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Randomized Controlled Trial

Effect of inulin supplementation on fecal and blood metabolome in alcohol use disorder patients: A randomised, controlled dietary intervention

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SUMMARY

Background and aims: Alcohol Use Disorder (AUD) is a psychiatric disorder characterized notably by gut microbial dysbiosis and insufficient dietary fiber (DF) intake. This study aims to investigate the effect of DF placebo-controlled intervention in patients suffering from AUD during a three-week period of alcohol withdrawal, in order to discover microbial-derived metabolites that could be involved in metabolic and behavioral status.

Methods: A randomized, double-blind, placebo-controlled study was performed with 50 AUD patients supplemented with inulin (prebiotic DF) or maltodextrin (placebo) during 17 days. Fecal microbiota composition, plasma and fecal metabolomics (liquid chromatography coupled to mass spectrometry), blood markers of inflammation and hepatic alterations, and psychological assessment (questionnaires) were analyzed before and after the intervention.

Results: Fecal metabolomics revealed 14 metabolites significantly modified by inulin versus placebo treatment (increased N8-acetylspermidine and decreased indole-3-butyric acid, 5-amino valeric acid betaine (5-AVAB) and bile acids). Thirteen plasma metabolites differentiated both treatments (higher levels of long-chain fatty acids, medium-chain acylcarnitines and sphingomyelin species, and reduced 3-methylhistidine by inulin versus placebo). Fecal *Lachnoclostridium* correlated with 6 of the identified fecal metabolites, whereas plasma lipidic moieties positively correlated with fecal *Ruminococcus torques* group and *Flavonifractor*. Interestingly, parameters reflecting liver alterations inversely correlated with sphingomyelin (SM 36:2).

Conclusions: Three weeks of inulin supplementation during alcohol withdrawal leads to specific and different changes in the plasma and fecal metabolome of AUD patients, some of these gut microbiota-related metabolites being correlated with liver function.

Abbreviations: AUD, Alcohol Use Disorder; BA, bile acids; AC, acylcarnitines; 5-AVAB, 5-amino-valeric acid betaine; DF, dietary fibers; Il-18, interleukin-18; LC-MS, liquid chromatography coupled to mass spectrometry; SCFA, short-chain fatty acids; SM, sphingomyelin.

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1. Introduction

Alcohol use disorder (AUD) is a chronic relapsing condition leading to significant health risks and challenges for individuals, their family and the society [1–3]. AUD is associated with various neuropsychiatric symptoms, including cognitive impairments, depression, and behavioral changes. Research has shown that chronic alcohol consumption can significantly impact the gut microbiota composition and function [1,4].

The gut microbiome is a complex ecosystem that plays a key role in various physiological processes [5]. A reduction in gut microbial diversity and a shift of gut bacteria involved in the control of gut permeability, inflammation and metabolism has been linked to the severity of neuropsychiatric symptoms such as depression, anxiety, alcohol craving and social impairment in AUD patients [6–8].

AUD is accompanied by nutritional imbalance, that includes a deficit in dietary fibers (DF) intake [2]. Fermentable DF respond to the definition of prebiotics when they can modulate the gut microbiome and thereby influence host metabolism, immunity and behaviour [9–11]. We have recently conducted a placebo-controlled intervention study in order to evaluate the relevance of inulin intervention to increase prebiotic DF intake during a three-weeks period of alcohol withdrawal [12]. This protocol allowed to show that inulin intake is well tolerated and modulates the gut microbiota in favor of bifidobacteria as compared to placebo. Inulin also improves the social behavior and increases the serum level of brain-derived neurotrophic factor in AUD patients [12].

Alcohol consumption clearly leads to alterations of the circulating metabolome, that concerns mainly complex lipids and amine-derivatives [13–16]. In humans, inulin supplementation has been shown to modulate blood metabolome and especially bile acid profile, amino acid metabolism and short-chain fatty acids (SCFA) in obesity [17–19]. However, the influence of inulin on metabolome has never been studied in AUD patients. Metabolomics analysis could be helpful in order to unravel which bacterial-derived metabolites could be implicated in biological and behavioural changes induced by prebiotic DF in this particular context. In this article, we report the non-targeted metabolomics data of plasma and fecal that characterizes AUD patients treated with inulin *versus* placebo during the period of alcohol withdrawal.

2. Methods

2.1. Study design & ethics

For this study, which aimed to investigate the effect of inulin supplementation on plasma and fecal metabolome, we took advantages of some psychological and biological data previously obtained from a randomized, double blind, placebo-controlled study in AUD patients [12,20]. This trial has been approved by the institutional ethics committee (N°2017/04JUL/354). All participants provided written informed consent and the trial was registered (ClinicalTrials.gov identifier: NCT03803709; dx.doi.org/10.17504/protocols.io.bvs2n6ge).

From October 2018 to December 2019 eligible participants were randomly assigned, in a 1:1 ratio, to daily intake of inulin (Fibruline®; Inulin group) or maltodextrin (Placebo group) using computer-generated random-number via the website http://www.randomization.com. Maltodextrin was used as placebo as it has the taste, odor, and texture than inulin and because it is a non-fermentable, digestible carbohydrate [21,22]. The randomization procedure was blinded to all persons involved in the study including the principal investigators. In order to reduce potential gastrointestinal side effects, the dose of inulin or maltodextrin increased gradually from 4 to 16 g per day during the 17 days of 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).

2.2. Participant selection

Eligible participants were AUD patients (mostly severe AUD), male or female aged 18–65 years old hospitalized for a 3-week highly standardized alcohol-detoxification program in Cliniques Universitaires Saint-Luc, Brussels, Belgium, Brussels, Belgium. AUD patients were diagnosed by a psychiatrist according to the *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition*. Participants had to speak French and actively consume alcohol until at least 48 h prior to admission.

The exclusion criteria were as follows: suffering from another addiction (except tobacco), inflammatory bowel disease, chronic inflammatory diseases (such as rheumatoid arthritis), cancer, obesity (BMI $\geq$30 kg/m2), diabetes, bariatric surgery, or severe cognitive impairment (Mini Mental State Examination (MMSE) < 24). Patients with known cirrhosis or significant hepatic fibrosis ($\geq$F2) detected by Fibroscan (>7.6 kPa) immediately after admission were also excluded from the study. Patients who had 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.

2.3. Metabolomics analysis

Non-targeted metabolomics analyses of fecal and plasma samples were performed using high-resolution liquid chromatography coupled with mass spectrometry (LC–MS) on the samples taken from placebo or inulin-supplemented AUD patients at the end of the withdrawal period T2. The details of the non-targeted analysis of the plasma metabolites are available in the publication by Leclercq et al. [4]. Fecal samples were thawed on ice, mixed with water to prepare a fecal slurry that was divided for separate extraction processes to be subjected for non-targeted metabolomics analysis using LC-quadrupole Time-of-Flight-MS instrument (Bruker Daltonic, Bremen, Germany) and targeted SCFA analysis using a solid-phase microextraction coupled gas chromatography mass spectrometer (Thermo Scientific, Wilmington, DE, USA). Methodological details of the sample processing, data acquisition and processing for both non-targeted and targeted metabolomics analysis are depicted in the supplementary file 1.

2.4. Statistical analysis

Graphs and statistical analyses were performed using R studio version 4.0.3., Graphpad Prism 8.0 and MetaboAnalyst 4.0 [23]. We first used partial least squares discriminant analysis (PLS-DA) to assess the features that best discriminated the study groups. A feature was considered discriminant if its VIP score was greater than 1.5. Then, univariate analyses were conducted using Mann–Whitney Wilcoxon test to compare the selected features between inulin and placebo. For all tests, $p < 0.05$ was considered as statistically significant. We have previously shown that inulin induced changes in gut microbiota composition, sociability, inflammation and some hepatic parameters [12,20]. In the present study, we therefore set out to investigate the link between inulin-modulated metabolites and these parameters. Therefore, Spearman correlations were performed in the inulin group for metabolites with a VIP score >1.5 and statistically significant different ($p < 0.05$) between the two groups.

3. Results

3.1. Clinical characteristics of the study participants

Fifty AUD patients undergoing elective alcohol withdrawal were recruited between October 2018 and December 2019. A total of 50 subjects were randomised, 25 to the placebo group and 25 to the inulin group. Of these, 21 subjects completed the study in the placebo group and 22 in the inulin group (Fig. 1). The clinical characteristics of the study participants are shown in Table 1. Both groups were comparable except for the DSM-5 AUD score (8.0 ± 1.9 in placebo vs 9.3 ± 1.3 in inulin group, $p = 0.01$), which all correspond anyway to high score of craving and severe AUD (DSM-5 $\geq$ 6 criteria). The number of alcohol withdrawal cures (2.5 ± 2.4 in placebo vs 1.4 ± 0.8 in inulin group, $p = 0.04$) were significantly different in both groups.

3.2. Effect of inulin supplementation on fecal metabolome

Non-targeted metabolomics analysis was performed on the fecal samples taken from placebo or inulin-supplemented AUD patients at T2. PLS-DA analysis revealed that the abundance of 15 annotated metabolites discriminated inulin and placebo groups (VIP score >1.5; Fig. 2a). Sixteen annotated metabolites were significantly different between inulin and placebo group in the fecal samples (Mann Whitney Wilcoxon; $p < 0.05$). The 14 annotated differential metabolites (Mann Whitney Wilcoxon $p < 0.05$ and sPLS-DA VIP score >1.5, Supplementary file 2) included bile acids (BA), gut-derived metabolites and fatty acids (FA) (Fig. 2b). Inulin decreased a set of 12 metabolites whereas it increased only 2 metabolites (N8-acetylspermidine and an unidentified metabolite with molecular formula $C_6H_{11}NO$) (Fig. 2b,c); N8-acetylspermidine being a gut-derived metabolite from polyamine family. Compared to placebo group, the fecal metabolite profiling of inulin supplemented patients was characterized by a decrease in amino-acid derived metabolites, such as indole-3-butyric acid, methyl-imidazoleacetic acid and 5-aminovaleric acid betaine (5-AVAB). Furthermore, we observed a decrease in secondary BA, namely deoxycholic acid, lithocholic acid, nutriacholic acid isomer 2 and ketodeoxycholic acid in inulin group compared to placebo. Finally, we observed a reduction in FA 18:1; O (hydroxyoctadecenoic acid), and amines (4-aminophenol, aniline, dimethylphenylamine, and zeatin) in patients supplemented with inulin compared with placebo (Fig. 2a,b,c).

SCFA were also measured in the feces by SPME-GC-MS. Fecal SCFA levels (acetate, propionate, butyrate, isobutyric acid, isovaleric acid and 2-methylbutyric acid) were not different in inulin and placebo groups (Supplementary Fig. 1).

3.3. Effect of inulin supplementation on plasma metabolome

The PLS-DA analysis of the plasma metabolome revealed 33 discriminant metabolites between the inulin and placebo groups with VIP >1.5 (Fig. 3a). Of these, 13 metabolites were significantly different based on Mann Whitney Wilcoxon test ($p < 0.05$) (Fig. 3b,c). Opposite to what was observed in the feces, inulin supplementation increased the majority of annotated metabolites, most of them being lipidic moieties. Inulin supplementation modified the lipid profile of AUD patients compared to placebo; inulin group was characterized by a higher levels of long-chain fatty acids, such as 16:0 (palmitic acid), 18:2 (linoleic acid), 18:3 (linolenic acid), 20:3; O, 22:5 (docosapentaenoic acid or DPA) and 22:6 (docosahexaenoic acid or DHA) fatty acids and most phospholipids holding these fatty acids. The medium-chain acylcarnitine, AC 10:2, and the sphingomyelin SM 36:2 were also higher in inulin group compared with placebo. Few other annotated metabolites were increased in the plasma upon inulin treatment *versus* placebo, such as unidentified compound with molecular formula $C_8H_{15}NO_2$ and cortisol while 3-methylhistidine was lower in the inulin-treated group compared with placebo (Fig. 3b,c).

3.4. Correlations between fecal and plasma metabolome and psychological and biological parameters

We previously observed that inulin supplementation during alcohol withdrawal period induced specific changes in gut microbiota composition, biological and psychological parameters [12,20]. We correlated those data with metabolomic profiles analysed in the present study. The relative abundance of *Bifidobacterium* – which was significantly increased by inulin-was significantly and positively correlated with both fecal metabolites that were increased upon inulin supplementation (N8-acetylspermidine and unidentified compound $C_6H_{11}NO$) (Fig. 4a). *Dorea*, which was decreased with inulin supplementation, was positively correlated with secondary bile acids such as deoxycholic acid, ketodeoxycholic acid and nutriacholic acid isomer 2 whereas *Desulfovibrio* were positively correlated with 5-AVAB, lithocholic acid, ketodeoxycholic acid and methylimidazolacetic acid. In contrast, *Lachnoclostridium* was negatively correlated with 5-AVAB, methylimidazolacetic acid, aminophenol and ketodeoxycholic acid, and positively with N8-acetylspermidine and $C_6H_{11}NO$. The indole 3-butyric acid, aniline, lithocholic acid, dimethylphenylamine and 4-aminophenol were also negatively correlated with *Bacteroides*. Many lipid metabolites that were increased in the plasma by inulin were positively correlated with *Ruminococcus torques* group and *Flavonifractor*. Haemophilus positively correlated with $C_8H_{15}NO_2$ and PC18:0_22:6 (Fig. 4b). Of note, plasma cortisol level was positively correlated with *Lachnoclostridium*.

ALT was positively correlated with fecal deoxycholic acid, nutriacholic acid isomer 2, a-aminophenol and indole-3-butyric acid whereas interleukin-18 (Il-18) was positively correlated with aniline (Fig. 4a). Regarding psychological outcomes, fecal 5-AVAB was positively correlated with the social activity score. Liver damages were assessed by measuring ALT, AST and M65, an epitope released from dying cells considered as a marker of hepatocyte death [24,25]. Correlation analysis of blood metabolome revealed that parameters reflecting liver alterations (ALT, AST, M65) inversely correlated with sphingomyelin SM 36:2 (Fig. 4b).

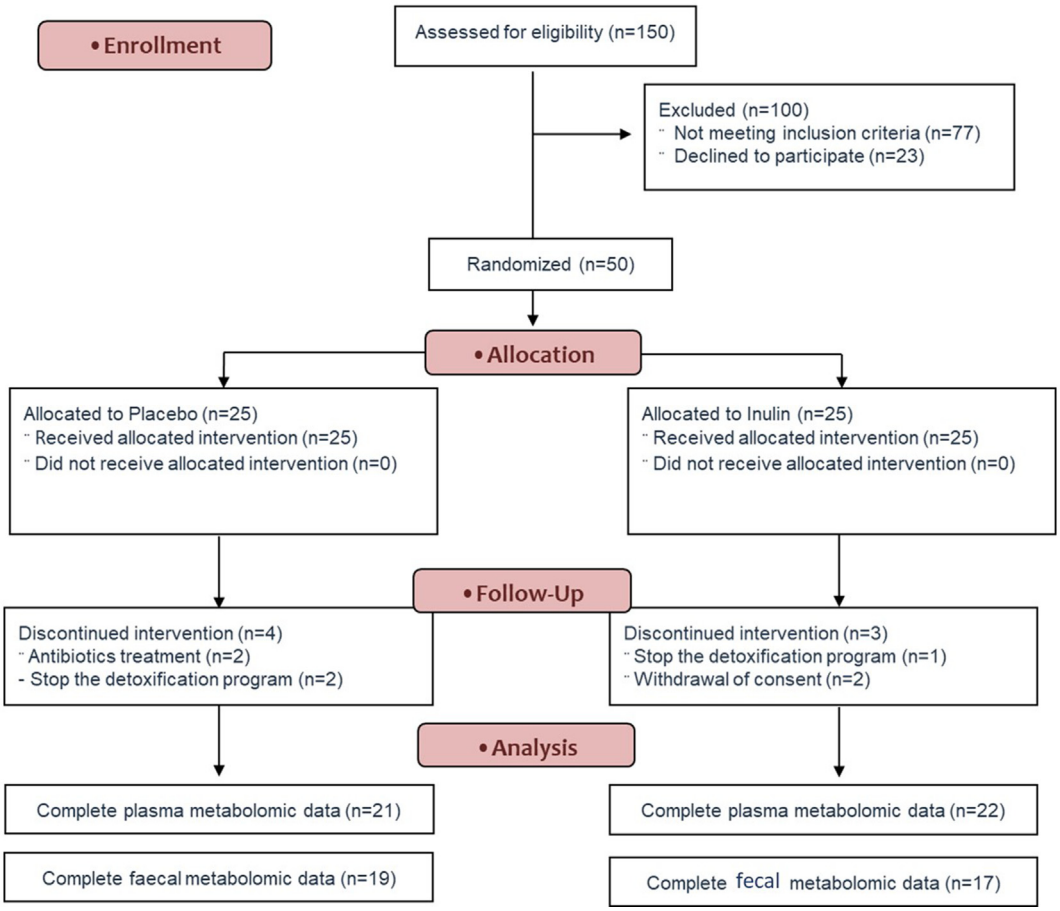

Fig. 1. Flow chart of Gut2Brain study.

Table 1
Baseline characteristics of study participants.

	Placebo n = 21	Inulin n = 22	[a]p
Sociodemographic characteristics			
Age (y)	48.8 ± 8.8	48.3 ± 9.8	0.88
Gender. n (%)			0.08
Male	16 (76.2)	11 (50.0)	
Female	5 (23.8)	11 (50.0)	
Marital status. n (%)			0.54
Couple/married	10 (47.6)	7 (31.8)	
Single	8 (38.1)	12 (54.5)	
Separated/divorced	3 (14.3)	3 (13.6)	
Educational level. n (%)			0.81
Primary	2 (19.5)	2 (8.0)	
Secondary	9 (40.0)	6 (28.0)	
Superior	12 (48.0)	14 (64.0)	
Clinical examination			
Weight (kg)	70.8 ± 10.2	73.4 ± 14.6	0.51
BMI (kg/m^2)	23.2 ± 3.5	24.4 ± 3.1	0.23
MMSE score	28.7 ± 1.2	27.7 ± 2.8	0.14
Smoking. n (%)	17 (80.9)	16 (72.7)	0.52
Alcohol history			
DSM-5 AUD score	8.0 ± 1.9	9.3 ± 1.3	0.01
Age of loss of control (y)	31.1 ± 10.7	31.7 ± 12.0	0.83
Number of alcohol withdrawal cures	2.5 ± 2.4	1.4 ± 0.8	0.04
Duration of drinking habit (y)	16.9 ± 10.3	16.5 ± 11.9	0.90
Alcohol consumption (g/d)	134.1 ± 54.7	152.7 ± 90.7	0.42

Values are means ± standard deviation.
AUD, Alcohol Use Disorder group; BMI, Body Mass Index; DSM-5, Diagnostic and Statistical Manual of Mental Disorders fifth edition; MMSE, Mini Mental State Examination.
[a] 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.

4. Discussion

The present study investigated the effects of inulin supplementation on the fecal and plasma metabolome in patients with AUD during a three-weeks period of alcohol withdrawal. The findings revealed that inulin treatment differentially affected fecal and plasma metabolomics *versus* placebo. In feces, inulin treatment mostly decreased metabolites like secondary bile acids, C18:1 FA, amines and amino-acid derived metabolites, whereas it increased N8-acetylspermidine. In the plasma, the changes in metabolites characterizing the inulin versus placebo treatments concerned mostly lipid metabolites and derivatives, namely an increase in saturated FAs, essential and long-chain polyunsaturated fatty acids (as free FA or as phospholipids), as well as in sphingomyelin (SM) and acylcarnitines. Next, we will discuss the potential links between those variations in metabolites and the impact of inulin on metabolism and behavior, taking into account the potential association with the modulation of the gut microbiome occurring upon inulin treatment.

In the fecal metabolome, the inulin group exhibited increased levels of N8-acetylspermidine. N8-acetylspermidine is the acetylation product of spermidine, one of the major polyamines, that has been linked to the maintenance of gut barrier function and the modulation of host–microbiota interactions in mice [26,27]. Gut microbiota is the primary contributor of polyamine production in the intestine [28]. We observed that this metabolite was positively correlated to the abundance of fecal *Bifidobacterium* and *Lachnoclostridium*. Of note, the change of bacteria Lachnospiraceae group

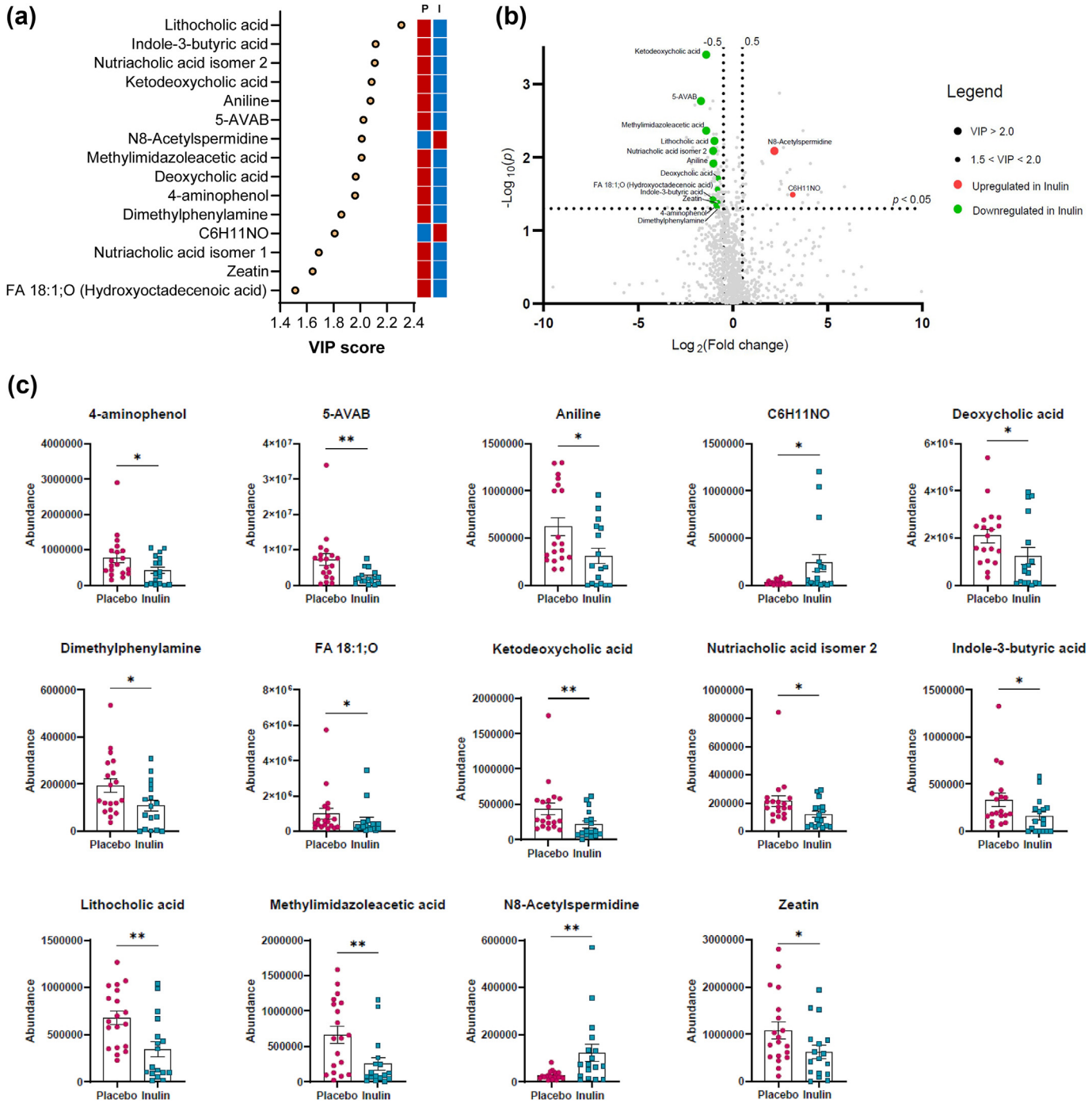

Fig. 2. Fecal metabolome of persons with AUD after three-weeks period of alcohol withdrawal in placebo group (P) and inulin group (I). Variable importance in projection (VIP) plots with the top discriminating fecal metabolites (VIP score >1.5) identified through PLS-DA analyses in descending order of importance (a). Red colour indicates higher level - blue colour indicates lower level after three-weeks period of alcohol withdrawal in placebo group (P) and inulin group (I). Volcano plot representing the fecal metabolomics comparison between inulin and placebo groups (b). The gray plot shows there is no difference, the red plots show up-regulated metabolites, while the green plots show down-regulated metabolites. Fecal metabolites significantly affected by inulin treatment (c). Values are mean ± SEM. (p values were issued from Mann Whitney Wilcoxon test; *p < 0.05, **p < 0.01). 5-AVAB, 5-amino-valeric acid betaine; FA, fatty acid

has already been associated to enhanced gut barrier function induced by spermidine [26]. In mice, pectin, a DF, stimulates the production of polyamines by intestinal bacteria [29]. Interestingly, Cruz-Pereira et al. have shown that levels of polyamine in the prefrontal cortex of aged mice are reduced by stress exposure, and that the fructooligosaccharides/inulin intervention recovered these levels [30].

Conversely, the fecal levels of indole-3-butyric acid and 5-AVAB, which are microbial-derived metabolites associated with gut health and immune modulation [31,32], were decreased in the inulin group. Indole-3-butyric acid is well known as an auxin naturally found in plant products and also produced by bacteria associated plant products that influences root development [33]. Several components of the diet can modulate the fecal and blood

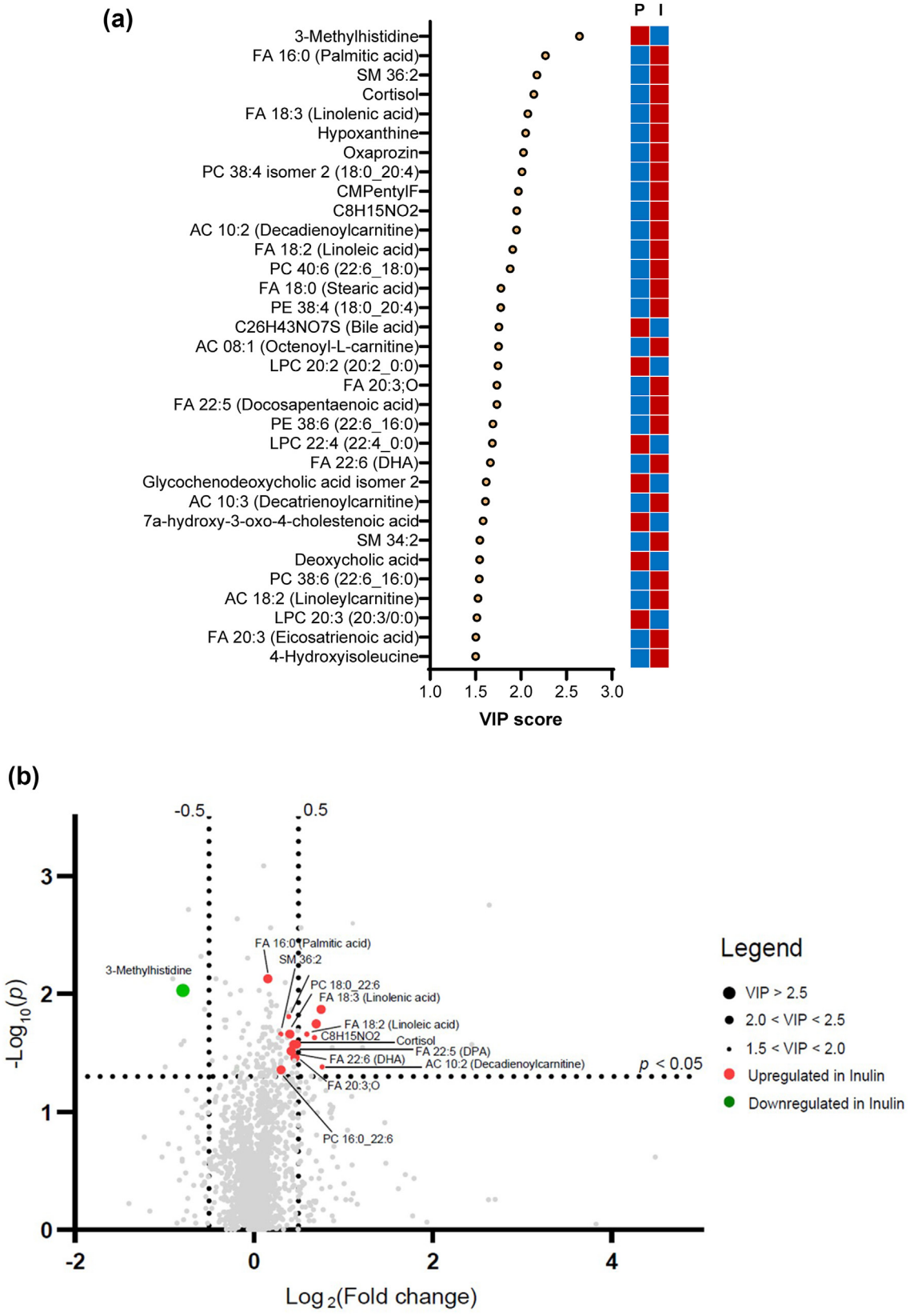

Fig. 3. Plasma metabolome of persons with AUD after three-weeks period of alcohol withdrawal in placebo group (P) and inulin group (I). Variable importance in projection (VIP) plots with the top discriminating plasma metabolites (VIP score >1.5) identified through PLS-DA analyses in descending order of importance (a). Red colour indicates higher level - blue colour indicates lower level after three-weeks period of alcohol withdrawal in placebo group (P) and inulin group (I). Volcano plot representing the plasma metabolomics comparison between inulin and placebo groups (b). The gray plot shows there is no difference, the red plots show up-regulated metabolites, while the green plots show down-regulated metabolites. Plasma metabolites significantly affected by inulin treatment (c). Values are mean ± SEM. (p values were issued from Mann Whitney Wilcoxon test; *p < 0.05, **p < 0.01). AC, Acylcarnitine; CMPentylF, 3-carboxy-4-methyl-5-pentyl-2-furanpropanoic acid; FA, Fatty acid; LPC, Lysophosphatidylcholine; PC, Phosphatidylcholine; PE, Phosphatidylethanolamine; SM, Sphingomyelin.

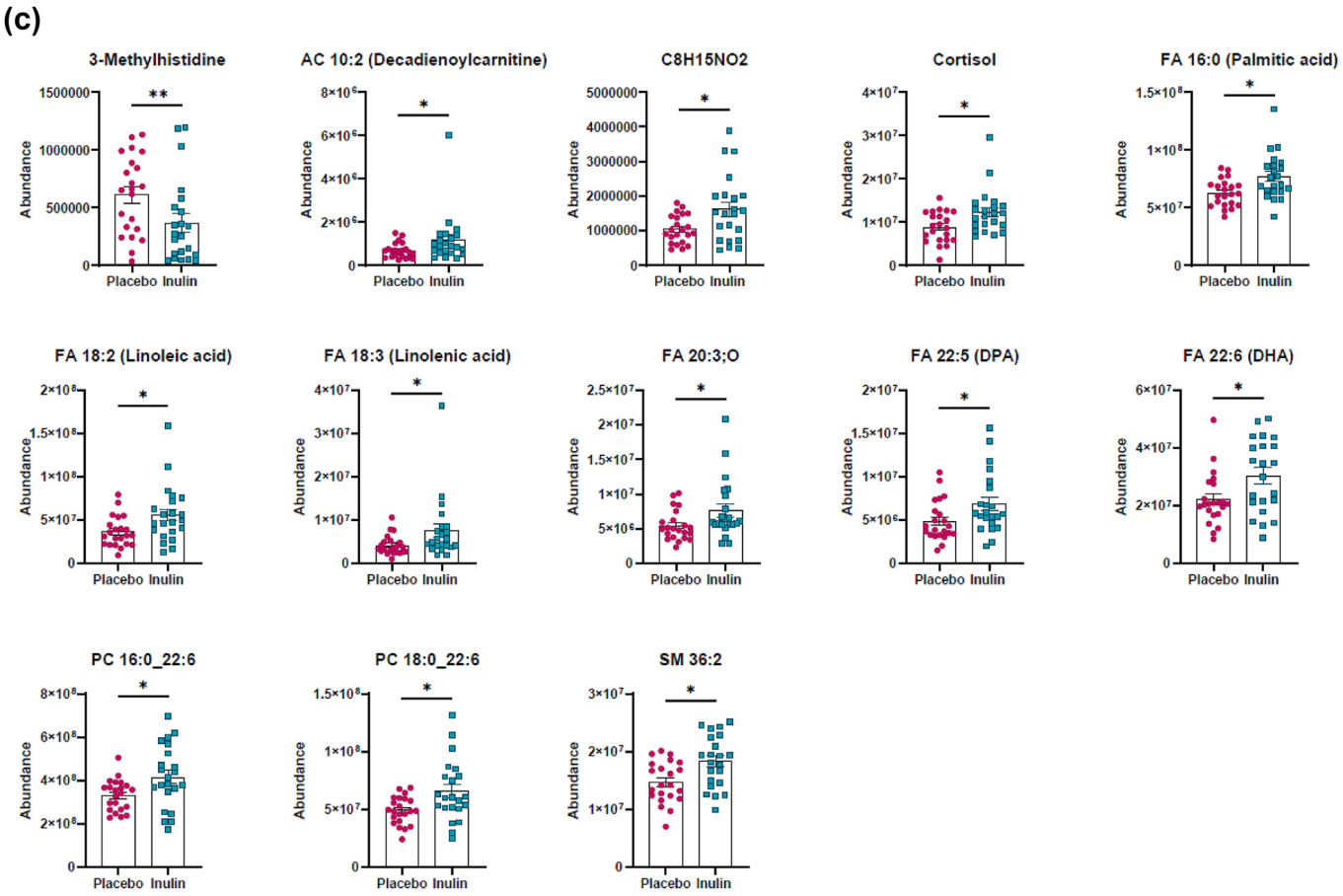

Fig. 3. Continued

metabolome. Patients recruited for this study were interviewed by a trained dietician using three non-consecutive 24-h dietary recall. During the second week of detoxification, the patients were asked to complete a food diary in which they registered all the food and drinks consumed during 3 defined days. We found that inulin supplementation had few impacts on nutrient intake except that AUD patients treated with inulin consumed less root-type vegetables as compared to placebo group [12]. Therefore, we cannot exclude that the lower level of indole-3-butyric acid are partly due to changes in specific food consumption in inulin-treated cohort. Indole-3-butyric acid is also a precursor of indole-acetic acid, the latest being produced by *Bifdobacterium*, *Lactilactobacillus*, *Clostridium*, and *Bacteroides* [34]. Inulin supplementation increase essentially the growth of bifidobacteria in AUD patients [12]. Nevertheless, there was a lack of correlation between indole-3-butyric acid and bifidobacteria. Interestingly, indole-3-butyric acid level in the plasma has been associated with increased risk of incident dementia [35]. The decrease in indole-3-butyric acid level by inulin would thus deserve to be further explored considering its potential interest in the context of mental health disorders. 5-AVAB inhibits β-oxidation of fatty acids by blocking L-carnitine intake via cell membrane carnitine transporter [32,36]. 5-AVAB can be produced by gut microbiota and low fecal levels could be linked to the altered fatty acid metabolism seen in the inulin group. We showed that this metabolite was negatively correlated with *Lachnoclostridium*. Lower levels of 5-AVAB could be beneficial in persons with AUD, since high circulating 5-AVAB levels have been linked to fatty liver disease and cognitive decline [37–40]. However, it is unclear by which mechanism fecal 5-AVAB levels could influence social behavior.

Notably, inulin decreased secondary bile acids in feces suggesting that inulin supplementation may have a significant impact on bile acid metabolism. Certain gut bacteria, particularly members of the Lachnospiraceae and Ruminococcaceae families, are known to be involved in the biotransformation of primary bile acids into secondary bile acids through deconjugation and dehydroxylation reactions [41,42]. Interestingly, we have shown in the same set of patients that there was a decrease in *Dorea* (member of the Lachnospiraceae family) and in an unknown genus from the Lachnospiraceae family in inulin-treated patients versus placebo. This effect could influence the composition and proportions of primary and secondary bile acid species in the gut. Coherently, and in line with previous work [43], we showed here that this decrease in *Dorea* correlated with the decrease in fecal secondary bile acids. One study showed that inulin and fructo-oligosaccharides supplementation in young men significantly altered the bile acid profile, in particular by reducing fecal deoxycholic acid [44]. Such an effect in the feces could contribute to a potential beneficial effect of inulin on gut integrity.

SCFAs are produced as a result of the fermentation of inulin in the colon. In our study, we did not find any effect of inulin supplementation on fecal SCFA. In humans, the effect of inulin supplementation on SCFAs has yielded heterogeneous results. Studies conducted on healthy or overweight/obese subjects have shown an increase in SCFAs with inulin supplementation [45–47], while other showed no effect or even a decrease in obese women [48,49].

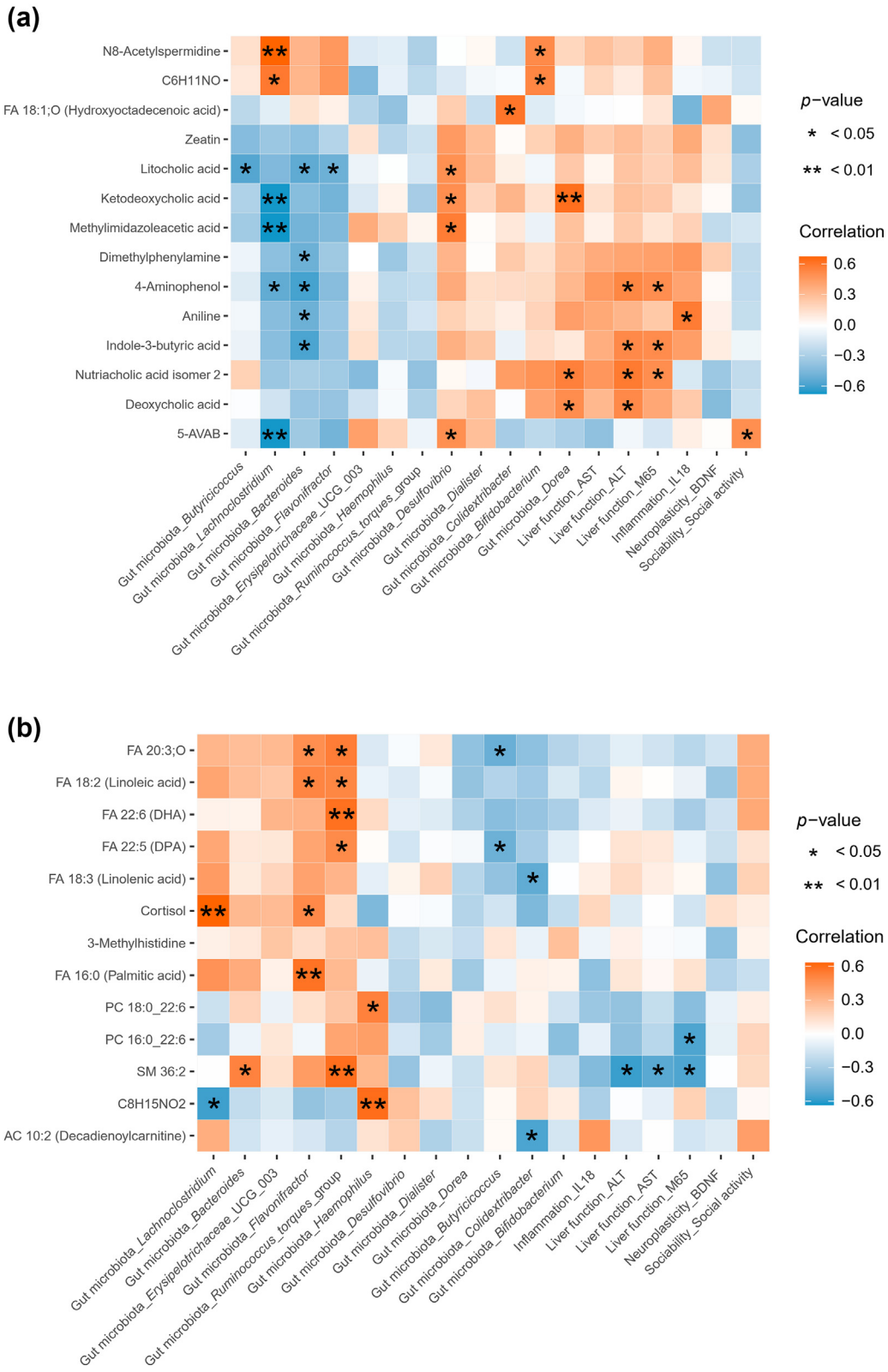

Fig. 4. Correlation between biological, psychological parameters and metabolome in inulin group after three-weeks period of alcohol withdrawal. Spearman rank correlations between metabolites significantly altered with inulin supplementation compare to placebo and biological and psychological parameters. Correlations with fecal metabolites (n = 17) (a). Correlations with fasting plasma metabolites (n = 22) (b). *p < 0.05, **p < 0.01. 5-AVAB, 5-amino-valeric acid betaine; AC, Acylcarnitine; FA, Fatty acid; PC, Phosphatidylcholine; SM, Sphingomyelin.

It therefore seems that the changes caused by the same prebiotic can vary considerably depending on the dose, the target population and the duration of supplementation. Furthermore, most SCFAs are rapidly absorbed in the colon and utilized by host tissues or consumed locally by epithelial cells in the colon [50]. Consequently, fecal SCFA levels may underestimate total SCFA production and do not accurately represent the metabolic activity of the gut microbiota.

Zeatin was reduced in inulin subjects compared with placebo. Zeatin is a phytohormone present in *Zea mays* whereas ribosylated zeata cytokinine, is mostly present in roots. Coherently, we showed that the placebo subjects consumed more roots and tubers than the inulin subjects [12].

Interestingly, inulin promotes an increase in polyunsaturated and saturated fatty acids in the plasma suggesting enhanced fatty acid mobilization and modified lipid metabolism. This effect could not be explained by changes in dietary habits occurring during the treatment period by inulin, since we did not show any significant changes in dietary intake of saturated or polyunsaturated fatty acids in the inulin treated group *versus* placebo [12]. It has been shown that inulin can modify lipid metabolism, notably by lowering cholesterol and triglyceride levels in the liver of rodents and in human plasma [51]. However, few studies have investigated the effect of inulin supplementation on fatty acid profile [52]. In a preclinical study in mice, it has been demonstrated that supplementation of high fat diet with inulin altered the amounts of several PUFAs in the liver including C18:0, C18:2, C18:3 result from the modulation of the expression of desaturases and elongases and/or of their activity [53]. Correlative analysis revealed that most of plasma level of lipidic moieties positively correlated with the level of fecal *R. torques* group and/or *Flavonifractor*.

A limitation of our study is the relatively small sample size, which may have lowered statistical power to detect certain effects. Despite this, our research innovates in investigating the impact of inulin supplementation on the metabolome of AUD patients. Future studies with larger sample sizes are essential to validate and expand upon our findings, allowing for a more comprehensive understanding of inulin effects and its clinical implications in AUD.

Establishing a direct causal link between specific microbiota changes and health outcomes remains a challenge [54,55]. To determine causal components (metabolites, taxa etc.) within the gut microbiota responsible for the physiological effects of DF such as inulin, disposing of human intervention trials with strong correlations between the gut microbiome (taxonomic and functional sides) and biological outcomes is the first but not sufficient step. To move from correlation to causation, complementary gnotobiotic animal studies that recapitulate keystone bacteria prone to interact with inulin, along with experiments comparing the effects of microbial-derived metabolites to placebo, can offer mechanistic evidence of how certain components of the gut microbiome, modulated by DF, can causally impact host physiology. Overall, even if correlation does not mean causation, our analyses reveal linkages between the metabolites identified as being modified by inulin and the biological parameters or sociability highlighted in our previous studies. Our data provide evidence that inulin may influence the metabolic potential of both host and microbiome, some of the effects pointed here - effect on plasma fatty acid profile and fecal bile acids - being potentially relevant for intestinal or metabolic health, outcomes which were not primarily analyzed in our cohort of AUD patients. In our study, metabolomic analysis was performed by LC-MS, whereas additional interesting lipidic moieties involved in metabolic and behavioral disorders could have been measured by applying NMR-based methods (i.e. lipoproteins), or more targeted assays to measure metabolites such as oxysterols or endocannabinoids. Several metabolites significantly modified by inulin *versus* placebo in AUD have not been identified yet. Thus, it is also possible that the observed effects of inulin on biological parameters and sociability are due to metabolites that have not been identified yet. One result which remains intriguing is the significant increase in serum cortisol by inulin, that merits further attention in particular in the context of mental disorders. Interestingly, one recent randomized control trial with inulin-type fructan included treatment showed an improvement of stress associated with working conditions [56].

We observe that several fecal metabolites decreased following inulin supplementation whereas there was an increase in plasma metabolites. The decrease in some metabolites might reflects an improved absorption upon inulin treatment, knowing that malabsorption is common in AUD [57]. While the observed changes in the fecal and plasma metabolome were quite selective, our findings provide insights into potential biomarkers of inulin supplementation that could influence metabolic pathways and gut–brain axis signaling in AUD patients during alcohol withdrawal. Further research is warranted to elucidate the specific microbial and metabolic alterations induced by inulin supplementation and their implications for the management of AUD and associated neuropsychiatric symptoms. The contribution of other types of DF in the management of metabolic and behavioral disorders in AUD would also be an interesting perspective of this work.

Data availability statement

The Supplementary file 2 is available from the authors upon request. 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 16 S rRNA gene sequencing reported in this paper is BioProject PRJNA745947 (SRA) and are available here https://www.ncbi.nlm.nih.gov/bioproject/PRJNA745947/.

Author contribution

Conceptualized and designed the study: SL, CA, PS, PdT, NMD.

Performed participant visits: CA, SL.

Performed data analysis, interpreted the data and drafted the manuscript: CA, HA, SL, QL, PdT, SLa, AMN, LBB, OK, KH, NMD.

Performed the analysis of the metabolomics data: HA, VK, OK, KH.

Contributed to ethics application; SL, PS, PdT, NMD.

All authors approved the final version of the manuscript.

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Declaration of competing interest

VK, OK and KH are founders of Afekta Technologies Ltd. The other authors report no financial interests or potential conflicts of interest.

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Appendix A. Supplementary data

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