caputo F, et al. IDENTIFICATION AND MANAGEMENT OF ALCOHOL WITHDRAWAL SYNDROME. Drugs 2015;75:353–65. https://doi.org/10.1007/s40265-015-0358-1.
[136] Weiss F, Parsons LH, Schulteis G, Hyytiä P, Lorang MT, Bloom FE, et al. Ethanol self-administration restores withdrawal-associated deficiencies in accumbal dopamine and 5-hydroxytryptamine release in dependent rats. J Neurosci 1996;16:3474–85.
[137] Rossetti ZL, Hmaidan Y, Gessa GL. Marked inhibition of mesolimbic dopamine release: a common feature of ethanol, morphine, cocaine and amphetamine abstinence in rats. Eur J Pharmacol 1992;221:227–34. https://doi.org/10.1016/0014-2999(92)90706-a.
[138] Swift RM. Drug therapy for alcohol dependence. N Engl J Med 1999;340:1482–90. https://doi.org/10.1056/NEJM199905133401907.

[139] Sachdeva A, Choudhary M, Chandra M. Alcohol Withdrawal Syndrome: Benzodiazepines and Beyond. J Clin Diagn Res 2015;9:VE01–7. https://doi.org/10.7860/JCDR/2015/13407.6538.
[140] Holbrook AM, Crowther R, Lotter A, Cheng C, King D. Meta-analysis of benzodiazepine use in the treatment of acute alcohol withdrawal. CMAJ 1999;160:649–55.
[141] Amato L, Minozzi S, Davoli M. Efficacy and safety of pharmacological interventions for the treatment of the Alcohol Withdrawal Syndrome. Cochrane Database of Systematic Reviews 2011. https://doi.org/10.1002/14651858.CD008537.pub2.
[142] Thomson AD. Mechanisms of vitamin deficiency in chronic alcohol misusers and the development of the Wernicke-Korsakoff syndrome. Alcohol Alcohol Suppl 2000;35:2–7. https://doi.org/10.1093/alcalc/35.supplement_1.2.
[143] Martin PR, Singleton CK, Hiller-Sturmhöfel S. The Role of Thiamine Deficiency in Alcoholic Brain Disease. Alcohol Res Health 2003;27:134–42.
[144] Hack JB, Hoffman RS. Thiamine before glucose to prevent Wernicke encephalopathy: examining the conventional wisdom. JAMA 1998;279:583–4. https://doi.org/10.1001/jama.279.8.583.
[145] Jonas DE, Amick HR, Feltner C, Bobashev G, Thomas K, Wines R, et al. Pharmacotherapy for adults with alcohol use disorders in outpatient settings: a systematic review and meta-analysis. JAMA 2014;311:1889–900. https://doi.org/10.1001/jama.2014.3628.
[146] Rösner S, Hackl-Herrwerth A, Leucht S, Vecchi S, Srisurapanont M, Soyka M. Opioid antagonists for alcohol dependence. Cochrane Database Syst Rev 2010:CD001867. https://doi.org/10.1002/14651858.CD001867.pub2.
[147] Mann K, Torup L, Sørensen P, Gual A, Swift R, Walker B, et al. Nalmefene for the management of alcohol dependence: review on its pharmacology, mechanism of action and meta-analysis on its clinical efficacy. Eur Neuropsychopharmacol 2016;26:1941–9. https://doi.org/10.1016/j.euroneuro.2016.10.008.
[148] Addolorato G, Caputo F, Capristo E, Domenicali M, Bernardi M, Janiri L, et al. Baclofen efficacy in reducing alcohol craving and intake: a preliminary double-blind randomized controlled study. Alcohol Alcohol 2002;37:504–8. https://doi.org/10.1093/alcalc/37.5.504.
[149] Addolorato G, Leggio L, Ferrulli A, Cardone S, Vonghia L, Mirijello A, et al. Effectiveness and safety of baclofen for maintenance of alcohol abstinence in alcohol-dependent patients with liver cirrhosis: randomised, double-blind controlled study. Lancet 2007;370:1915–22. https://doi.org/10.1016/S0140-6736(07)61814-5.
[150] Garbutt JC, Kampov-Polevoy AB, Gallop R, Kalka-Juhl L, Flannery BA. Efficacy and safety of baclofen for alcohol dependence: a randomized, double-blind, placebo-controlled trial. Alcohol Clin Exp Res 2010;34:1849–57. https://doi.org/10.1111/j.1530-0277.2010.01273.x.
[151] Minozzi S, Saulle R, Rösner S. Baclofen for alcohol use disorder. Cochrane Database Syst Rev 2018;11:CD012557. https://doi.org/10.1002/14651858.CD012557.pub2.
[152] Laaksonen E, Koski-Jännes A, Salaspuro M, Ahtinen H, Alho H. A randomized, multicentre, open-label, comparative trial of disulfiram, naltrexone and

acamprosate in the treatment of alcohol dependence. Alcohol Alcohol 2008;43:53–61. https://doi.org/10.1093/alcalc/agm136.
[153] JOHNSON BA. UPDATE ON NEUROPHARMACOLOGICAL TREATMENTS FOR ALCOHOLISM: SCIENTIFIC BASIS AND CLINICAL FINDINGS. Biochem Pharmacol 2008;75:34–56. https://doi.org/10.1016/j.bcp.2007.08.005.
[154] Lloyd-Price J, Abu-Ali G, Huttenhower C. The healthy human microbiome. Genome Med 2016;8:51. https://doi.org/10.1186/s13073-016-0307-y.
[155] Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol 2016;14:e1002533. https://doi.org/10.1371/journal.pbio.1002533.
[156] Grice EA, Segre JA. The Human Microbiome: Our Second Genome. Annu Rev Genom Hum Genet 2012;13:151–70. https://doi.org/10.1146/annurev-genom-090711-163814.
[157] Hillman ET, Lu H, Yao T, Nakatsu CH. Microbial Ecology along the Gastrointestinal Tract. Microbes and Environments 2017;32:300–13. https://doi.org/10.1264/jsme2.ME17017.
[158] Berg R. The indigenous gastrointestinal microflora. Trends in Microbiology 1996;4:430–5. https://doi.org/10.1016/0966-842X(96)10057-3.
[159] Avelar Rodriguez D, Ryan PM, Toro Monjaraz EM, Ramirez Mayans JA, Quigley EM. Small Intestinal Bacterial Overgrowth in Children: A State-Of-The-Art Review. Front Pediatr 2019;7:363. https://doi.org/10.3389/fped.2019.00363.
[160] Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proc Natl Acad Sci U S A 2010;107:11971–5. https://doi.org/10.1073/pnas.1002601107.
[161] Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. Microbiol Mol Biol Rev 2017;81. https://doi.org/10.1128/MMBR.00036-17.
[162] Reyman M, van Houten MA, van Baarle D, Bosch AATM, Man WH, Chu MLJN, et al. Impact of delivery mode-associated gut microbiota dynamics on health in the first year of life. Nat Commun 2019;10:4997. https://doi.org/10.1038/s41467-019-13014-7.
[163] Derrien M, Alvarez A-S, de Vos WM. The Gut Microbiota in the First Decade of Life. Trends in Microbiology 2019;27:997–1010. https://doi.org/10.1016/j.tim.2019.08.001.
[164] Cheng J, Palva AM, de Vos WM, Satokari R. Contribution of the Intestinal Microbiota to Human Health: From Birth to 100 Years of Age. In: Dobrindt U, Hacker JH, Svanborg C, editors. Between Pathogenicity and Commensalism, vol. 358, Berlin, Heidelberg: Springer Berlin Heidelberg; 2011, p. 323–46. https://doi.org/10.1007/82_2011_189.
[165] Cryan JF, O'Riordan KJ, Cowan CSM, Sandhu KV, Bastiaanssen TFS, Boehme M, et al. The Microbiota-Gut-Brain Axis. Physiol Rev 2019;99:1877–2013. https://doi.org/10.1152/physrev.00018.2018.

[166] Jakobsson HE, Jernberg C, Andersson AF, Sjölund-Karlsson M, Jansson JK, Engstrand L. Short-Term Antibiotic Treatment Has Differing Long-Term Impacts on the Human Throat and Gut Microbiome. PLOS ONE 2010;5:e9836. https://doi.org/10.1371/journal.pone.0009836.
[167] Maurice CF, Haiser HJ, Turnbaugh PJ. Xenobiotics shape the physiology and gene expression of the active human gut microbiome. Cell 2013;152:39–50. https://doi.org/10.1016/j.cell.2012.10.052.
[168] Vieira-Silva S, Falony G, Belda E, Nielsen T, Aron-Wisnewsky J, Chakaroun R, et al. Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. Nature 2020;581:310–5. https://doi.org/10.1038/s41586-020-2269-x.
[169] Cussotto S, Strain CR, Fouhy F, Strain RG, Peterson VL, Clarke G, et al. Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function. Psychopharmacology 2019;236:1671–85. https://doi.org/10.1007/s00213-018-5006-5.
[170] Jackson MA, Goodrich JK, Maxan M-E, Freedberg DE, Abrams JA, Poole AC, et al. Proton pump inhibitors alter the composition of the gut microbiota. Gut 2016;65:749–56. https://doi.org/10.1136/gutjnl-2015-310861.
[171] Imhann F, Bonder MJ, Vila AV, Fu J, Mujagic Z, Vork L, et al. Proton pump inhibitors affect the gut microbiome. Gut 2016;65:740–8. https://doi.org/10.1136/gutjnl-2015-310376.
[172] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505:559–63. https://doi.org/10.1038/nature12820.
[173] Zinöcker MK, Lindseth IA. The Western Diet–Microbiome-Host Interaction and Its Role in Metabolic Disease. Nutrients 2018;10:365. https://doi.org/10.3390/nu10030365.
[174] Statovci D, Aguilera M, MacSharry J, Melgar S. The Impact of Western Diet and Nutrients on the Microbiota and Immune Response at Mucosal Interfaces. Frontiers in Immunology 2017;8:838. https://doi.org/10.3389/fimmu.2017.00838.
[175] Singh RK, Chang H-W, Yan D, Lee KM, Ucmak D, Wong K, et al. Influence of diet on the gut microbiome and implications for human health. J Transl Med 2017;15:73. https://doi.org/10.1186/s12967-017-1175-y.
[176] Merra G, Noce A, Marrone G, Cintoni M, Tarsitano MG, Capacci A, et al. Influence of Mediterranean Diet on Human Gut Microbiota. Nutrients 2020;13:7. https://doi.org/10.3390/nu13010007.
[177] Martínez-González MA, Gea A, Ruiz-Canela M. The Mediterranean Diet and Cardiovascular Health. Circ Res 2019;124:779–98. https://doi.org/10.1161/CIRCRESAHA.118.313348.
[178] Mentella MC, Scaldaferri F, Ricci C, Gasbarrini A, Miggiano GAD. Cancer and Mediterranean Diet: A Review. Nutrients 2019;11:2059. https://doi.org/10.3390/nu11092059.
[179] Gardener H, Caunca MR. Mediterranean Diet in Preventing Neurodegenerative Diseases. Curr Nutr Rep 2018;7:10–20. https://doi.org/10.1007/s13668-018-0222-5.

[180] Tung J, Barreiro LB, Burns MB, Grenier J-C, Lynch J, Grieneisen LE, et al. Social networks predict gut microbiome composition in wild baboons. ELife 2015;4:e05224. https://doi.org/10.7554/eLife.05224.
[181] Dill-McFarland KA, Tang Z-Z, Kemis JH, Kerby RL, Chen G, Palloni A, et al. Close social relationships correlate with human gut microbiota composition. Sci Rep 2019;9:703. https://doi.org/10.1038/s41598-018-37298-9.
[182] Claesson MJ, Clooney AG, O'Toole PW. A clinician's guide to microbiome analysis. Nat Rev Gastroenterol Hepatol 2017;14:585–95. https://doi.org/10.1038/nrgastro.2017.97.
[183] Krautkramer KA, Fan J, Bäckhed F. Gut microbial metabolites as multi-kingdom intermediates. Nat Rev Microbiol 2021;19:77–94. https://doi.org/10.1038/s41579-020-0438-4.
[184] Cummings JH, Macfarlane GT, Englyst HN. Prebiotic digestion and fermentation. Am J Clin Nutr 2001;73:415S-420S. https://doi.org/10.1093/ajcn/73.2.415s.
[185] Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. Gut 1987;28:1221–7. https://doi.org/10.1136/gut.28.10.1221.
[186] den Besten G, van Eunen K, Groen AK, Venema K, Reijngoud DJ, Bakker BM. The role of short-chain fatty acids in the interplay between diet, gut microbiota, and host energy metabolism. J Lipid Res 2013;54:2325–40. https://doi.org/10.1194/jlr.R036012.
[187] Belenguer A, Duncan SH, Calder AG, Holtrop G, Louis P, Lobley GE, et al. Two routes of metabolic cross-feeding between Bifidobacterium adolescentis and butyrate-producing anaerobes from the human gut. Appl Environ Microbiol 2006;72:3593–9. https://doi.org/10.1128/AEM.72.5.3593-3599.2006.
[188] Kanauchi O, Iwanaga T, Mitsuyama K, Saiki T, Tsuruta O, Noguchi K, et al. Butyrate from bacterial fermentation of germinated barley foodstuff preserves intestinal barrier function in experimental colitis in the rat model. J Gastroenterol Hepatol 1999;14:880–8. https://doi.org/10.1046/j.1440-1746.1999.01971.x.
[189] Rios-Covian D, Gueimonde M, Duncan SH, Flint HJ, de los Reyes-Gavilan CG. Enhanced butyrate formation by cross-feeding between Faecalibacterium prausnitzii and Bifidobacterium adolescentis. FEMS Microbiology Letters 2015;362. https://doi.org/10.1093/femsle/fnv176.
[190] Ríos-Covián D, Ruas-Madiedo P, Margolles A, Gueimonde M, de los Reyes-Gavilán CG, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Health. Front Microbiol 2016;7. https://doi.org/10.3389/fmicb.2016.00185.
[191] Macfarlane S, Macfarlane GT. Regulation of short-chain fatty acid production. Proc Nutr Soc 2003;62:67–72. https://doi.org/10.1079/PNS2002207.
[192] Dalile B, Van Oudenhove L, Vervliet B, Verbeke K. The role of short-chain fatty acids in microbiota-gut-brain communication. Nat Rev Gastroenterol Hepatol 2019;16:461–78. https://doi.org/10.1038/s41575-019-0157-3.
[193] Frost G, Sleeth ML, Sahuri-Arisoylu M, Lizarbe B, Cerdan S, Brody L, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nat Commun 2014;5:3611. https://doi.org/10.1038/ncomms4611.

[194] Kim SW, Hooker JM, Otto N, Win K, Muench L, Shea C, et al. Whole-body pharmacokinetics of HDAC inhibitor drugs, butyric acid, valproic acid and 4-phenylbutyric acid measured with carbon-11 labeled analogs by PET. Nucl Med Biol 2013;40:912–8. https://doi.org/10.1016/j.nucmedbio.2013.06.007.
[195] Song WS, Nielson BR, Banks KP, Bradley YC. Normal organ standard uptake values in carbon-11 acetate PET imaging. Nucl Med Commun 2009;30:462–5. https://doi.org/10.1097/MNM.0b013e32832aa7ce.
[196] Seltzer MA, Jahan SA, Sparks R, Stout DB, Satyamurthy N, Dahlbom M, et al. Radiation dose estimates in humans for (11)C-acetate whole-body PET. J Nucl Med 2004;45:1233–6.
[197] S M, Gt M. Regulation of short-chain fatty acid production. The Proceedings of the Nutrition Society 2003;62. https://doi.org/10.1079/PNS2002207.
[198] Windey K, De Preter V, Verbeke K. Relevance of protein fermentation to gut health. Mol Nutr Food Res 2012;56:184–96. https://doi.org/10.1002/mnfr.201100542.
[199] Diether N, Willing B. Microbial Fermentation of Dietary Protein: An Important Factor in Diet–Microbe–Host Interaction. Microorganisms 2019;7:19. https://doi.org/10.3390/microorganisms7010019.
[200] Aguirre M, Eck A, Koenen ME, Savelkoul PHM, Budding AE, Venema K. Diet drives quick changes in the metabolic activity and composition of human gut microbiota in a validated in vitro gut model. Research in Microbiology 2016;167:114–25. https://doi.org/10.1016/j.resmic.2015.09.006.
[201] Pugin B, Barcik W, Westermann P, Heider A, Wawrzyniak M, Hellings P, et al. A wide diversity of bacteria from the human gut produces and degrades biogenic amines. Microbial Ecology in Health and Disease 2017;28:1353881. https://doi.org/10.1080/16512235.2017.1353881.
[202] Tofalo R, Cocchi S, Suzzi G. Polyamines and Gut Microbiota. Front Nutr 2019;6:16. https://doi.org/10.3389/fnut.2019.00016.
[203] Hirano R, Shirasawa H, Kurihara S. Health-Promoting Effects of Dietary Polyamines. Med Sci (Basel) 2021;9:8. https://doi.org/10.3390/medsci9010008.
[204] Sridharan GV, Choi K, Klemashevich C, Wu C, Prabakaran D, Pan LB, et al. Prediction and quantification of bioactive microbiota metabolites in the mouse gut. Nat Commun 2014;5:5492. https://doi.org/10.1038/ncomms6492.
[205] Smith EA, Macfarlane GT. Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. J Appl Bacteriol 1996;81:288–302. https://doi.org/10.1111/j.1365-2672.1996.tb04331.x.
[206] Macfarlane GT, Cummings JH, Allison C. Protein degradation by human intestinal bacteria. J Gen Microbiol 1986;132:1647–56. https://doi.org/10.1099/00221287-132-6-1647.
[207] Wu GD, Chen J, Hoffmann C, Bittinger K, Chen Y-Y, Keilbaugh SA, et al. Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes. Science 2011;334:105–8. https://doi.org/10.1126/science.1208344.

[208] Zimmer J, Lange B, Frick J-S, Sauer H, Zimmermann K, Schwiertz A, et al. A vegan or vegetarian diet substantially alters the human colonic faecal microbiota. Eur J Clin Nutr 2012;66:53–60. https://doi.org/10.1038/ejcn.2011.141.
[209] Davila AM, Blachier F, Gotteland M, Andriamihaja M, Benetti PH, Sanz Y, et al. Intestinal luminal nitrogen metabolism: role of the gut microbiota and consequences for the host. Pharmacol Res 2013;68:95–107. https://doi.org/10.1016/j.phrs.2012.11.005.
[210] Said HM. Intestinal absorption of water-soluble vitamins in health and disease. Biochemical Journal 2011;437:357–72. https://doi.org/10.1042/BJ20110326.
[211] Magnúsdóttir S, Ravcheev D, de Crécy-Lagard V, Thiele I. Systematic genome assessment of B-vitamin biosynthesis suggests co-operation among gut microbes. Frontiers in Genetics 2015;6:148. https://doi.org/10.3389/fgene.2015.00148.
[212] Rowland I, Gibson G, Heinken A, Scott K, Swann J, Thiele I, et al. Gut microbiota functions: metabolism of nutrients and other food components. Eur J Nutr 2018;57:1–24. https://doi.org/10.1007/s00394-017-1445-8.
[213] Gustafsson BE, Daft FS, McDaniel EG, Smith JC, Fitzgerald RJ. Effects of Vitamin K-Active Compounds and Intestinal Microorganisms in Vitamin K-Deficient Germfree Rats. The Journal of Nutrition 1962;78:461–8. https://doi.org/10.1093/jn/78.4.461.
[214] Molinero N, Ruiz L, Sánchez B, Margolles A, Delgado S. Intestinal Bacteria Interplay With Bile and Cholesterol Metabolism: Implications on Host Physiology. Front Physiol 2019;10:185. https://doi.org/10.3389/fphys.2019.00185.
[215] Ridlon JM, Kang D-J, Hylemon PB. Bile salt biotransformations by human intestinal bacteria. J Lipid Res 2006;47:241–59. https://doi.org/10.1194/jlr.R500013-JLR200.
[216] Gérard C, Vidal H. Impact of Gut Microbiota on Host Glycemic Control. Frontiers in Endocrinology 2019;10:29. https://doi.org/10.3389/fendo.2019.00029.
[217] Déchelotte P, Breton J, Trotin-Picolo C, Grube B, Erlenbeck C, Bothe G, et al. The Probiotic Strain H. alvei HA4597® Improves Weight Loss in Overweight Subjects under Moderate Hypocaloric Diet: A Proof-of-Concept, Multicenter Randomized, Double-Blind Placebo-Controlled Study. Nutrients 2021;13:1902. https://doi.org/10.3390/nu13061902.
[218] Han H, Yi B, Zhong R, Wang M, Zhang S, Ma J, et al. From gut microbiota to host appetite: gut microbiota-derived metabolites as key regulators. Microbiome 2021;9:162. https://doi.org/10.1186/s40168-021-01093-y.
[219] Covasa M, Stephens RW, Toderean R, Cobuz C. Intestinal Sensing by Gut Microbiota: Targeting Gut Peptides. Front Endocrinol 2019;10:82. https://doi.org/10.3389/fendo.2019.00082.
[220] Torres-Fuentes C, Golubeva AV, Zhdanov AV, Wallace S, Arboleya S, Papkovsky DB, et al. Short-chain fatty acids and microbiota metabolites attenuate ghrelin receptor signaling. The FASEB Journal 2019;33:13546–59. https://doi.org/10.1096/fj.201901433R.
[221] Leeuwendaal NK, Cryan JF, Schellekens H. Gut peptides and the microbiome: focus on ghrelin. Current Opinion in Endocrinology, Diabetes & Obesity 2021;28:243–52. https://doi.org/10.1097/MED.0000000000000616.

[222] Dominique M, Lucas N, Legrand R, Bouleté I-M, Bôle-Feysot C, Deroissart C, et al. Effects of Bacterial CLPB Protein Fragments on Food Intake and PYY Secretion. Nutrients 2021;13:2223. https://doi.org/10.3390/nu13072223.
[223] Schauber J. Expression of the cathelicidin LL-37 is modulated by short chain fatty acids in colonocytes: relevance of signalling pathways. Gut 2003;52:735–41. https://doi.org/10.1136/gut.52.5.735.
[224] Bilotta AJ, Cong Y. Gut microbiota metabolite regulation of host defenses at mucosal surfaces: implication in precision medicine. Precision Clinical Medicine 2019;2:110–9. https://doi.org/10.1093/pcmedi/pbz008.
[225] Wrzosek L, Miquel S, Noordine M-L, Bouet S, Chevalier-Curt MJ, Robert V, et al. Bacteroides thetaiotaomicron and Faecalibacterium prausnitziiinfluence the production of mucus glycans and the development of goblet cells in the colonic epithelium of a gnotobiotic model rodent. BMC Biol 2013;11:61. https://doi.org/10.1186/1741-7007-11-61.
[226] Burger-van Paassen N, Vincent A, Puiman PJ, van der Sluis M, Bouma J, Boehm G, et al. The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. Biochemical Journal 2009;420:211–9. https://doi.org/10.1042/BJ20082222.
[227] Belkaid Y, Hand TW. Role of the microbiota in immunity and inflammation. Cell 2014;157:121–41. https://doi.org/10.1016/j.cell.2014.03.011.
[228] Patten DA, Collett A. Exploring the immunomodulatory potential of microbial-associated molecular patterns derived from the enteric bacterial microbiota. Microbiology (Reading) 2013;159:1535–44. https://doi.org/10.1099/mic.0.064717-0.
[229] Chu H, Mazmanian SK. Innate immune recognition of the microbiota promotes host-microbial symbiosis. Nat Immunol 2013;14:668–75. https://doi.org/10.1038/ni.2635.
[230] Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux J-J, et al. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 2008;105:16731–6. https://doi.org/10.1073/pnas.0804812105.
[231] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer R-J. Review article: the role of butyrate on colonic function. Aliment Pharmacol Ther 2008;27:104–19. https://doi.org/10.1111/j.1365-2036.2007.03562.x.
[232] Wlodarska M, Luo C, Kolde R, d'Hennezel E, Annand JW, Heim CE, et al. Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. Cell Host & Microbe 2017;22:25-37.e6. https://doi.org/10.1016/j.chom.2017.06.007.
[233] Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027–31. https://doi.org/10.1038/nature05414.
[234] Thingholm LB, Rühlemann MC, Koch M, Fuqua B, Laucke G, Boehm R, et al. Obese Individuals with and without Type 2 Diabetes Show Different Gut Microbial Functional Capacity and Composition. Cell Host Microbe 2019;26:252-264.e10. https://doi.org/10.1016/j.chom.2019.07.004.

[235] Sokol H, Seksik P, Rigottier-Gois L, Lay C, Lepage P, Podglajen I, et al. Specificities of the fecal microbiota in inflammatory bowel disease. Inflamm Bowel Dis 2006;12:106–11. https://doi.org/10.1097/01.MIB.0000200323.38139.c6.
[236] Manichanh C, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, et al. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. Gut 2006;55:205–11. https://doi.org/10.1136/gut.2005.073817.
[237] Pasteur L. Observations relatives à la note précédente de M. Duclaux CR Acad Sci 1885;100:68.
[238] Bäckhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718–23. https://doi.org/10.1073/pnas.0407076101.
[239] Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. PNAS 2007;104:979–84. https://doi.org/10.1073/pnas.0605374104.
[240] Rabot S, Membrez M, Bruneau A, Gérard P, Harach T, Moser M, et al. Germ-free C57BL/6J mice are resistant to high-fat-diet-induced insulin resistance and have altered cholesterol metabolism. FASEB J 2010;24:4948–59. https://doi.org/10.1096/fj.10-164921.
[241] Luczynski P, McVey Neufeld K-A, Oriach CS, Clarke G, Dinan TG, Cryan JF. Growing up in a Bubble: Using Germ-Free Animals to Assess the Influence of the Gut Microbiota on Brain and Behavior. Int J Neuropsychopharmacol 2016;19. https://doi.org/10.1093/ijnp/pyw020.
[242] Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. Front Physiol 2011;2:94. https://doi.org/10.3389/fphys.2011.00094.
[243] Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 2011;23:255–64, e119. https://doi.org/10.1111/j.1365-2982.2010.01620.x.
[244] Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT, Philpott DJ, et al. Bacterial infection causes stress-induced memory dysfunction in mice. Gut 2011;60:307–17. https://doi.org/10.1136/gut.2009.202515.
[245] Stensballe LG, Simonsen J, Jensen SM, Bønnelykke K, Bisgaard H. Use of antibiotics during pregnancy increases the risk of asthma in early childhood. J Pediatr 2013;162:832-838.e3. https://doi.org/10.1016/j.jpeds.2012.09.049.
[246] Kummeling I, Stelma FF, Dagnelie PC, Snijders BEP, Penders J, Huber M, et al. Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: the KOALA Birth Cohort Study. Pediatrics 2007;119:e225-231. https://doi.org/10.1542/peds.2006-0896.
[247] Hviid A, Svanström H, Frisch M. Antibiotic use and inflammatory bowel diseases in childhood. Gut 2011;60:49–54. https://doi.org/10.1136/gut.2010.219683.
[248] Rasmussen SH, Shrestha S, Bjerregaard LG, Ängquist LH, Baker JL, Jess T, et al. Antibiotic exposure in early life and childhood overweight and obesity: A systematic review and meta-analysis. Diabetes Obes Metab 2018;20:1508–14. https://doi.org/10.1111/dom.13230.

[249] Slykerman RF, Thompson J, Waldie KE, Murphy R, Wall C, Mitchell EA. Antibiotics in the first year of life and subsequent neurocognitive outcomes. Acta Paediatr 2017;106:87–94. https://doi.org/10.1111/apa.13613.
[250] Kelly JR, Borre Y, O' Brien C, Patterson E, El Aidy S, Deane J, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res 2016;82:109–18. https://doi.org/10.1016/j.jpsychires.2016.07.019.
[251] Leclercq S, Le Roy T, Furgiuele S, Coste V, Bindels LB, Leyrolle Q, et al. Gut Microbiota-Induced Changes in β-Hydroxybutyrate Metabolism Are Linked to Altered Sociability and Depression in Alcohol Use Disorder. Cell Rep 2020;33:108238. https://doi.org/10.1016/j.celrep.2020.108238.
[252] Zhu F, Guo R, Wang W, Ju Y, Wang Q, Ma Q, et al. Transplantation of microbiota from drug-free patients with schizophrenia causes schizophrenia-like abnormal behaviors and dysregulated kynurenine metabolism in mice. Mol Psychiatry 2020;25:2905–18. https://doi.org/10.1038/s41380-019-0475-4.
[253] Arnoriaga-Rodríguez M, Mayneris-Perxachs J, Burokas A, Contreras-Rodríguez O, Blasco G, Coll C, et al. Obesity Impairs Short-Term and Working Memory through Gut Microbial Metabolism of Aromatic Amino Acids. Cell Metabolism 2020;32:548-560.e7. https://doi.org/10.1016/j.cmet.2020.09.002.
[254] Boehme M, Guzzetta KE, Bastiaanssen TFS, van de Wouw M, Moloney GM, Gual-Grau A, et al. Microbiota from young mice counteracts selective age-associated behavioral deficits. Nat Aging 2021;1:666–76. https://doi.org/10.1038/s43587-021-00093-9.
[255] Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. Brain Behav Immun 2011;25:397–407. https://doi.org/10.1016/j.bbi.2010.10.023.
[256] Rincel M, Darnaudéry M. Maternal separation in rodents: a journey from gut to brain and nutritional perspectives. Proc Nutr Soc 2020;79:113–32. https://doi.org/10.1017/S0029665119000958.
[257] Moloney RD, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the Microbiota-Gut-Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome. CNS Neurosci Ther 2016;22:102–17. https://doi.org/10.1111/cns.12490.
[258] Ilchmann-Diounou H, Menard S. Psychological Stress, Intestinal Barrier Dysfunctions, and Autoimmune Disorders: An Overview. Frontiers in Immunology 2020;11:1823. https://doi.org/10.3389/fimmu.2020.01823.
[259] Yoshikawa K, Kurihara C, Furuhashi H, Takajo T, Maruta K, Yasutake Y, et al. Psychological stress exacerbates NSAID-induced small bowel injury by inducing changes in intestinal microbiota and permeability via glucocorticoid receptor signaling. J Gastroenterol 2017;52:61–71. https://doi.org/10.1007/s00535-016-1205-1.
[260] Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu X-N, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress

response in mice. J Physiol 2004;558:263–75. https://doi.org/10.1113/jphysiol.2004.063388.
[261] Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 2010;170:1179–88. https://doi.org/10.1016/j.neuroscience.2010.08.005.
[262] Eutamene H, Bueno L. Role of probiotics in correcting abnormalities of colonic flora induced by stress. Gut 2007;56:1495–7. https://doi.org/10.1136/gut.2007.124040.
[263] Gareau MG, Jury J, MacQueen G, Sherman PM, Perdue MH. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 2007;56:1522–8. https://doi.org/10.1136/gut.2006.117176.
[264] Breit S, Kupferberg A, Rogler G, Hasler G. Vagus Nerve as Modulator of the Brain–Gut Axis in Psychiatric and Inflammatory Disorders. Frontiers in Psychiatry 2018;9:44. https://doi.org/10.3389/fpsyt.2018.00044.
[265] Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. PNAS 2011;108:16050–5. https://doi.org/10.1073/pnas.1102999108.
[266] Sgritta M, Dooling SW, Buffington SA, Momin EN, Francis MB, Britton RA, et al. Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder. Neuron 2019;101:246-259.e6. https://doi.org/10.1016/j.neuron.2018.11.018.
[267] Leclercq S, de Timary P, Delzenne NM, Stärkel P. The link between inflammation, bugs, the intestine and the brain in alcohol dependence. Transl Psychiatry 2017;7:e1048–e1048. https://doi.org/10.1038/tp.2017.15.
[268] Kelly JR, Kennedy PJ, Cryan JF, Dinan TG, Clarke G, Hyland NP. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Front Cell Neurosci 2015;9:392. https://doi.org/10.3389/fncel.2015.00392.
[269] Takeuchi O, Akira S. Pattern Recognition Receptors and Inflammation. Cell 2010;140:805–20. https://doi.org/10.1016/j.cell.2010.01.022.
[270] Akira S, Uematsu S, Takeuchi O. Pathogen Recognition and Innate Immunity. Cell 2006;124:783–801. https://doi.org/10.1016/j.cell.2006.02.015.
[271] Levy M, Blacher E, Elinav E. Microbiome, metabolites and host immunity. Curr Opin Microbiol 2017;35:8–15. https://doi.org/10.1016/j.mib.2016.10.003.
[272] Peng L, Li Z-R, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. J Nutr 2009;139:1619–25. https://doi.org/10.3945/jn.109.104638.
[273] Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015;18:965–77. https://doi.org/10.1038/nn.4030.

[274] Silva YP, Bernardi A, Frozza RL. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Frontiers in Endocrinology 2020;11:25. https://doi.org/10.3389/fendo.2020.00025.
[275] Lee B-H, Kim H, Park S-H, Kim Y-K. Decreased plasma BDNF level in depressive patients. Journal of Affective Disorders 2007;101:239–44. https://doi.org/10.1016/j.jad.2006.11.005.
[276] Matsumoto M, Kibe R, Ooga T, Aiba Y, Sawaki E, Koga Y, et al. Cerebral low-molecular metabolites influenced by intestinal microbiota: a pilot study. Front Syst Neurosci 2013;7:9. https://doi.org/10.3389/fnsys.2013.00009.
[277] Matsumoto M, Kibe R, Ooga T, Aiba Y, Kurihara S, Sawaki E, et al. Impact of Intestinal Microbiota on Intestinal Luminal Metabolome. Sci Rep 2012;2:233. https://doi.org/10.1038/srep00233.
[278] Gacias M, Gaspari S, Santos P-MG, Tamburini S, Andrade M, Zhang F, et al. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. ELife n.d.;5. https://doi.org/10.7554/eLife.13442.
[279] Rothhammer V, Borucki DM, Tjon EC, Takenaka MC, Chao C-C, Ardura-Fabregat A, et al. Microglial control of astrocytes in response to microbial metabolites. Nature 2018;557:724–8. https://doi.org/10.1038/s41586-018-0119-x.
[280] Vuong HE, Pronovost GN, Williams DW, Coley EJL, Siegler EL, Qiu A, et al. The maternal microbiome modulates fetal neurodevelopment in mice. Nature 2020;586:281–6. https://doi.org/10.1038/s41586-020-2745-3.
[281] Mancini A, Campagna F, Amodio P, Tuohy KM. Gut : liver : brain axis: the microbial challenge in the hepatic encephalopathy. Food Funct 2018;9:1373–88. https://doi.org/10.1039/c7fo01528c.
[282] Hsuchou H, Pan W, Kastin AJ. The fasting polypeptide FGF21 can enter brain from blood. Peptides 2007;28:2382–6. https://doi.org/10.1016/j.peptides.2007.10.007.
[283] Bookout AL, de Groot MHM, Owen BM, Lee S, Gautron L, Lawrence HL, et al. FGF21 regulates circadian behavior and metabolism by acting on the nervous system. Nat Med 2013;19:1147–52. https://doi.org/10.1038/nm.3249.
[284] Potthoff MJ. A new frontier in FGF21 biology. Nat Rev Endocrinol 2017;13:74–6. https://doi.org/10.1038/nrendo.2016.206.
[285] Nanji AA, Khettry U, Sadrzadeh SM, Yamanaka T. Severity of liver injury in experimental alcoholic liver disease. Correlation with plasma endotoxin, prostaglandin E2, leukotriene B4, and thromboxane B2. Am J Pathol 1993;142:367–73.
[286] Bode C, Kugler V, Bode JC. Endotoxemia in patients with alcoholic and non-alcoholic cirrhosis and in subjects with no evidence of chronic liver disease following acute alcohol excess. J Hepatol 1987;4:8–14. https://doi.org/10.1016/s0168-8278(87)80003-x.
[287] Parlesak A, Schäfer C, Schütz T, Bode JC, Bode C. Increased intestinal permeability to macromolecules and endotoxemia in patients with chronic alcohol abuse in different stages of alcohol-induced liver disease. J Hepatol 2000;32:742–7. https://doi.org/10.1016/s0168-8278(00)80242-1.

[288] Adachi Y, Moore LE, Bradford BU, Gao W, Thurman RG. Antibiotics prevent liver injury in rats following long-term exposure to ethanol. Gastroenterology 1995;108:218–24. https://doi.org/10.1016/0016-5085(95)90027-6.
[289] Nanji AA, Khettry U, Sadrzadeh SM. Lactobacillus feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). Proc Soc Exp Biol Med 1994;205:243–7. https://doi.org/10.3181/00379727-205-43703.
[290] Forsyth CB, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. Lactobacillus GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. Alcohol 2009;43:163–72. https://doi.org/10.1016/j.alcohol.2008.12.009.
[291] Bajaj JS. Alcohol, liver disease and the gut microbiota. Nat Rev Gastroenterol Hepatol 2019;16:235–46. https://doi.org/10.1038/s41575-018-0099-1.
[292] Kirpich IA, Solovieva NV, Leikhter SN, Shidakova NA, Lebedeva OV, Sidorov PI, et al. Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: a pilot study. Alcohol 2008;42:675–82. https://doi.org/10.1016/j.alcohol.2008.08.006.
[293] Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, et al. Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. Proc Natl Acad Sci USA 2014;111:E4485-4493. https://doi.org/10.1073/pnas.1415174111.
[294] Mutlu EA, Gillevet PM, Rangwala H, Sikaroodi M, Naqvi A, Engen PA, et al. Colonic microbiome is altered in alcoholism. American Journal of Physiology-Gastrointestinal and Liver Physiology 2012;302:G966–78. https://doi.org/10.1152/ajpgi.00380.2011.
[295] Bjørkhaug ST, Aanes H, Neupane SP, Bramness JG, Malvik S, Henriksen C, et al. Characterization of gut microbiota composition and functions in patients with chronic alcohol overconsumption. Gut Microbes 2019;10:663–75. https://doi.org/10.1080/19490976.2019.1580097.
[296] Bjarnason I, Peters TJ, Wise RJ. The leaky gut of alcoholism: possible route of entry for toxic compounds. Lancet 1984;1:179–82. https://doi.org/10.1016/s0140-6736(84)92109-3.
[297] Keshavarzian A, Fields JZ, Vaeth J, Holmes EW. The differing effects of acute and chronic alcohol on gastric and intestinal permeability. Am J Gastroenterol 1994;89:2205–11.
[298] Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, et al. Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. Gut Microbes 2020;12:1782157. https://doi.org/10.1080/19490976.2020.1782157.
[299] Beaumont M, Neyrinck AM, Olivares M, Rodriguez J, de Rocca Serra A, Roumain M, et al. The gut microbiota metabolite indole alleviates liver inflammation in mice. FASEB J 2018:fj201800544. https://doi.org/10.1096/fj.201800544.
[300] Hendrikx T, Schnabl B. Indoles: metabolites produced by intestinal bacteria capable of controlling liver disease manifestation. Journal of Internal Medicine 2019;286:32–40. https://doi.org/10.1111/joim.12892.

[301] Leclercq S, De Saeger C, Delzenne N, de Timary P, Stärkel P. Role of inflammatory pathways, blood mononuclear cells, and gut-derived bacterial products in alcohol dependence. Biol Psychiatry 2014;76:725–33. https://doi.org/10.1016/j.biopsych.2014.02.003.
[302] Leclercq S, Stärkel P, Delzenne NM, de Timary P. The gut microbiota: A new target in the management of alcohol dependence? Alcohol 2019;74:105–11. https://doi.org/10.1016/j.alcohol.2018.03.005.
[303] Delzenne NM, Neyrinck AM, Bäckhed F, Cani PD. Targeting gut microbiota in obesity: effects of prebiotics and probiotics. Nat Rev Endocrinol 2011;7:639–46. https://doi.org/10.1038/nrendo.2011.126.
[304] Leggio L, Ferrulli A, Cardone S, Nesci A, Miceli A, Malandrino N, et al. Ghrelin system in alcohol-dependent subjects: role of plasma ghrelin levels in alcohol drinking and craving. Addict Biol 2012;17:452–64. https://doi.org/10.1111/j.1369-1600.2010.00308.x.
[305] Leggio L, Zywiak WH, Fricchione SR, Edwards SM, de la Monte SM, Swift RM, et al. Intravenous ghrelin administration increases alcohol craving in alcohol-dependent heavy drinkers: a preliminary investigation. Biol Psychiatry 2014;76:734–41. https://doi.org/10.1016/j.biopsych.2014.03.019.
[306] Schellekens H, Finger BC, Dinan TG, Cryan JF. Ghrelin signalling and obesity: at the interface of stress, mood and food reward. Pharmacol Ther 2012;135:316–26. https://doi.org/10.1016/j.pharmthera.2012.06.004.
[307] Schellekens H, Dinan TG, Cryan JF. Ghrelin at the interface of obesity and reward. Vitam Horm 2013;91:285–323. https://doi.org/10.1016/B978-0-12-407766-9.00013-4.
[308] Stone LA, Harmatz ES, Goosens KA. Ghrelin as a Stress Hormone: Implications for Psychiatric Illness. Biol Psychiatry 2020;88:531–40. https://doi.org/10.1016/j.biopsych.2020.05.013.
[309] Addolorato G, Capristo E, Leggio L, Ferrulli A, Abenavoli L, Malandrino N, et al. Relationship Between Ghrelin Levels, Alcohol Craving, and Nutritional Status in Current Alcoholic Patients. Alcoholism: Clinical and Experimental Research 2006;30:1933–7. https://doi.org/10.1111/j.1530-0277.2006.00238.x.
[310] Badaoui A, De Saeger C, Duchemin J, Gihousse D, De Timary P, Stärkel P. Alcohol dependence is associated with reduced plasma and fundic ghrelin levels: ALCOHOLISM AND GHRELIN. European Journal of Clinical Investigation 2008;38:397–403. https://doi.org/10.1111/j.1365-2362.2008.01947.x.
[311] Koopmann A, von der Goltz C, Grosshans M, Dinter C, Vitale M, Wiedemann K, et al. The association of the appetitive peptide acetylated ghrelin with alcohol craving in early abstinent alcohol dependent individuals. Psychoneuroendocrinology 2012;37:980–6. https://doi.org/10.1016/j.psyneuen.2011.11.005.
[312] Koopmann A, Bach P, Schuster R, Bumb JM, Vollstädt-Klein S, Reinhard I, et al. Ghrelin modulates mesolimbic reactivity to alcohol cues in alcohol-addicted subjects: a functional imaging study. Addict Biol 2019;24:1066–76. https://doi.org/10.1111/adb.12651.
[313] Leclercq S, Schwarz M, Delzenne NM, Stärkel P, de Timary P. Alterations of kynurenine pathway in alcohol use disorder and abstinence: a link with gut

microbiota, peripheral inflammation and psychological symptoms. Transl Psychiatry 2021;11:1–9. https://doi.org/10.1038/s41398-021-01610-5.
[314] Arnaout B, Petrakis IL. Diagnosing Co-Morbid Drug Use in Patients With Alcohol Use Disorders. Alcohol Res Health 2008;31:148–54.
[315] Weinberger AH, Pacek LR, Giovenco D, Galea S, Zvolensky MJ, Gbedemah M, et al. Cigarette Use Among Individuals with Alcohol Use Disorders in the United States, 2002 to 2016: Trends Overall and by Race/Ethnicity. Alcohol Clin Exp Res 2019;43:79–90. https://doi.org/10.1111/acer.13922.
[316] Lien L, Bolstad I, Bramness JG. Smoking among inpatients in treatment for substance use disorders: prevalence and effect on mental health and quality of life. BMC Psychiatry 2021;21:1–9. https://doi.org/10.1186/s12888-021-03252-9.
[317] Zmora N, Suez J, Elinav E. You are what you eat: diet, health and the gut microbiota. Nat Rev Gastroenterol Hepatol 2019;16:35–56. https://doi.org/10.1038/s41575-018-0061-2.
[318] Gentile CL, Weir TL. The gut microbiota at the intersection of diet and human health. Science 2018;362:776–80. https://doi.org/10.1126/science.aau5812.
[319] Fluhr L, Mor U, Kolodziejczyk AA, Dori-Bachash M, Leshem A, Itav S, et al. Gut microbiota modulates weight gain in mice after discontinued smoke exposure. Nature 2021;600:713–9. https://doi.org/10.1038/s41586-021-04194-8.
[320] Carbone EA, D'Amato P, Vicchio G, De Fazio P, Segura-Garcia C. A systematic review on the role of microbiota in the pathogenesis and treatment of eating disorders. Eur Psychiatry 2020;64:e2. https://doi.org/10.1192/j.eurpsy.2020.109.
[321] Gorwood P, Blanchet-Collet C, Chartrel N, Duclos J, Dechelotte P, Hanachi M, et al. New Insights in Anorexia Nervosa. Front Neurosci 2016;10:256. https://doi.org/10.3389/fnins.2016.00256.
[322] Ding K, Chen J, Zhan W, Zhang S, Chen Y, Long S, et al. Microbiome Links Cigarette Smoke-Induced Chronic Obstructive Pulmonary Disease and Dietary Fiber via the Gut-Lung Axis: A Narrative Review. COPD 2021:1–8. https://doi.org/10.1080/15412555.2021.2019208.
[323] Navarro-Tapia E, Almeida-Toledano L, Sebastiani G, Serra-Delgado M, García-Algar Ó, Andreu-Fernández V. Effects of Microbiota Imbalance in Anxiety and Eating Disorders: Probiotics as Novel Therapeutic Approaches. Int J Mol Sci 2021;22:2351. https://doi.org/10.3390/ijms22052351.
[324] Clauss M, Gérard P, Mosca A, Leclerc M. Interplay Between Exercise and Gut Microbiome in the Context of Human Health and Performance. Frontiers in Nutrition 2021;8:305. https://doi.org/10.3389/fnut.2021.637010.
[325] Berding K, Cryan JF. Microbiota-targeted interventions for mental health. Curr Opin Psychiatry 2021. https://doi.org/10.1097/YCO.0000000000000758.
[326] Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 2014;11:506–14. https://doi.org/10.1038/nrgastro.2014.66.
[327] Ciorba MA. A Gastroenterologist's Guide to Probiotics. Clin Gastroenterol Hepatol 2012;10:960–8. https://doi.org/10.1016/j.cgh.2012.03.024.

[328] Wu S-I, Wu C-C, Tsai P-J, Cheng L-H, Hsu C-C, Shan I-K, et al. Psychobiotic Supplementation of PS128TM Improves Stress, Anxiety, and Insomnia in Highly Stressed Information Technology Specialists: A Pilot Study. Frontiers in Nutrition 2021;8:130. https://doi.org/10.3389/fnut.2021.614105.
[329] Ma T, Jin H, Kwok L-Y, Sun Z, Liong M-T, Zhang H. Probiotic consumption relieved human stress and anxiety symptoms possibly via modulating the neuroactive potential of the gut microbiota. Neurobiol Stress 2021;14:100294. https://doi.org/10.1016/j.ynstr.2021.100294.
[330] Eskandarzadeh S, Effatpanah M, Khosravi-Darani K, Askari R, Hosseini AF, Reisian M, et al. Efficacy of a multispecies probiotic as adjunctive therapy in generalized anxiety disorder: a double blind, randomized, placebo-controlled trial. Nutr Neurosci 2021;24:102–8. https://doi.org/10.1080/1028415X.2019.1598669.
[331] Mörkl S, Butler MI, Holl A, Cryan JF, Dinan TG. Probiotics and the Microbiota-Gut-Brain Axis: Focus on Psychiatry. Curr Nutr Rep 2020;9:171–82. https://doi.org/10.1007/s13668-020-00313-5.
[332] Martín R, Bermúdez-Humarán LG, Langella P. Searching for the Bacterial Effector: The Example of the Multi-Skilled Commensal Bacterium Faecalibacterium prausnitzii. Frontiers in Microbiology 2018;9:346. https://doi.org/10.3389/fmicb.2018.00346.
[333] Ueda A, Shinkai S, Shiroma H, Taniguchi Y, Tsuchida S, Kariya T, et al. Identification of Faecalibacterium prausnitzii strains for gut microbiome-based intervention in Alzheimer's-type dementia. Cell Reports Medicine 2021;2:100398. https://doi.org/10.1016/j.xcrm.2021.100398.
[334] Socała K, Doboszewska U, Szopa A, Serefko A, Włodarczyk M, Zielińska A, et al. The role of microbiota-gut-brain axis in neuropsychiatric and neurological disorders. Pharmacological Research 2021;172:105840. https://doi.org/10.1016/j.phrs.2021.105840.
[335] Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, et al. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med 2019;25:1096–103. https://doi.org/10.1038/s41591-019-0495-2.
[336] Salminen S, Collado MC, Endo A, Hill C, Lebeer S, Quigley EMM, et al. The International Scientific Association of Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of postbiotics. Nat Rev Gastroenterol Hepatol 2021;18:649–67. https://doi.org/10.1038/s41575-021-00440-6.
[337] Es C, A V, A P, Dj M, Kg M, Se Z-V, et al. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut 2015;64. https://doi.org/10.1136/gutjnl-2014-307913.
[338] Dalile B, Vervliet B, Bergonzelli G, Verbeke K, Van Oudenhove L. Colon-delivered short-chain fatty acids attenuate the cortisol response to psychosocial stress in healthy men: a randomized, placebo-controlled trial. Neuropsychopharmacol 2020. https://doi.org/10.1038/s41386-020-0732-x.
[339] van Beurden YH, de Groot PF, van Nood E, Nieuwdorp M, Keller JJ, Goorhuis A. Complications, effectiveness, and long term follow-up of fecal microbiota transfer

by nasoduodenal tube for treatment of recurrent Clostridium difficile infection. United European Gastroenterol J 2017;5:868–79. https://doi.org/10.1177/2050640616678099.

[340] Chapman BC, Moore HB, Overbey DM, Morton AP, Harnke B, Gerich ME, et al. Fecal microbiota transplant in patients with Clostridium difficile infection: A systematic review. J Trauma Acute Care Surg 2016;81:756–64. https://doi.org/10.1097/TA.0000000000001195.

[341] van Nood E, Vrieze A, Nieuwdorp M, Fuentes S, Zoetendal EG, de Vos WM, et al. Duodenal Infusion of Donor Feces for Recurrent *Clostridium difficile*. N Engl J Med 2013;368:407–15. https://doi.org/10.1056/NEJMoa1205037.

[342] Allegretti JR, Kassam Z, Mullish BH, Chiang A, Carrellas M, Hurtado J, et al. Effects of Fecal Microbiota Transplantation With Oral Capsules in Obese Patients. Clin Gastroenterol Hepatol 2020;18:855-863.e2. https://doi.org/10.1016/j.cgh.2019.07.006.

[343] Kootte RS, Levin E, Salojärvi J, Smits LP, Hartstra AV, Udayappan SD, et al. Improvement of Insulin Sensitivity after Lean Donor Feces in Metabolic Syndrome Is Driven by Baseline Intestinal Microbiota Composition. Cell Metab 2017;26:611-619.e6. https://doi.org/10.1016/j.cmet.2017.09.008.

[344] Kang D-W, Adams JB, Gregory AC, Borody T, Chittick L, Fasano A, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome 2017;5:10. https://doi.org/10.1186/s40168-016-0225-7.

[345] Bajaj JS, Gavis EA, Fagan A, Wade JB, Thacker LR, Fuchs M, et al. A Randomized Clinical Trial of Fecal Microbiota Transplant for Alcohol Use Disorder. Hepatology 2021;73:1688–700. https://doi.org/10.1002/hep.31496.

[346] Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. J Nutr 1995;125:1401–12. https://doi.org/10.1093/jn/125.6.1401.

[347] Bindels LB, Delzenne NM, Cani PD, Walter J. Towards a more comprehensive concept for prebiotics. Nat Rev Gastroenterol Hepatol 2015;12:303–10. https://doi.org/10.1038/nrgastro.2015.47.

[348] Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, et al. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. Nat Rev Gastroenterol Hepatol 2017;14:491–502. https://doi.org/10.1038/nrgastro.2017.75.

[349] Scott KP, Grimaldi R, Cunningham M, Sarbini SR, Wijeyesekera A, Tang MLK, et al. Developments in understanding and applying prebiotics in research and practice—an ISAPP conference paper. J Appl Microbiol 2020;128:934–49. https://doi.org/10.1111/jam.14424.

[350] Van Loo J, Coussement P, De Leenheer L, Hoebregs H, Smits G. On the presence of Inulin and Oligofructose as natural ingredients in the western diet. Critical Reviews in Food Science and Nutrition 1995;35:525–52. https://doi.org/10.1080/10408399509527714.

[351] Roberfroid MB. Inulin-Type Fructans: Functional Food Ingredients. The Journal of Nutrition 2007;137:2493S-2502S. https://doi.org/10.1093/jn/137.11.2493S.
[352] Wilson B, Whelan K. Prebiotic inulin-type fructans and galacto-oligosaccharides: definition, specificity, function, and application in gastrointestinal disorders. J Gastroenterol Hepatol 2017;32 Suppl 1:64–8. https://doi.org/10.1111/jgh.13700.
[353] Roberfroid M, Gibson GR, Hoyles L, McCartney AL, Rastall R, Rowland I, et al. Prebiotic effects: metabolic and health benefits. Br J Nutr 2010;104 Suppl 2:S1-63. https://doi.org/10.1017/S0007114510003363.
[354] Le Bastard Q, Chapelet G, Javaudin F, Lepelletier D, Batard E, Montassier E. The effects of inulin on gut microbial composition: a systematic review of evidence from human studies. Eur J Clin Microbiol Infect Dis 2020;39:403–13. https://doi.org/10.1007/s10096-019-03721-w.
[355] Reimer RA, Willis HJ, Tunnicliffe JM, Park H, Madsen KL, Soto-Vaca A. Inulin-type fructans and whey protein both modulate appetite but only fructans alter gut microbiota in adults with overweight/obesity: A randomized controlled trial. Mol Nutr Food Res 2017;61:1700484. https://doi.org/10.1002/mnfr.201700484.
[356] Nicolucci AC, Hume MP, Martínez I, Mayengbam S, Walter J, Reimer RA. Prebiotics Reduce Body Fat and Alter Intestinal Microbiota in Children Who Are Overweight or With Obesity. Gastroenterology 2017;153:711–22. https://doi.org/10.1053/j.gastro.2017.05.055.
[357] Vandeputte D, Falony G, Vieira-Silva S, Wang J, Sailer M, Theis S, et al. Prebiotic inulin-type fructans induce specific changes in the human gut microbiota. Gut 2017;66:1968–74. https://doi.org/10.1136/gutjnl-2016-313271.
[358] Healey G, Murphy R, Butts C, Brough L, Whelan K, Coad J. Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study. Br J Nutr 2018;119:176–89. https://doi.org/10.1017/S0007114517003440.
[359] Rodriguez J, Hiel S, Neyrinck AM, Le Roy T, Pötgens SA, Leyrolle Q, et al. Discovery of the gut microbial signature driving the efficacy of prebiotic intervention in obese patients. Gut 2020;69:1975–87. https://doi.org/10.1136/gutjnl-2019-319726.
[360] de Preter V, Vanhoutte T, Huys G, Swings J, Rutgeerts P, Verbeke K. Baseline microbiota activity and initial bifidobacteria counts influence responses to prebiotic dosing in healthy subjects. Aliment Pharmacol Ther 2008;27:504–13. https://doi.org/10.1111/j.1365-2036.2007.03588.x.
[361] Bouhnik Y, Raskine L, Champion K, Andrieux C, Penven S, Jacobs H, et al. Prolonged administration of low-dose inulin stimulates the growth of bifidobacteria in humans. Nutrition Research 2007;27:187–93. https://doi.org/10.1016/j.nutres.2007.01.013.
[362] Bouhnik Y, Raskine L, Simoneau G, Paineau D, Bornet F. The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: a dose-response relationship study in healthy humans. Nutr J 2006;5:8. https://doi.org/10.1186/1475-2891-5-8.
[363] Hiel S, Gianfrancesco MA, Rodriguez J, Portheault D, Leyrolle Q, Bindels LB, et al. Link between gut microbiota and health outcomes in inulin -treated obese

patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. Clinical Nutrition 2020:S0261561420301606. https://doi.org/10.1016/j.clnu.2020.04.005.

[364] Bouhnik Y, Vahedi K, Achour L, Attar A, Salfati J, Pochart P, et al. Short-Chain Fructo-Oligosaccharide Administration Dose-Dependently Increases Fecal Bifidobacteria in Healthy Humans. The Journal of Nutrition 1999;129:113–6. https://doi.org/10.1093/jn/129.1.113.

[365] Gill SK, Rossi M, Bajka B, Whelan K. Dietary fibre in gastrointestinal health and disease. Nat Rev Gastroenterol Hepatol 2021;18:101–16. https://doi.org/10.1038/s41575-020-00375-4.

[366] Micka A, Siepelmeyer A, Holz A, Theis S, Schön C. Effect of consumption of chicory inulin on bowel function in healthy subjects with constipation: a randomized, double-blind, placebo-controlled trial. International Journal of Food Sciences and Nutrition 2017;68:82–9. https://doi.org/10.1080/09637486.2016.1212819.

[367] Soret R, Chevalier J, De Coppet P, Poupeau G, Derkinderen P, Segain JP, et al. Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. Gastroenterology 2010;138:1772–82. https://doi.org/10.1053/j.gastro.2010.01.053.

[368] Stone-Dorshow T, Levitt MD. Gaseous response to ingestion of a poorly absorbed fructo-oligosaccharide sweetener. The American Journal of Clinical Nutrition 1987;46:61–5. https://doi.org/10.1093/ajcn/46.1.61.

[369] Kruse H-P, Kleessen B, Blaut M. Effects of inulin on faecal bifidobacteria in human subjects. Br J Nutr 1999;82:375–82. https://doi.org/10.1017/S0007114599001622.

[370] Wilson B, Rossi M, Dimidi E, Whelan K. Prebiotics in irritable bowel syndrome and other functional bowel disorders in adults: a systematic review and meta-analysis of randomized controlled trials. Am J Clin Nutr 2019. https://doi.org/10.1093/ajcn/nqy376.

[371] Hiel S, Bindels LB, Pachikian BD, Kalala G, Broers V, Zamariola G, et al. Effects of a diet based on inulin-rich vegetables on gut health and nutritional behavior in healthy humans. The American Journal of Clinical Nutrition 2019;109:1683–95. https://doi.org/10.1093/ajcn/nqz001.

[372] Bonnema AL, Kolberg LW, Thomas W, Slavin JL. Gastrointestinal Tolerance of Chicory Inulin Products. Journal of the American Dietetic Association 2010;110:865–8. https://doi.org/10.1016/j.jada.2010.03.025.

[373] Stewart ML, Timm DA, Slavin JL. Fructooligosaccharides exhibit more rapid fermentation than long-chain inulin in an in vitro fermentation system. Nutrition Research 2008;28:329–34. https://doi.org/10.1016/j.nutres.2008.02.014.

[374] Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50:2374–83. https://doi.org/10.1007/s00125-007-0791-0.

[375] Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PGB, Neyrinck AM, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. Gut 2013;62:1112–21. https://doi.org/10.1136/gutjnl-2012-303304.

[376] Delzenne NM, Daubioul C, Neyrinck A, Lasa M, Taper HS. Inulin and oligofructose modulate lipid metabolism in animals: review of biochemical events and future prospects. British Journal of Nutrition 2002;87:S255–9. https://doi.org/10.1079/BJN/2002545.
[377] Delzenne NM, Williams CM. Prebiotics and lipid metabolism. Curr Opin Lipidol 2002;13:61–7. https://doi.org/10.1097/00041433-200202000-00009.
[378] Beylot M. Effects of inulin-type fructans on lipid metabolism in man and in animal models. Br J Nutr 2005;93:S163–8. https://doi.org/10.1079/BJN20041339.
[379] Cani PD, Neyrinck AM, Maton N, Delzenne NM. Oligofructose promotes satiety in rats fed a high-fat diet: involvement of glucagon-like Peptide-1. Obes Res 2005;13:1000–7. https://doi.org/10.1038/oby.2005.117.
[380] Cani PD, Dewever C, Delzenne NM. Inulin-type fructans modulate gastrointestinal peptides involved in appetite regulation (glucagon-like peptide-1 and ghrelin) in rats. Br J Nutr 2004;92:521–6. https://doi.org/10.1079/bjn20041225.
[381] Cani PD, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy human: a pilot study. Eur J Clin Nutr 2006;60:567–72. https://doi.org/10.1038/sj.ejcn.1602350.
[382] Daubioul C, Rousseau N, Demeure R, Gallez B, Taper H, Declerck B, et al. Dietary fructans, but not cellulose, decrease triglyceride accumulation in the liver of obese Zucker fa/fa rats. J Nutr 2002;132:967–73. https://doi.org/10.1093/jn/132.5.967.
[383] Busserolles J, Gueux E, Rock E, Demigné C, Mazur A, Rayssiguier Y. Oligofructose protects against the hypertriglyceridemic and pro-oxidative effects of a high fructose diet in rats. J Nutr 2003;133:1903–8. https://doi.org/10.1093/jn/133.6.1903.
[384] Weitkunat K, Schumann S, Petzke KJ, Blaut M, Loh G, Klaus S. Effects of dietary inulin on bacterial growth, short-chain fatty acid production and hepatic lipid metabolism in gnotobiotic mice. J Nutr Biochem 2015;26:929–37. https://doi.org/10.1016/j.jnutbio.2015.03.010.
[385] Daubioul CA, Taper HS, De Wispelaere LD, Delzenne NM. Dietary oligofructose lessens hepatic steatosis, but does not prevent hypertriglyceridemia in obese zucker rats. J Nutr 2000;130:1314–9. https://doi.org/10.1093/jn/130.5.1314.
[386] Daubioul CA, Horsmans Y, Lambert P, Danse E, Delzenne NM. Effects of oligofructose on glucose and lipid metabolism in patients with nonalcoholic steatohepatitis: results of a pilot study. Eur J Clin Nutr 2005;59:723–6. https://doi.org/10.1038/sj.ejcn.1602127.
[387] Farhangi MA, Javid AZ, Dehghan P. The effect of enriched chicory inulin on liver enzymes, calcium homeostasis and hematological parameters in patients with type 2 diabetes mellitus: A randomized placebo-controlled trial. Prim Care Diabetes 2016;10:265–71. https://doi.org/10.1016/j.pcd.2015.10.009.
[388] Cani PD, Possemiers S, Van de Wiele T, Guiot Y, Everard A, Rottier O, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut 2009;58:1091–103. https://doi.org/10.1136/gut.2008.165886.

[389] Ohland CL, MacNaughton WK. Probiotic bacteria and intestinal epithelial barrier function. American Journal of Physiology-Gastrointestinal and Liver Physiology 2010;298:G807–19. https://doi.org/10.1152/ajpgi.00243.2009.
[390] Yang X, He F, Zhang Y, Xue J, Li K, Zhang X, et al. Inulin Ameliorates Alcoholic Liver Disease via Suppressing LPS-TLR4-Mψ Axis and Modulating Gut Microbiota in Mice. Alcohol Clin Exp Res 2019;43:411–24. https://doi.org/10.1111/acer.13950.
[391] Wang Z, Zhang X, Zhu L, Yang X, He F, Wang T, et al. Inulin alleviates inflammation of alcoholic liver disease via SCFAs-inducing suppression of M1 and facilitation of M2 macrophages in mice. Int Immunopharmacol 2020;78:106062. https://doi.org/10.1016/j.intimp.2019.106062.
[392] Byrne CS, Chambers ES, Alhabeeb H, Chhina N, Morrison DJ, Preston T, et al. Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. Am J Clin Nutr 2016;104:5–14. https://doi.org/10.3945/ajcn.115.126706.
[393] Smith AP, Sutherland D, Hewlett P. An Investigation of the Acute Effects of Oligofructose-Enriched Inulin on Subjective Wellbeing, Mood and Cognitive Performance. Nutrients 2015;7:8887–96. https://doi.org/10.3390/nu7115441.
[394] Smith AP. The concept of well-being: relevance to nutrition research. Br J Nutr 2005;93 Suppl 1:S1-5. https://doi.org/10.1079/bjn20041351.
[395] Azpiroz F, Dubray C, Bernalier-Donadille A, Cardot J-M, Accarino A, Serra J, et al. Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. Neurogastroenterology & Motility 2017;29:e12911. https://doi.org/10.1111/nmo.12911.
[396] Leyrolle Q, Cserjesi R, D.G.H. Mulders M, Zamariola G, Hiel S, Gianfrancesco MA, et al. Prebiotic effect on mood in obese patients is determined by the initial gut microbiota composition: A randomized, controlled trial. Brain, Behavior, and Immunity 2021;94:289–98. https://doi.org/10.1016/j.bbi.2021.01.014.
[397] Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PWJ. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology (Berl) 2015;232:1793–801. https://doi.org/10.1007/s00213-014-3810-0.
[398] Buigues C, Fernández-Garrido J, Pruimboom L, Hoogland AJ, Navarro-Martínez R, Martínez-Martínez M, et al. Effect of a Prebiotic Formulation on Frailty Syndrome: A Randomized, Double-Blind Clinical Trial. Int J Mol Sci 2016;17:E932. https://doi.org/10.3390/ijms17060932.
[399] Anastasovska J, Arora T, Canon GJS, Parkinson JRC, Touhy K, Gibson GR, et al. Fermentable Carbohydrate Alters Hypothalamic Neuronal Activity and Protects Against the Obesogenic Environment. Obesity 2012;20:1016–23. https://doi.org/10.1038/oby.2012.6.
[400] Yen C-H, Wang C-H, Wu W-T, Chen H-L. Fructo-oligosaccharide improved brain β-amyloid, β-secretase, cognitive function, and plasma antioxidant levels in D-galactose-treated Balb/cJ mice. Nutr Neurosci 2017;20:228–37. https://doi.org/10.1080/1028415X.2015.1110952.

[401] Hoffman JD, Yanckello LM, Chlipala G, Hammond TC, McCulloch SD, Parikh I, et al. Dietary inulin alters the gut microbiome, enhances systemic metabolism and reduces neuroinflammation in an APOE4 mouse model. PLoS One 2019;14:e0221828. https://doi.org/10.1371/journal.pone.0221828.

[402] Boehme M, Wouw M van de, Bastiaanssen TFS, Olavarría-Ramírez L, Lyons K, Fouhy F, et al. Mid-life microbiota crises: middle age is associated with pervasive neuroimmune alterations that are reversed by targeting the gut microbiome. Molecular Psychiatry 2019:1. https://doi.org/10.1038/s41380-019-0425-1.

[403] Burokas A, Arboleya S, Moloney RD, Peterson VL, Murphy K, Clarke G, et al. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry 2017;82:472–87. https://doi.org/10.1016/j.biopsych.2016.12.031.

[404] Sun J, Liu S, Ling Z, Wang F, Ling Y, Gong T, et al. Fructooligosaccharides Ameliorating Cognitive Deficits and Neurodegeneration in APP/PS1 Transgenic Mice through Modulating Gut Microbiota. J Agric Food Chem 2019;67:3006–17. https://doi.org/10.1021/acs.jafc.8b07313.

[405] Messaoudi M, Rozan P, Nejdi A, Hidalgo S, Desor D. Behavioural and cognitive effects of oligofructose-enriched inulin in rats. British Journal of Nutrition 2005;93:S27–30. https://doi.org/10.1079/BJN20041348.

[406] American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. American Psychiatric Association; 2013. https://doi.org/10.1176/appi.books.9780890425596.

[407] Beaton GH, Milner J, McGuire V, Feather TE, Little JA. Source of variance in 24-hour dietary recall data: implications for nutrition study design and interpretation. Carbohydrate sources, vitamins, and minerals. Am J Clin Nutr 1983;37:986–95. https://doi.org/10.1093/ajcn/37.6.986.

[408] Favier J-C, Ireland-Ripert J, Toque C, Feinberg M. Répertoire général des aliments : table de composition = Composition tables. Paris: Technique & Documentation; 1995.

[409] Superior Health Council. Dietary guidelines for the Belgian adult population. Brussels: 2019.

[410] Samieri C, Jutand MA, Feart C, Capuron L, Letenneur L, Barberger-Gateau P. Dietary patterns derived by hybrid clustering method in older people: association with cognition, mood, and self-rated health. J Am Diet Assoc 2008;108:1461–71. https://doi.org/10.1016/j.jada.2008.06.437.

[411] Kalala G, Kambashi B, Everaert N, Beckers Y, Richel A, Pachikian B, et al. Characterization of fructans and dietary fibre profiles in raw and steamed vegetables. Int J Food Sci Nutr 2018;69:682–9. https://doi.org/10.1080/09637486.2017.1412404.

[412] Monteiro CA, Cannon G, Levy R, Moubarac J-C, Jaime P, Martins AP, et al. NOVA. The star shines bright. World Nutrition 2016;7:28–38.

[413] Neyrinck AM, Rodriguez J, Vinoy S, Maquet V, Walter J, Bischoff SC, et al. The FiberTAG project: Tagging dietary fibre intake by measuring biomarkers related to the gut microbiota and their interest for health. Nutr Bull 2020;45:59–65. https://doi.org/10.1111/nbu.12416.

[414] Strunk KK, Lane FC. The Beck Depression Inventory, Second Edition (BDI-II): A Cross-Sample Structural Analysis. Measurement and Evaluation in Counseling and Development 2016:0748175616664010. https://doi.org/10.1177/0748175616664010.
[415] Ansseau M, Besson J, Lejoyeux M, Pinto E, Landry U, Cornes M, et al. A French Translation of the Obsessive-Compulsive Drinking Scale for Craving in Alcohol-Dependent Patients: A Validation Study in Belgium, France, and Switzerland. EAR 2000;6:51–6. https://doi.org/10.1159/000019010.
[416] Smets EM, Garssen B, Bonke B, De Haes JC. The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. J Psychosom Res 1995;39:315–25.
[417] Carpenter JS, Andrykowski MA. Psychometric evaluation of the pittsburgh sleep quality index. Journal of Psychosomatic Research 1998;45:5–13. https://doi.org/10.1016/S0022-3999(97)00298-5.
[418] Sobell LC, Sobell MB. Timeline Follow-Back. In: Litten RZ, Allen JP, editors. Measuring Alcohol Consumption, Totowa, NJ: Humana Press; 1992, p. 41–72. https://doi.org/10.1007/978-1-4612-0357-5_3.
[419] Orrell M, Yard P, Handysides J, Schapira R. Validity and reliability of the Health of the Nation Outcome Scales in psychiatric patients in the community. Br J Psychiatry 1999;174:409–12. https://doi.org/10.1192/bjp.174.5.409.
[420] Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975;12:189–98.
[421] Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther 1997;11:395–402. https://doi.org/10.1046/j.1365-2036.1997.142318000.x.
[422] Blake MR, Raker JM, Whelan K. Validity and reliability of the Bristol Stool Form Scale in healthy adults and patients with diarrhoea-predominant irritable bowel syndrome. Aliment Pharmacol Ther 2016;44:693–703. https://doi.org/10.1111/apt.13746.
[423] González I, Cao K-AL, Davis MJ, Déjean S. Visualising associations between paired 'omics' data sets. BioData Mining 2012;5:19. https://doi.org/10.1186/1756-0381-5-19.
[424] Manari AP, Preedy VR, Peters TJ. Nutritional intake of hazardous drinkers and dependent alcoholics in the UK. Addict Biol 2003;8:201–10. https://doi.org/10.1080/1355621031000117437.
[425] Thomson M, Fulton M, Elton RA, Brown S, Wood DA, Oliver MF. Alcohol consumption and nutrient intake in middle-aged Scottish men. Am J Clin Nutr 1988;47:139–45. https://doi.org/10.1093/ajcn/47.1.139.
[426] Jerlhag E. Gut-brain axis and addictive disorders: A review with focus on alcohol and drugs of abuse. Pharmacology & Therapeutics 2018. https://doi.org/10.1016/j.pharmthera.2018.11.005.
[427] Leggio L, Addolorato G, Cippitelli A, Jerlhag E, Kampov-Polevoy AB, Swift RM. Role of feeding-related pathways in alcohol dependence: A focus on sweet preference,

NPY, and ghrelin. Alcohol Clin Exp Res 2011;35:194–202. https://doi.org/10.1111/j.1530-0277.2010.01334.x.
[428] Mehta S, Baruah A, Das S, Avinash P, Chetia D, Gupta D. Leptin levels in alcohol dependent patients and their relationship with withdrawal and craving. Asian Journal of Psychiatry 2020;51:101967. https://doi.org/10.1016/j.ajp.2020.101967.
[429] Kiefer F, Jahn H, Jaschinski M, Holzbach R, Wolf K, Naber D, et al. Leptin: a modulator of alcohol craving? Biological Psychiatry 2001;49:782–7. https://doi.org/10.1016/S0006-3223(01)01081-2.
[430] Edington J. Problems of nutritional assessment in the community. Proc Nutr Soc 1999;58:47–51. https://doi.org/10.1079/pns19990007.
[431] Konttinen H, Männistö S, Sarlio-Lähteenkorva S, Silventoinen K, Haukkala A. Emotional eating, depressive symptoms and self-reported food consumption. A population-based study. Appetite 2010;54:473–9. https://doi.org/10.1016/j.appet.2010.01.014.
[432] Kim SY, Breslow RA, Ahn J, Salem N. Alcohol Consumption and Fatty Acid Intakes in the 2001?2002 National Health and Nutrition Examination Survey. Alcoholism Clin Exp Res 2007;31:1407–14. https://doi.org/10.1111/j.1530-0277.2007.00442.x.
[433] Borsonelo EC, Galduróz JCF. The role of polyunsaturated fatty acids (PUFAs) in development, aging and substance abuse disorders: Review and propositions. Prostaglandins, Leukotrienes and Essential Fatty Acids 2008;78:237–45. https://doi.org/10.1016/j.plefa.2008.03.005.
[434] Liao Y, Xie B, Zhang H, He Q, Guo L, Subramaniapillai M, et al. Efficacy of omega-3 PUFAs in depression: A meta-analysis. Transl Psychiatry 2019;9:190. https://doi.org/10.1038/s41398-019-0515-5.
[435] Larrieu T, Layé S. Food for Mood: Relevance of Nutritional Omega-3 Fatty Acids for Depression and Anxiety. Front Physiol 2018;9. https://doi.org/10.3389/fphys.2018.01047.
[436] Hussain G, Schmitt F, Loeffler J-P, Gonzalez de Aguilar J-L. Fatting the brain: a brief of recent research. Front Cell Neurosci 2013;7:144. https://doi.org/10.3389/fncel.2013.00144.
[437] Buydens-Branchey L, Branchey M. Long-chain n-3 polyunsaturated fatty acids decrease feelings of anger in substance abusers. Psychiatry Res 2008;157:95–104. https://doi.org/10.1016/j.psychres.2007.01.004.
[438] Adjibade M, Julia C, Allès B, Touvier M, Lemogne C, Srour B, et al. Prospective association between ultra-processed food consumption and incident depressive symptoms in the French NutriNet-Santé cohort. BMC Medicine 2019;17:78. https://doi.org/10.1186/s12916-019-1312-y.
[439] Gómez-Donoso C, Sánchez-Villegas A, Martínez-González MA, Gea A, Mendonça R de D, Lahortiga-Ramos F, et al. Ultra-processed food consumption and the incidence of depression in a Mediterranean cohort: the SUN Project. Eur J Nutr 2020;59:1093–103. https://doi.org/10.1007/s00394-019-01970-1.
[440] Grochowski C, Blicharska E, Baj J, Mierzwińska A, Brzozowska K, Forma A, et al. Serum iron, Magnesium, Copper, and Manganese Levels in Alcoholism: A Systematic Review. Molecules 2019;24. https://doi.org/10.3390/molecules24071361.

[441] Barve S, Chen S-Y, Kirpich I, Watson WH, McClain C. Development, Prevention, and Treatment of Alcohol-Induced Organ Injury: The Role of Nutrition. Alcohol Res 2017;38:289–302.
[442] Torres SJ, Nowson CA, Worsley A. Dietary electrolytes are related to mood. Br J Nutr 2008;100:1038–45. https://doi.org/10.1017/S0007114508959201.
[443] Thi Thu Nguyen T, Miyagi S, Tsujiguchi H, Kambayashi Y, Hara A, Nakamura H, et al. Association between Lower Intake of Minerals and Depressive Symptoms among Elderly Japanese Women but Not Men: Findings from Shika Study. Nutrients 2019;11:389. https://doi.org/10.3390/nu11020389.
[444] Fawehinmi TO, Ilomäki J, Voutilainen S, Kauhanen J. Alcohol Consumption and Dietary Patterns: The FinDrink Study. PLoS ONE 2012;7:e38607. https://doi.org/10.1371/journal.pone.0038607.
[445] Reynolds A, Mann J, Cummings J, Winter N, Mete E, Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. Lancet 2019. https://doi.org/10.1016/S0140-6736(18)31809-9.
[446] Stephen AM, Champ MM-J, Cloran SJ, Fleith M, van Lieshout L, Mejborn H, et al. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. Nutr Res Rev 2017;30:149–90. https://doi.org/10.1017/S095442241700004X.
[447] Buffington SA, Di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial Reconstitution Reverses Maternal Diet-Induced Social and Synaptic Deficits in Offspring. Cell 2016;165:1762–75. https://doi.org/10.1016/j.cell.2016.06.001.
[448] Degroote S, Hunting DJ, Baccarelli AA, Takser L. Maternal gut and fetal brain connection: Increased anxiety and reduced social interactions in Wistar rat offspring following peri-conceptional antibiotic exposure. Prog Neuropsychopharmacol Biol Psychiatry 2016;71:76–82. https://doi.org/10.1016/j.pnpbp.2016.06.010.
[449] Aslam H, Green J, Jacka FN, Collier F, Berk M, Pasco J, et al. Fermented foods, the gut and mental health: a mechanistic overview with implications for depression and anxiety. Nutritional Neuroscience 2020;23:659–71. https://doi.org/10.1080/1028415X.2018.1544332.
[450] Woo J, Lynn H, Lau WY, Leung J, Lau E, Wong SYS, et al. Nutrient intake and psychological health in an elderly Chinese population. Int J Geriatr Psychiatry 2006;21:1036–43. https://doi.org/10.1002/gps.1603.
[451] Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejía JL, Hansen LH, et al. A prebiotic intervention study in children with autism spectrum disorders (ASDs). Microbiome 2018;6:133. https://doi.org/10.1186/s40168-018-0523-3.
[452] St-Onge M-P, Roberts A, Shechter A, Choudhury AR. Fiber and Saturated Fat Are Associated with Sleep Arousals and Slow Wave Sleep. Journal of Clinical Sleep Medicine 2016;12:19–24. https://doi.org/10.5664/jcsm.5384.
[453] van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, O'Sullivan O, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol (Lond) 2018;596:4923–44. https://doi.org/10.1113/JP276431.

[454] Sarkar A, Harty S, Johnson KV-A, Moeller AH, Carmody RN, Lehto SM, et al. The role of the microbiome in the neurobiology of social behaviour. Biol Rev Camb Philos Soc 2020. https://doi.org/10.1111/brv.12603.
[455] Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. Psychobiotics and the Manipulation of Bacteria–Gut–Brain Signals. Trends in Neurosciences 2016;39:763–81. https://doi.org/10.1016/j.tins.2016.09.002.
[456] Chae W, Ju YJ, Shin J, Jang S-I, Park E-C. Association between eating behaviour and diet quality: eating alone vs. eating with others. Nutr J 2018;17:117. https://doi.org/10.1186/s12937-018-0424-0.
[457] Williams R, Rollo M, Schumacher T, Collins C. Diet Quality Scores of Australian Adults Who Have Completed the Healthy Eating Quiz. Nutrients 2017;9:880. https://doi.org/10.3390/nu9080880.
[458] Roberfroid M. Dietary fiber, inulin, and oligofructose: A review comparing their physiological effects. Critical Reviews in Food Science and Nutrition 1993;33:103–48. https://doi.org/10.1080/10408399309527616.
[459] Silveira ALM, Ferreira AVM, de Oliveira MC, Rachid MA, da Cunha Sousa LF, dos Santos Martins F, et al. Preventive rather than therapeutic treatment with high fiber diet attenuates clinical and inflammatory markers of acute and chronic DSS-induced colitis in mice. Eur J Nutr 2017;56:179–91. https://doi.org/10.1007/s00394-015-1068-x.
[460] Fedorak RN, Madsen KL. Probiotics and the Management of Inflammatory Bowel Disease: Inflammatory Bowel Diseases 2004;10:286–99. https://doi.org/10.1097/00054725-200405000-00018.
[461] Hamer HM, Jonkers D, Venema K, Vanhoutvin S, Troost FJ, Brummer R-J. Review article: the role of butyrate on colonic function: REVIEW: ROLE OF BUTYRATE ON COLONIC FUNCTION. Alimentary Pharmacology & Therapeutics 2007;27:104–19. https://doi.org/10.1111/j.1365-2036.2007.03562.x.
[462] Galobardes B, Morabia A, Bernstein MS. Diet and socioeconomic position: does the use of different indicators matter? Int J Epidemiol 2001;30:334–40. https://doi.org/10.1093/ije/30.2.334.
[463] Temme E, Huybrechts I, Vandevijvere S, De Henauw S, Leveque A, Kornitzer M, et al. Energy and macronutrient intakes in Belgium: results from the first National Food Consumption Survey. Br J Nutr 2010;103:1823–9. https://doi.org/10.1017/S0007114510000085.
[464] Rustad JK, Stern TA, Prabhakar M, Musselman D. Risk factors for alcohol relapse following orthotopic liver transplantation: a systematic review. Psychosomatics 2015;56:21–35. https://doi.org/10.1016/j.psym.2014.09.006.
[465] Quoilin C, Wilhelm E, Maurage P, de Timary P, Duque J. Deficient inhibition in alcohol-dependence: let's consider the role of the motor system! Neuropsychopharmacology 2018;43:1851–8. https://doi.org/10.1038/s41386-018-0074-0.
[466] Schachter J, Martel J, Lin C-S, Chang C-J, Wu T-R, Lu C-C, et al. Effects of obesity on depression: A role for inflammation and the gut microbiota. Brain Behav Immun 2018;69:1–8. https://doi.org/10.1016/j.bbi.2017.08.026.

[467] Torres-Fuentes C, Schellekens H, Dinan TG, Cryan JF. The microbiota-gut-brain axis in obesity. Lancet Gastroenterol Hepatol 2017;2:747–56. https://doi.org/10.1016/S2468-1253(17)30147-4.
[468] Boulangé CL, Neves AL, Chilloux J, Nicholson JK, Dumas M-E. Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome Medicine 2016;8:42. https://doi.org/10.1186/s13073-016-0303-2.
[469] Li Q, Han Y, Dy ABC, Hagerman RJ. The Gut Microbiota and Autism Spectrum Disorders. Frontiers in Cellular Neuroscience 2017;11:120. https://doi.org/10.3389/fncel.2017.00120.
[470] Pascal V, Pozuelo M, Borruel N, Casellas F, Campos D, Santiago A, et al. A microbial signature for Crohn's disease. Gut 2017;66:813–22. https://doi.org/10.1136/gutjnl-2016-313235.
[471] Broglio K. Randomization in Clinical Trials: Permuted Blocks and Stratification. JAMA 2018;319:2223. https://doi.org/10.1001/jama.2018.6360.
[472] Moos RH, Moos BS. Rates and predictors of relapse after natural and treated remission from alcohol use disorders. Addiction 2006;101:212–22. https://doi.org/10.1111/j.1360-0443.2006.01310.x.
[473] Maisto SA, Witkiewitz K, Moskal D, Wilson AD. Is the Construct of Relapse Heuristic, and Does It Advance Alcohol Use Disorder Clinical Practice? J Stud Alcohol Drugs 2016;77:849–58. https://doi.org/10.15288/jsad.2016.77.849.
[474] Neyrinck AM, Nazare J-A, Rodriguez J, Jottard R, Dib S, Sothier M, et al. Development of a Repertoire and a Food Frequency Questionnaire for Estimating Dietary Fiber Intake Considering Prebiotics: Input from the FiberTAG Project. Nutrients 2020;12:2824. https://doi.org/10.3390/nu12092824.
[475] Deehan EC, Duar RM, Armet AM, Perez-Muñoz ME, Jin M, Walter J. Modulation of the Gastrointestinal Microbiome with Nondigestible Fermentable Carbohydrates To Improve Human Health. Microbiology Spectrum 2017;5:5.5.04. https://doi.org/10.1128/microbiolspec.BAD-0019-2017.
[476] Makki K, Deehan EC, Walter J, Bäckhed F. The Impact of Dietary Fiber on Gut Microbiota in Host Health and Disease. Cell Host Microbe 2018;23:705–15. https://doi.org/10.1016/j.chom.2018.05.012.
[477] Marzorati M, Vilchez-Vargas R, Bussche JV, Truchado P, Jauregui R, El Hage RA, et al. High-fiber and high-protein diets shape different gut microbial communities, which ecologically behave similarly under stress conditions, as shown in a gastrointestinal simulator. Molecular Nutrition & Food Research 2017;61:1600150. https://doi.org/10.1002/mnfr.201600150.
[478] Torre DL, Verbeke K, Dalile B. Dietary fibre and the gut–brain axis: microbiota-dependent and independent mechanisms of action. Gut Microbiome 2021;2. https://doi.org/10.1017/gmb.2021.3.
[479] Bijkerk CJ, de Wit NJ, Muris JWM, Whorwell PJ, Knottnerus JA, Hoes AW. Soluble or insoluble fibre in irritable bowel syndrome in primary care? Randomised placebo controlled trial. BMJ 2009;339:b3154. https://doi.org/10.1136/bmj.b3154.
[480] Kim C-S, Byeon S, Shin D-M. Sources of Dietary Fiber Are Differently Associated with Prevalence of Depression. Nutrients 2020;12:2813. https://doi.org/10.3390/nu12092813.

[481] Silk DBA, Davis A, Vulevic J, Tzortzis G, Gibson GR. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. Aliment Pharmacol Ther 2009;29:508–18. https://doi.org/10.1111/j.1365-2036.2008.03911.x.

[482] Raulo A, Allen BE, Troitsky T, Husby A, Firth JA, Coulson T, et al. Social networks strongly predict the gut microbiota of wild mice. ISME J 2021;15:2601–13. https://doi.org/10.1038/s41396-021-00949-3.

[483] Johnson KV-A. Gut microbiome composition and diversity are related to human personality traits. Human Microbiome Journal 2020;15:100069. https://doi.org/10.1016/j.humic.2019.100069.

[484] Desbonnet L, Clarke G, Shanahan F, Dinan TG, Cryan JF. Microbiota is essential for social development in the mouse. Mol Psychiatry 2014;19:146–8. https://doi.org/10.1038/mp.2013.65.

[485] Wang Y, Li N, Yang J-J, Zhao D-M, Chen B, Zhang G-Q, et al. Probiotics and fructo-oligosaccharide intervention modulate the microbiota-gut brain axis to improve autism spectrum reducing also the hyper-serotonergic state and the dopamine metabolism disorder. Pharmacological Research 2020;157:104784. https://doi.org/10.1016/j.phrs.2020.104784.

[486] Mitchell LK, Davies PSW. Pre- and probiotics in the management of children with autism and gut issues: a review of the current evidence. Eur J Clin Nutr 2021:1–9. https://doi.org/10.1038/s41430-021-01027-9.

[487] Sherwin E, Bordenstein SR, Quinn JL, Dinan TG, Cryan JF. Microbiota and the social brain. Science 2019;366. https://doi.org/10.1126/science.aar2016.

[488] Wu W-L, Adame MD, Liou C-W, Barlow JT, Lai T-T, Sharon G, et al. Microbiota regulate social behaviour via stress response neurons in the brain. Nature 2021;595:409–14. https://doi.org/10.1038/s41586-021-03669-y.

[489] de Timary P, Luts A, Hers D, Luminet O. Absolute and relative stability of alexithymia in alcoholic inpatients undergoing alcohol withdrawal: relationship to depression and anxiety. Psychiatry Res 2008;157:105–13. https://doi.org/10.1016/j.psychres.2006.12.008.

[490] Thorberg FA, Young RM, Sullivan KA, Lyvers M. Alexithymia and alcohol use disorders: a critical review. Addict Behav 2009;34:237–45. https://doi.org/10.1016/j.addbeh.2008.10.016.

[491] Cruise KE, Becerra R. Alexithymia and problematic alcohol use: A critical update. Addictive Behaviors 2018;77:232–46. https://doi.org/10.1016/j.addbeh.2017.09.025.

[492] Peterson K, Malouff J, Thorsteinsson EB. A meta-analytic investigation of emotional intelligence and alcohol involvement. Subst Use Misuse 2011;46:1726–33. https://doi.org/10.3109/10826084.2011.618962.

[493] Riley H, Schutte NS. Low emotional intelligence as a predictor of substance-use problems. J Drug Educ 2003;33:391–8. https://doi.org/10.2190/6DH9-YT0M-FT99-2X05.

[494] Mohagheghi A, Amiri S, Mousavi Rizi S, Safikhanlou S. Emotional Intelligence Components in Alcohol Dependent and Mentally Healthy Individuals. The Scientific World Journal 2015;2015:e841039. https://doi.org/10.1155/2015/841039.

[495] Maurage F, de Timary P, Tecco JM, Lechantre S, Samson D. Theory of Mind Difficulties in Patients with Alcohol Dependence: Beyond the Prefrontal Cortex Dysfunction Hypothesis. Alcoholism: Clinical and Experimental Research 2015;39:980–8. https://doi.org/10.1111/acer.12717.

[496] Rupp CI, Derntl B, Osthaus F, Kemmler G, Fleischhacker WW. Impact of Social Cognition on Alcohol Dependence Treatment Outcome: Poorer Facial Emotion Recognition Predicts Relapse/Dropout. Alcoholism: Clinical and Experimental Research 2017;41:2197–206. https://doi.org/10.1111/acer.13522.

[497] Vich Vila A, Collij V, Sanna S, Sinha T, Imhann F, Bourgonje AR, et al. Impact of commonly used drugs on the composition and metabolic function of the gut microbiota. Nat Commun 2020;11:362. https://doi.org/10.1038/s41467-019-14177-z.

[498] Hooks KB, O'Malley MA. Dysbiosis and Its Discontents. MBio 2017;8:e01492-17. https://doi.org/10.1128/mBio.01492-17.

[499] Brüssow H. Problems with the concept of gut microbiota dysbiosis. Microbial Biotechnology 2020;13:423–34. https://doi.org/10.1111/1751-7915.13479.

[500] Tuohy KM, Finlay RK, Wynne AG, Gibson GR. A Human Volunteer Study on the Prebiotic Effects of HP-Inulin—Faecal Bacteria Enumerated Using Fluorescent In Situ Hybridisation (FISH). Anaerobe 2001;7:113–8. https://doi.org/10.1006/anae.2001.0368.

[501] Kolida S, Meyer D, Gibson GR. A double-blind placebo-controlled study to establish the bifidogenic dose of inulin in healthy humans. Eur J Clin Nutr 2007;61:1189–95. https://doi.org/10.1038/sj.ejcn.1602636.

[502] Claesson MJ, Jeffery IB, Conde S, Power SE, O'Connor EM, Cusack S, et al. Gut microbiota composition correlates with diet and health in the elderly. Nature 2012;488:178–84. https://doi.org/10.1038/nature11319.

[503] Cuevas-Sierra A, Milagro FI, Aranaz P, Martínez JA, Riezu-Boj JI. Gut Microbiota Differences According to Ultra-Processed Food Consumption in a Spanish Population. Nutrients 2021;13:2710. https://doi.org/10.3390/nu13082710.

[504] Zhao L, Zhang F, Ding X, Wu G, Lam YY, Wang X, et al. Gut bacteria selectively promoted by dietary fibers alleviate type 2 diabetes. Science 2018;359:1151–6. https://doi.org/10.1126/science.aao5774.

[505] Miranda M, Morici JF, Zanoni MB, Bekinschtein P. Brain-Derived Neurotrophic Factor: A Key Molecule for Memory in the Healthy and the Pathological Brain. Frontiers in Cellular Neuroscience 2019;13:363. https://doi.org/10.3389/fncel.2019.00363.

[506] BINDER DK, SCHARFMAN HE. Brain-derived Neurotrophic Factor. Growth Factors 2004;22:123–31. https://doi.org/10.1080/08977190410001723308.

[507] Park H, Poo M. Neurotrophin regulation of neural circuit development and function. Nat Rev Neurosci 2013;14:7–23. https://doi.org/10.1038/nrn3379.

[508] Nunes PV, Nascimento CF, Kim HK, Andreazza AC, Brentani HP, Suemoto CK, et al. Low brain-derived neurotrophic factor levels in post-mortem brains of older adults with depression and dementia in a large clinicopathological sample. J Affect Disord 2018;241:176–81. https://doi.org/10.1016/j.jad.2018.08.025.

[509] Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G, Aubry J-M. Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 2002;109:143–8. https://doi.org/10.1016/s0165-1781(02)00005-7.

[510] Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder. Neurobiology of Disease 2015;77:220–7. https://doi.org/10.1016/j.nbd.2015.03.011.

[511] F O, F H, Fb S, F PR, Al T, Jn S, et al. Brain-derived neurotrophic factor in substance use disorders: A systematic review and meta-analysis. Drug and Alcohol Dependence 2018;193. https://doi.org/10.1016/j.drugalcdep.2018.08.036.

[512] Levchuk LA, Meeder EMG, Roschina OV, Loonen AJM, Boiko AS, Michalitskaya EV, et al. Exploring Brain Derived Neurotrophic Factor and Cell Adhesion Molecules as Biomarkers for the Transdiagnostic Symptom Anhedonia in Alcohol Use Disorder and Comorbid Depression. Frontiers in Psychiatry 2020;11:296. https://doi.org/10.3389/fpsyt.2020.00296.

[513] Girard M, Carrier P, Loustaud-Ratti V, Nubukpo P. BDNF levels and liver stiffness in subjects with alcohol use disorder: Evaluation after alcohol withdrawal. The American Journal of Drug and Alcohol Abuse 2021;47:191–8. https://doi.org/10.1080/00952990.2020.1833211.

[514] Savignac HM, Corona G, Mills H, Chen L, Spencer JPE, Tzortzis G, et al. Prebiotic feeding elevates central brain derived neurotrophic factor, N-methyl-d-aspartate receptor subunits and d-serine. Neurochemistry International 2013;63:756–64. https://doi.org/10.1016/j.neuint.2013.10.006.

[515] Morshedi M, Saghafi-Asl M, Hosseinifard E-S. The potential therapeutic effects of the gut microbiome manipulation by synbiotic containing-Lactobacillus plantarum on neuropsychological performance of diabetic rats. Journal of Translational Medicine 2020;18:18. https://doi.org/10.1186/s12967-019-02169-y.

[516] Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The Intestinal Microbiota Affect Central Levels of Brain-Derived Neurotropic Factor and Behavior in Mice. Gastroenterology 2011;141:599-609.e3. https://doi.org/10.1053/j.gastro.2011.04.052.

[517] Haghighat N, Rajabi S, Mohammadshahi M. Effect of synbiotic and probiotic supplementation on serum brain-derived neurotrophic factor level, depression and anxiety symptoms in hemodialysis patients: a randomized, double-blinded, clinical trial. Nutr Neurosci 2021;24:490–9. https://doi.org/10.1080/1028415X.2019.1646975.

[518] Stilling RM, van de Wouw M, Clarke G, Stanton C, Dinan TG, Cryan JF. The neuropharmacology of butyrate: The bread and butter of the microbiota-gut-brain axis? Neurochemistry International 2016;99:110–32. https://doi.org/10.1016/j.neuint.2016.06.011.

[519] Gejl AK, Enevold C, Bugge A, Andersen MS, Nielsen CH, Andersen LB. Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. Sci Rep 2019;9:9655. https://doi.org/10.1038/s41598-019-45976-5.

[520] Polyakova M, Schlögl H, Sacher J, Schmidt-Kassow M, Kaiser J, Stumvoll M, et al. Stability of BDNF in Human Samples Stored Up to 6 Months and Correlations of Serum and EDTA-Plasma Concentrations. International Journal of Molecular Sciences 2017;18:1189. https://doi.org/10.3390/ijms18061189.

[521] Robinson MW, Harmon C, O'Farrelly C. Liver immunology and its role in inflammation and homeostasis. Cell Mol Immunol 2016;13:267–76. https://doi.org/10.1038/cmi.2016.3.

[522] Martín-Mateos R, Albillos A. The Role of the Gut-Liver Axis in Metabolic Dysfunction-Associated Fatty Liver Disease. Frontiers in Immunology 2021;12:1265. https://doi.org/10.3389/fimmu.2021.660179.

[523] Kerner A, Avizohar O, Sella R, Bartha P, Zinder O, Markiewicz W, et al. Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2005;25:193–7. https://doi.org/10.1161/01.ATV.0000148324.63685.6a.

[524] Thomes PG, Rasineni K, Saraswathi V, Kharbanda KK, Clemens DL, Sweeney SA, et al. Natural Recovery by the Liver and Other Organs after Chronic Alcohol Use. Alcohol Res 2021;41:05. https://doi.org/10.35946/arcr.v41.1.05.

[525] Lane BP, Lieber CS. Ultrastructural alterations in human hepatocytes following ingestion of ethanol with adequate diets. Am J Pathol 1966;49:593–603.

[526] Lackner C, Spindelboeck W, Haybaeck J, Douschan P, Rainer F, Terracciano L, et al. Histological parameters and alcohol abstinence determine long-term prognosis in patients with alcoholic liver disease. J Hepatol 2017;66:610–8. https://doi.org/10.1016/j.jhep.2016.11.011.

[527] Oliva F, Nibbio G, Vizzuso P, Jaretti Sodano A, Ostacoli L, Carletto S, et al. Gender Differences in Anxiety and Depression before and after Alcohol Detoxification: Anxiety and Depression as Gender-Related Predictors of Relapse. EAR 2018;24:163–72. https://doi.org/10.1159/000490046.

[528] Petit G, Luminet O, Uva MC de S, Monhonval P, Leclercq S, Spilliaert Q, et al. Gender Differences in Affects and Craving in Alcohol-Dependence: A Study During Alcohol Detoxification. Alcoholism: Clinical and Experimental Research 2017;41:421–31. https://doi.org/10.1111/acer.13292.

[529] Guy J, Peters MG. Liver Disease in Women: The Influence of Gender on Epidemiology, Natural History, and Patient Outcomes. Gastroenterology & Hepatology 2013;9:633.

[530] Malhi GS, Bell E, Morris G, Hamilton A. The delay in response to antidepressant therapy: A window of opportunity? Aust N Z J Psychiatry 2020;54:127–9. https://doi.org/10.1177/0004867419900313.

[531] Stout RL, Kelly JF, Magill M, Pagano ME. Association Between Social Influences and Drinking Outcomes Across Three Years. J Stud Alcohol Drugs 2012;73:489–97.

[532] Yan AW, Fouts DE, Brandl J, Stärkel P, Torralba M, Schott E, et al. Enteric dysbiosis associated with a mouse model of alcoholic liver disease. Hepatology 2011;53:96–105. https://doi.org/10.1002/hep.24018.

[533] Ferrere G, Wrzosek L, Cailleux F, Turpin W, Puchois V, Spatz M, et al. Fecal microbiota manipulation prevents dysbiosis and alcohol-induced liver injury in mice. J Hepatol 2017;66:806–15. https://doi.org/10.1016/j.jhep.2016.11.008.
[534] Beukema M, Faas MM, de Vos P. The effects of different dietary fiber pectin structures on the gastrointestinal immune barrier: impact via gut microbiota and direct effects on immune cells. Exp Mol Med 2020;52:1364–76. https://doi.org/10.1038/s12276-020-0449-2.
[535] Larsen N, Bussolo de Souza C, Krych L, Barbosa Cahú T, Wiese M, Kot W, et al. Potential of Pectins to Beneficially Modulate the Gut Microbiota Depends on Their Structural Properties. Frontiers in Microbiology 2019;10:223. https://doi.org/10.3389/fmicb.2019.00223.
[536] Olano-Martin E, Gibson GR, Rastell RA. Comparison of the in vitro bifidogenic properties of pectins and pectic-oligosaccharides. J Appl Microbiol 2002;93:505–11. https://doi.org/10.1046/j.1365-2672.2002.01719.x.
[537] Chang B, Sang L, Wang Y, Tong J, Zhang D, Wang B. The protective effect of VSL#3 on intestinal permeability in a rat model of alcoholic intestinal injury. BMC Gastroenterol 2013;13:151. https://doi.org/10.1186/1471-230X-13-151.
[538] Pinto-Sanchez MI, Hall GB, Ghajar K, Nardelli A, Bolino C, Lau JT, et al. Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. Gastroenterology 2017;153:448-459.e8. https://doi.org/10.1053/j.gastro.2017.05.003.
[539] Moludi J, Khedmatgozar H, Nachvak SM, Abdollahzad H, Moradinazar M, Sadeghpour tabaei A. The effects of co-administration of probiotics and prebiotics on chronic inflammation, and depression symptoms in patients with coronary artery diseases: a randomized clinical trial. Nutritional Neuroscience 2021:1–10. https://doi.org/10.1080/1028415X.2021.1889451.
[540] Pandey KavitaR, Naik SureshR, Vakil BabuV. Probiotics, prebiotics and synbiotics- a review. J Food Sci Technol 2015;52:7577–87. https://doi.org/10.1007/s13197-015-1921-1.
[541] Swanson KS, Gibson GR, Hutkins R, Reimer RA, Reid G, Verbeke K, et al. The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of synbiotics. Nat Rev Gastroenterol Hepatol 2020;17:687–701. https://doi.org/10.1038/s41575-020-0344-2.
[542] Cani PD. Human gut microbiome: hopes, threats and promises. Gut 2018;67:1716–25. https://doi.org/10.1136/gutjnl-2018-316723.
[543] Gao B, Emami A, Nath S, Schnabl B. Microbial Products and Metabolites Contributing to Alcohol-Related Liver Disease. Mol Nutr Food Res 2021;65:2000023. https://doi.org/10.1002/mnfr.202000023.
[544] Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell 2016;165:1332–45. https://doi.org/10.1016/j.cell.2016.05.041.
[545] Hartmann P, Hochrath K, Horvath A, Chen P, Seebauer CT, Llorente C, et al. Modulation of the intestinal bile acid/farnesoid X receptor/fibroblast growth factor 15 axis improves alcoholic liver disease in mice. Hepatology 2018;67:2150–66. https://doi.org/10.1002/hep.29676.

[546] Zhu X, Huang J, Huang S, Wen Y, Lan X, Wang X, et al. Combining Metabolomics and Interpretable Machine Learning to Reveal Plasma Metabolic Profiling and Biological Correlates of Alcohol-Dependent Inpatients: What About Tryptophan Metabolism Regulation? Frontiers in Molecular Biosciences 2021;8:1061. https://doi.org/10.3389/fmolb.2021.760669.

[547] Voutilainen T, Kärkkäinen O. Changes in the Human Metabolome Associated With Alcohol Use: A Review. Alcohol and Alcoholism 2019;54:225–34. https://doi.org/10.1093/alcalc/agz030.

[548] Axelson M, Mörk B, Sjövall J. Ethanol has an acute effect on bile acid biosynthesis in man. FEBS Lett 1991;281:155–9. https://doi.org/10.1016/0014-5793(91)80382-d.

[549] Kakiyama G, Hylemon PB, Zhou H, Pandak WM, Heuman DM, Kang DJ, et al. Colonic inflammation and secondary bile acids in alcoholic cirrhosis. Am J Physiol Gastrointest Liver Physiol 2014;306:G929-937. https://doi.org/10.1152/ajpgi.00315.2013.

[550] Bajaj JS, Kakiyama G, Zhao D, Takei H, Fagan A, Hylemon P, et al. Continued Alcohol Misuse in Human Cirrhosis is Associated with an Impaired Gut–Liver Axis. Alcoholism: Clinical and Experimental Research 2017;41:1857–65. https://doi.org/10.1111/acer.13498.

[551] Jin M, Kalainy S, Baskota N, Chiang D, Deehan EC, McDougall C, et al. Faecal microbiota from patients with cirrhosis has a low capacity to ferment non-digestible carbohydrates into short-chain fatty acids. Liver Int 2019;39:1437–47. https://doi.org/10.1111/liv.14106.

[552] Smirnova E, Puri P, Muthiah MD, Daitya K, Brown R, Chalasani N, et al. Fecal Microbiome Distinguishes Alcohol Consumption From Alcoholic Hepatitis But Does Not Discriminate Disease Severity. Hepatology 2020;72:271–86. https://doi.org/10.1002/hep.31178.

[553] Bouhnik Y, Flourié B, Riottot M, Bisetti N, Gailing MF, Guibert A, et al. Effects of fructo-oligosaccharides ingestion on fecal bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. Nutr Cancer 1996;26:21–9. https://doi.org/10.1080/01635589609514459.

[554] Causey JL, Feirtag JM, Gallaher DD, Tungland BC, Slavin JL. Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. Nutrition Research 2000;20:191–201. https://doi.org/10.1016/S0271-5317(99)00152-9.

[555] Brighenti F, Casiraghi MC, Canzi E, Ferrari A. Effect of consumption of a ready-to-eat breakfast cereal containing inulin on the intestinal milieu and blood lipids in healthy male volunteers. Eur J Clin Nutr 1999;53:726–33. https://doi.org/10.1038/sj.ejcn.1600841.

[556] van Dokkum W, Wezendonk B, Srikumar TS, van den Heuvel EG. Effect of nondigestible oligosaccharides on large-bowel functions, blood lipid concentrations and glucose absorption in young healthy male subjects. Eur J Clin Nutr 1999;53:1–7. https://doi.org/10.1038/sj.ejcn.1600668.

[557] Salazar N, Dewulf EM, Neyrinck AM, Bindels LB, Cani PD, Mahillon J, et al. Inulin-type fructans modulate intestinal Bifidobacterium species populations and

decrease fecal short-chain fatty acids in obese women. Clinical Nutrition 2015;34:501–7. https://doi.org/10.1016/j.clnu.2014.06.001.

[558] Rahat-Rozenbloom S, Fernandes J, Cheng J, Gloor GB, Wolever TMS. The acute effects of inulin and resistant starch on postprandial serum short-chain fatty acids and second-meal glycemic response in lean and overweight humans. Eur J Clin Nutr 2017;71:227–33. https://doi.org/10.1038/ejcn.2016.248.

[559] van der Beek CM, Canfora EE, Kip AM, Gorissen SHM, Olde Damink SWM, van Eijk HM, et al. The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. Metabolism 2018;87:25–35. https://doi.org/10.1016/j.metabol.2018.06.009.

[560] Tarini J, Wolever TMS. The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. Appl Physiol Nutr Metab 2010;35:9–16. https://doi.org/10.1139/H09-119.

[561] Gacias M, Gaspari S, Santos P-MG, Tamburini S, Andrade M, Zhang F, et al. Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. Elife 2016;5. https://doi.org/10.7554/eLife.13442.

[562] Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155:1451–63. https://doi.org/10.1016/j.cell.2013.11.024.

[563] Needham BD, Adame MD, Serena G, Rose DR, Preston GM, Conrad MC, et al. Plasma and Fecal Metabolite Profiles in Autism Spectrum Disorder. Biol Psychiatry 2021;89:451–62. https://doi.org/10.1016/j.biopsych.2020.09.025.

[564] Laursen MF, Sakanaka M, von Burg N, Mörbe U, Andersen D, Moll JM, et al. Bifidobacterium species associated with breastfeeding produce aromatic lactic acids in the infant gut. Nat Microbiol 2021;6:1367–82. https://doi.org/10.1038/s41564-021-00970-4.

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