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Prebiotic effect on mood in obese patients is determined by the initial gut microbiota composition: A randomized, controlled trial

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ABSTRACT

Background and aims: Metabolic and behavioural diseases, which are often related to obesity, have been associated to alterations of the gut microbiota considered as an interesting therapeutic target. We have analyzed in a cohort of obese patients treated with prebiotic inulin versus placebo the potential link between gut microbiota changes occurring upon intervention and their effect on psychological parameters (mood and cognition).
Methods: A randomized, single-blinded, multicentric, placebo-controlled trial was conducted in 106 obese patients assigned to two groups: prebiotic *versus* placebo, who received respectively 16 g/d of native inulin or maltodextrin combined with dietary advice to consume inulin-rich or -poor vegetables for 3 months as well as to restrict caloric intake. Anthropometric measurements, food intake, psychological questionnaires, serum measures, and fecal microbiome sequencing were performed before and after the intervention.
Results: Inulin supplementation in obese subjects had moderate beneficial effect on emotional competence and cognitive flexibility. However, an exploratory analysis revealed that some patients exhibiting specific microbial signature -elevated *Coprococcus* levels at baseline- were more prone to benefit from prebiotic supplementation in terms of mood. Positive responders toward inulin intervention in term of mood also displayed worse metabolic and inflammatory profiles at baseline (increased levels of IL-8, insulin resistance and adiposity).
Conclusion: This study shows that inulin intake can be helpful to improve mood in obese subjects exhibiting a specific microbial profile. The present work highlights some microbial, metabolic and inflammatory features (IL-8, insulin resistance) which can predict or mediate the beneficial effects of inulin on behaviour in obesity.
Food4gut, clinicaltrial.gov: NCT03852069, https://clinicaltrials.gov/ct2/show/NCT03852069.

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1. Introduction

The obesity prevalence continues to increase, with 39% of adults currently being overweight and 13% obese worldwide (WHO, 2016). Obesity is associated with co-morbidities such as diabetes, cardiovascular disease and cancer (Nyberg et al., 2018). Psychiatric disorders and especially depression are more common in obese than in lean individuals; in addition, treatment efficacy of these diseases is affected by obesity (Capuron et al., 2017; Kloiber et al., 2007; Luppino et al., 2010). Low-grade inflammation, perturbed glucose homeostasis or higher adiposity can contribute to psychological disorders (Capuron et al., 2017; Schachter et al., 2018). Another potential biological contributor to behavioural disturbances seen in obesity is the gut microbiota, composed of thousands of billions of microorganisms that produce neuro-active metabolites. It has been shown that the gut microbiota can influence brain and behaviour in many ways (Cryan et al., 2019; Delzenne et al., 2020; Torres-Fuentes et al., 2017). Obesity is associated with an altered composition of gut microbiota (Thingholm et al., 2019) and it was discovered that transferring gut microbiota from high fat-fed mice to non-obese mice leads to an anxiety-like behaviour in the recipients (Bruce-Keller et al., 2015). Targeting the gut microbiota thus appears as a promising approach to improve metabolic and psychological health in the context of obesity.

Prebiotics are food components that are selectively used by host microorganisms and that confer health benefits (Gibson et al., 2017). Some studies have tested the effect of prebiotics on human behaviour in healthy populations, elderly subjects and in patients with irritable bowel syndrome (IBS) (Desmedt et al., 2019). Its potential impact on behaviour associated with metabolic diseases including obesity remains unknown (Desmedt et al., 2019). A few studies have shown mood and/or cognitive benefits of prebiotics (Azpiroz et al., 2017; Berding et al., 2020; Childs et al., 2014; Costabile et al., 2010; Schmidt et al., 2015; Silk et al., 2009). Inulin is a prebiotic dietary fiber that promotes the growth of some beneficial bacteria, mostly *Bifidobacterium*, and *Lactobacillus* (Costabile et al., 2010; Dewulf et al., 2013; Healey et al., 2018; Slavin and Feirtag, 2011; Vandeputte et al., 2017). Treatment of healthy volunteers or IBS patients with bacteria of these two genera alone or in combination led to beneficial effects on mood (Allen et al., 2016; Pinto-Sanchez et al., 2017; Steenbergen et al., 2015). Preclinical studies showed that inulin alleviates neuroinflammation in middle-aged and APOE4 transgenic mice (Boehme et al., 2019; Hoffman et al., 2019).

Targeting the gut microbiota with inulin-type prebiotic to improve psychological health of obese subjects thus may hold interest. In the present study, we report data from a multicentre single-blind placebo-controlled intervention trial in obese subjects treated with inulin-rich food together with native inulin supplementation for three months. One of the secondary outcomes of this Food4gut study was to test the efficacy of the prebiotic intervention on behaviour. Mood, eating behaviour and cognition screening was done before and after the intervention in the participants. We tested the relevance of basal gut microbiota composition to explain variability in the response of psychological variables toward inulin. The present work paves the way to identify targeted population for personalized nutritional intervention in the context of obesity and mood disorders.

2. Material and methods

2.1. Subjects

Male and female subjects were recruited in three university hospitals in Belgium (Hôpital Erasme in Brussels, Centre Hospitalier Universitaire in Liège and Cliniques universitaires Saint-Luc Brussels). This study was a 3-month long, multicentric, single-blind, placebo-controlled trial (details provided in supplementary methods). The duration of the supplementation as well as the type of placebo (maltodextrin) were chosen based on previous studies from our lab and others (Dewulf et al., 2013; Nicolucci et al., 2017; Reimer et al., 2017). The inclusion criteria were: BMI > 30 kg/m^2, age 18–65 years, Caucasian ethnicity, presence of at least one metabolic obesity-related disorder (prediabetes/diabetes, dyslipidemia, hypertension, fatty liver disease). The exclusion criteria were: use of antibiotics, pro/prebiotics, dietary fibers or any molecule that modifies intestinal transit time within 6 weeks before starting the study, pregnancy in progress or planned within 6 months, psychiatric problems and/or use of antipsychotics, recent (<6 weeks) or current particular diets (e.g., vegetarian, vegan, high-protein, high-fiber diet), excessive alcohol consumption (presented in Supplementary Table 1, excessive alcohol consumption was defined by a consumption above 3 units/day), type 1 diabetes, general dislike for vegetables. The recruitment was conducted from January 2016 to May 2018. One hundred and fifty subjects were randomized. One hundred and forty-six received allocated intervention and 106 were included in the analysis (see more details in flow diagram). Of the 106 participants (placebo n = 55, prebiotic n = 51), 94 had psychological assessments and 86 individuals had both the gut microbiota sequencing and behavioural tests (Supplementary Fig. 1). Following screening, participants were randomly assigned to the prebiotic or placebo arm (random sequence generated using MS Excel®, simple randomization). Randomization sequences were not revealed to the study staff. Subjects and research staff (excepted dieticians who provided dietary advices and recipes books) were blinded to the treatments. For each university hospital center, one person was responsible for enrolling and assigning participants to interventions. This study was approved by the "Comité d'éthique Hospitalo-facultaire de Saint-Luc". Written informed consents were obtained from all participants before inclusion in the study. The trial was registered at ClinicalTrials.gov under identification number NCT03852069.

2.2. Dietary intervention

The subjects consumed either 16 g/d native inulin (extracted from chicory root, Cosucra, Belgium) or 16 g/d maltodextrin (Cargill, Belgium), provided in identical packaging. During the first week, patients were asked to ingest half the dose to allow adaptation to the fiber. Patients received a cookbook with recipes based on vegetables either rich or poor in inulin and were advised to consume at least one recipe per day. Telephone follow-up was performed three times during the study (after 2 weeks, 1 month and 2 months of treatment) to verify compliance and enquire about possible side effects. Participants met a dietician before the study and monthly during the intervention. At baseline, the dietician calculated energy expenditure of the participants in order to prescribe a hypocaloric diet corresponding to −30% of their calculated energy expenditure. We previously report that most subjects achieved a substantial decrease in energy intake (no statistical differences between groups: Placebo 16.3%; prebiotic 20.6%) (Hiel et al., 2020). Using one-week recall questionnaires dietary energy and nutrient intake was evaluated at baseline and monthly, using the Nubel Pro program (Nubel asbl, Belgium).

2.3. Blood parameters

Fasting glycaemia, HbA1c, liver enzymes, and lipids were measured in the hospital laboratory. The remainder of plasma was centrifuged at 2000 × g for 10 min at 4 °C and frozen at −80 °C. Insulin levels were measured by ELISA (Mercodia, Uppsala, Sweden). Dipeptidyl-peptidase IV (DPP-IV) activity was assessed as previously described (Olivares et al., 2018). Cytokines levels (Il-1β; IL-8; IL-12p70; IL-17a; MCP1; TNFα; IFNγ) were determined by multiplex immunoassay (Milliplex Human Cytokine, Merck Millipore) with Luminex technology (BioplexH, Bio-rad, Belgium). C-reactive protein (CRP) levels were measured using the quantikine ELISA (Ref: DCRP00, R&D Systems, Minneapolis, USA).

2.4. Gut microbiota composition

Stool samples were collected at baseline and after 3 months of intervention and stored at room temperature with a DNA stabilizer (Stratec biomolecular, Berlin, Germany) for maximum three days, then transferred to −80 °C. Genomic DNA was extracted using a PSP® spin stool DNA kit (Stratec biomolecular). Sequencing and subsequent bioinformatics were performed as previously described (Pötgens et al., 2018) and were described in supplementary methods. The 16S rDNA sequencing dataset generated and analyzed for this study can be found in the SRA database (project ID: PRJNA595949). center-log transformed data (clr) were calculated and used for analysis (Gloor et al., 2017) Metagenomics predictions based on ASV were generated using PICRUSt2 (Douglas et al., 2020). The mean weighted NSTI was 0,152.

2.5. Psychological measures

Participants were tested twice using questionnaires and cognitive tests during their study visits. Participants were asked to answer the semi-structured interview, fill out questionnaires, and perform cognitive tasks on a computer before and after the intervention. Mood and emotion regulation abilities of participants were assessed using the Positive and Negative Affect Schedule (PANAS; NA and PA, negative and positive affect respectively), the Profile of Emotional Competences (PEC. TOT, total, INTRA, intrapersonal; INTER, interpersonal) and the Scale of Positive and Negative Experience (SPANE, NE and PE, negative and positive emotion respectively; BE, Balance emotion) (Brasseur et al., 2013; Diener et al., 2010; Watson et al., 1988). Cognitive tasks were used to test flexibility, working memory and inhibition (Rogers and Monsell, 1995). Testing order was the same and lasted 2–3 h. A well-trained scientist conducted each session in French. The detailed procedures to collect personal information and assess mood, emotional competences and cognition are presented in supplementary methods.

2.6. Statistical analyses

The number of subjects for each analysis is detailed in the supplementary Fig. 1. R Software (version 3.5.1, MixOmics package), JMP Pro 14 and Graphpad Prism 8.0 were used for analyses. Primary outcome of the study was the change of the gut microbiota composition after inulin intervention. Secondary outcomes were changes in anthropometric, biological and psychological parameters. In the present study, we focused on the psychological outcomes. The sample size (number of volunteers) was determined with JMP software before trial initiation based on the primary endpoint of the trial. The calculation of sample size are made to observe an effect size of 2.0 for the relative abundance of *Bifidobacterium* genus taking into account an alpha of 0.05 and a power of 80% based on changes observed in our previous study in obese subjects with inulin (Dewulf et al., 2013). Group differences in baseline characteristics were assessed using χ_2-tests for categorical variables and parametric t-tests or Wilcoxon test for quantitative variables. For all comparisons, we tested the normality and homoscedasticity. Between group differences were analyzed by mixed model repeated measures ANOVA with time and treatment as fixed factors and patient and hospital as random factors. Analyses were adjusted for age, gender, educational level, family status, alcohol intake and smoking. Within group analyses were done using Tukey post-hoc test when a time effect or the interaction (time × treatment) were significant ($p < 0.05$, mixed model).

The selection of positive and negative responders in the prebiotic group was based on the positivity score (difference between positive and negative mood assessed by PANAS): responders had a positive delta reflecting improved mood during the study, while negative-responders displayed no changes or decreased balance between positive and negative affect. In order to discriminate the two groups we conducted firstly a partial least square discriminant analysis (PLS-DA) allowing us to highlight variables accounting for the differences between groups based on the VIP score. Then we conducted unidimensional analysis on these selected variables. The second stratification was based on *Coprococcus* levels at baseline to explore whether specific gut microbiome feature can predict the behavioural response toward inulin supplementation. We evaluated the effectiveness of prebiotic in High vs Low *Coprococcus* group in order to test whether *Coprococcus* levels can predict the response toward inulin supplementation. Participants were allocated to "Low" and "High" *Coprococcus* based on median *Coprococcus* levels (supplementary Fig. 1, n = 48 and 49 respectively). To explore the relationship between the evolution of the positivity score and the ones of selected biological and microbial variables we used Spearman's correlation. Data are expressed as mean ± SD. Statistical significance was $p < 0.05$.

3. Results

3.1. Prebiotic intervention lead to moderate improvements of mood and cognition in obese subjects

Baseline mood and cognitive parameters were not different between prebiotic and placebo groups except for inhibition (number of errors) which was better in prebiotic group (Supplementary Table 1). Baseline alcohol consumption was significantly higher in the placebo group (Supplementary Table 1).

The emotional competence (PEC TOT) was differentially modulated by the treatment (interaction <0.05). Indeed, emotional competence, even if within group comparisons were not significant, tend to increase in prebiotic group while it tends to decrease in placebo (Table 1). Within-group comparisons revealed a significant decrease in negative emotion measured by the Scale of Positive and Negative Experience (SPANE-NE), improved flexibility (decrease of Z-score and reaction time), only in the prebiotic group (Table 1).

3.2. The response toward prebiotic supplementation depends on baseline gut microbiota profiles

We recently showed that the effect of inulin supplementation on biological outcomes is influenced by gut microbiota profile before treatment (Rodriguez et al., 2020). In order to identify individuals whose mood could benefit from inulin, we conducted an exploratory analysis to test whether baseline bacteria levels predict the effect of inulin supplementation on psychological parameters. To do so, we analyzed in the prebiotic group the change in "positivity score" (difference between positive and negative affect measured by PANAS) to distinguish positive (improved mood, n = 21) and negative responders (no change or worsening of mood, n = 19) (Fig. 1A). Using PLS-DA on biological parameters and gut microbiota composition at baseline, we selected variables with higher VIP score (>1.5) to distinguish the two populations (Fig. 1B). Unidimensional analysis revealed that among genera, only the baseline level of *Coprococcus* and *Lactobacillus* were significantly different between positive and negative responders (Fig. 1C). Of note, *Lactobacillus* genus was present only in 41% of individuals, whereas *Coprococcus* was present in >90% of individuals. This leads us to conclude that the level of *Coprococcus* is more suitable to predict the behavioural response toward inulin supplementation. Positive responders had higher plasma IL-8 levels and HOMA-IR (indicating greater insulin resistance) at baseline. Mood was more disturbed in responders at baseline as evidenced by a lower "positivity score" (Fig. 1D).

Positive responders to inulin in terms of mood also exhibited differences in post-treatment microbial and biological parameters (Fig. 2 A). Five genera had a VIP score >1.5 and among them, Mann-Whitney tests revealed that two were significantly different between positive and negative responders: the increases of *Bifidobacterium* and *Haemophilus* were larger (Fig. 2B). Of note, α and β-diversity were not different at baseline nor after inulin-treatment (data not shown). Positive

Table 1
Psychological parameters before and after 3 months of intervention in patients receiving placebo and prebiotic.[1]

		Placebo			Prebiotic			Between group comparison		
		Baseline	3 months	Within group	Baseline	3 months	Within group	Time	Treatment	Inter
Mood	PANAS PA	31.7 ± 1.42	30.0 ± 1.48	NS	30.6 ± 1.56	31.0 ± 1.60	NS	NS	NS	NS
	PANAS NA	16.2 ± 1.38	14.6 ± 1.45	NS	14.2 ± 1.47	14.2 ± 1.50	NS	NS	NS	NS
	PEC TOT	3.40 ± 0.07	3.36 ± 0.07	NS	3.33 ± 0.08	3.42 ± 0.08	NS	NS	NS	0.039
	PEC INTRA	3.34 ± 0.09	3.31 ± 0.09	NS	3.20 ± 0.09	3.31 ± 0.09	NS	NS	NS	NS
	PEC INTER	3.41 ± 0.08	3.37 ± 0.08	NS	3.42 ± 0.10	3.47 ± 0.10	NS	NS	NS	NS
	SPANE.PE	19.4 ± 0.96	18.7 ± 1.02	NS	19.0 ± 0.97	19.4 ± 0.98	NS	NS	NS	NS
	SPANE.NE	11.7 ± 0.64	10.7 ± 0.67	NS	12.5 ± 0.73	10.6 ± 0.74	0.006	<0.001	NS	NS
	SPANE.BE	7.80 ± 1.33	7.93 ± 1.39	NS	6.54 ± 1.52	8.7 ± 1.55	NS	NS	NS	NS
Cognition	Flexibility Z-score	0.01 ± 0.19	−0.17 ± 0.18	NS	0.23 ± 0.18	−0.18 ± 0.18	0.003	<0.001	NS	NS
	Working Memory Z-score	0.09 ± 0.19	−0.15 ± 0.19	NS	0.19 ± 0.19	0.0001 ± 0.19	NS	0.003	NS	NS
	Inhibition Z-score	0.13 ± 0.21	0.07 ± 0.21	NS	−0.02 ± 0.21	−0.22 ± 0.21	NS	NS	NS	NS
	Flexibility RT, ms	2140 ± 1867	1867 ± 129	NS	2445 ± 136	1900 ± 143	<0.001	<0.001	NS	NS
	Flexibility errors	7.19 ± 2.15	6.93 ± 2.25	NS	6.53 ± 2.29	3.98 ± 2.39	NS	NS	NS	NS
	Working memory RT, ms	4798 ± 336	4280 ± 354	NS	5168 ± 365	4437 ± 383	NS	0.007	NS	NS
	Working memory errors	7.73 ± 0.83	6.87 ± 0.85	NS	7.21 ± 0.89	7.49 ± 0.91	NS	NS	NS	NS
	Inhibition RT, ms	773 ± 25.5	765 ± 26.2	NS	757 ± 26.7	751 ± 27.5	NS	NS	NS	NS
	Inhibition errors	71.6 ± 6.16	73.04 ± 6.32	NS	61.2 ± 6.56	57.0 ± 6.74	NS	NS	NS	NS

[1] Values are means ± SD. Between-groups variations were analyzed by mixed model Anova and within-group variations by HSD Tukey post-hoc test. Random effects: Subject and Hospital. Adjustments for age, gender, tobacco, alcohol intake (units per week), educational level (years) and family statue (living alone or not). * within group comparisons were made using Tukey post-hoc test. PANAS, Positive and Negative Affect Schedule; PEC, Profile of Emotional Competences; SPANE, Scale of Positive and Negative Experience; PA, positive affect; NA, negative affect INTRA, intra-personal; INTER, inter-personal; TOT, total, RT, reaction time.

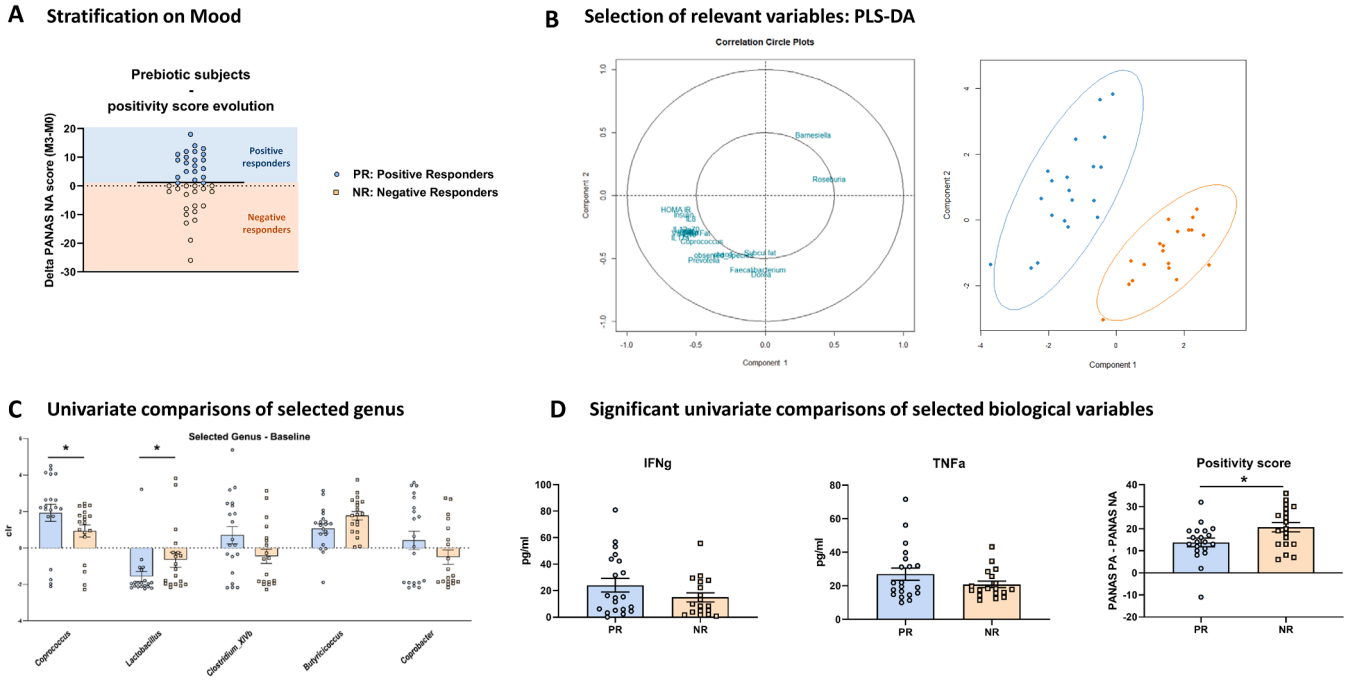

Fig. 1. A. Defining positive (n = 21) and negative responders (n = 19) based on the change in positivity score (difference between the positive and negative score in PANAS). The horizontal full line represent the median value. B. Partial least square discriminant analysis to highlight variables (gut microbes and biological parameters) responsible for the segregation between positive and negative responders at baseline (VIP score > 1.5). C. Univariate analysis (Mann-Whitney or parametric *t*-test) of the selected microbial (C) and biological (D) variables (n = 19–21 for positive and 18–19 for negative responders) at baseline.

responders to inulin in terms of mood also exhibited differences in post-treatment biological parameters (Fig. 2B). Among the selected variables, there were significant differences for IL8, subcutaneous fat and DPPIV whereas non-significant differences were observed for HOMA-IR, IL10, IL17a, IL1β and IFNγ despite an elevated VIP score. IL-8 significantly increased upon prebiotic intervention in positive responders compared to negative responders. Positive responders exhibited a greater decrease in DPP-IV and subcutaneous fat mass compared to negative responders. Finally, we tested whether the selected biological and microbial variables were associated with the positivity score and we found significant correlation of this score with *Bifidobacterium*, *Haemophilus*, IL-8 and subcutaneous fat mass (Fig. 2C).

3.3. Potential microbial functional characteristics of positive responders

To investigate the potential differences in gut microbiome functions between positive and negative responders, we used a PICRUSt2 approach (Douglas et al., 2020). Sparse PLS-DA (sPLS-DA) allows to select the main pathways modulated differently between positive and negative responders (Fig. 3A). The two pathways with the higher VIP

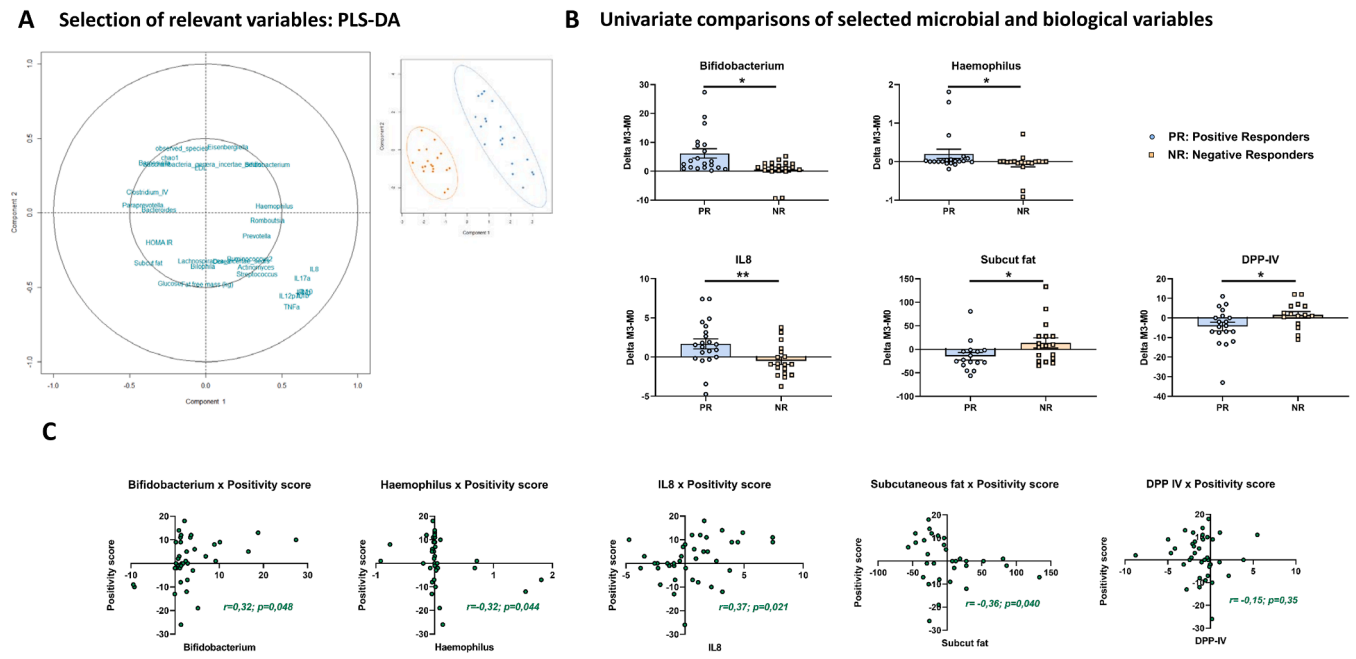

Fig. 2. A. Partial least square discriminant analysis to highlight variables (gut microbes and biological parameters) responsible for the segregation between positive and negative responders during the prebiotic intervention. B. Graphical representation of the statistically significant univariate analysis (Mann-Whitney or parametric *t*-test) of the evolution of preselected variables (VIP score > 1.5; n = 19–21 for positive responders and 18–19 for negative responders) between the start (M0) and end of the intervention (M3). C. Spearman's correlation between the evolution (M3-M0) of bacterial levels and the positive mood (positivity score) in inulin-treated individuals.

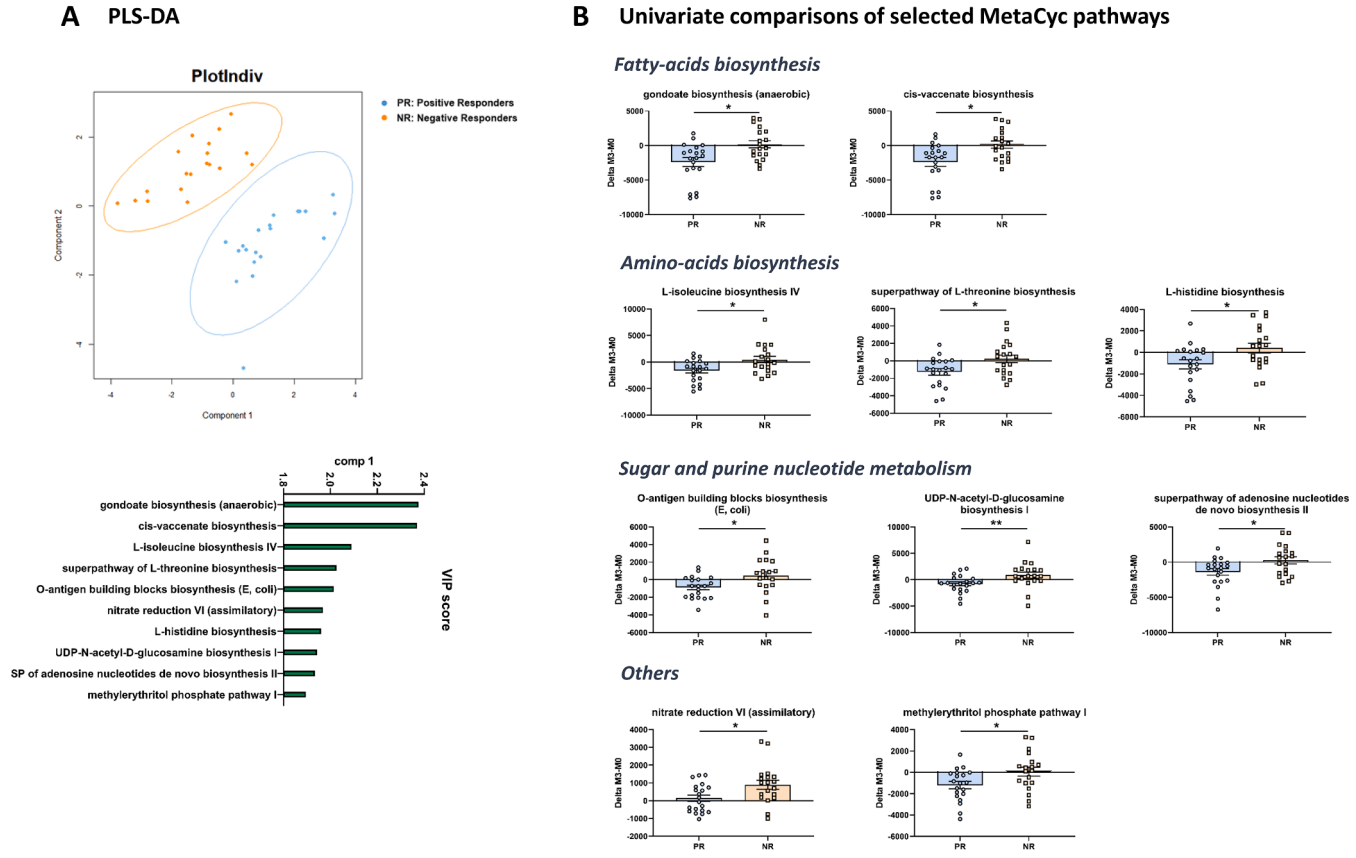

Fig. 3. A. Partial least square discriminant analysis to highlight MetaCyc pathways responsible for the segregation between positive and negative responders during the prebiotic intervention. VIP scores are presented at the bottom. B. Univariate analysis (Mann-Whitney test) of the evolution of preselected variables (VIP score > 1.8; n = 19–20 for negative and positive responders respectively) between the start (M0) and end of the intervention (M3).

score are associated with unsaturated fatty-acids metabolism in bacteria, namely the gondoate and the *cis*-vaccenate (Fig. 3). Among the ten most important pathways (VIP score > 1.9, Fig. 3A) several are associated with amino-acids metabolism (Isoleucine, Threonine, Histidine). The remaining pathways were related to nitrogen metabolism, sugar and purine nucleotide metabolism as well as terpenoid metabolism (Fig. 3B). All the aforementioned pathways are decreased in positive responders while we observed no changes or an increased in negative responders ($p < 0.05$). At baseline, there were not any differences in Metabolic pathways (MetaCyc pathways; Supplementary Fig. 2).

3.4. Baseline profile of participants according to Coprococcus levels

After stratification of participants according to basal *Coprococcus* levels, no significant differences were observed between the two groups regarding socioeconomic variables (Supplementary Table 2). There were no differences between High and Low *Coprococcus* group at baseline in any of the clinical, biological nor nutritional variables that were measured (Table 2). Regarding psychological variables, the High *Coprococcus* group displayed a lower score in the PANAS PA and the positivity score (Supplementary Table 2). We used PLS-DA to select variables accounting for the difference between groups in gut microbiota composition (VIP score > 1.5). Unidimensional analysis revealed that the High *Coprococcus* group had higher levels of *Dorea*, *Collinsella* and lower *Clostridium* XIVa, *Fusobacterium* (Supplementary Fig. 3). Despite an elevated VIP score, the Shannon index was not significantly higher in the High *Coprococcus* group.

3.5. Beneficial effects of prebiotic supplementation on mood in participants with higher baseline Coprococcus levels

The stratification of patients following baseline *Coprococcus* level revealed that beneficial effect of inulin occurred only in High *Coprococcus* group. Prebiotic supplementation improved emotional competence (PEC Total) and mood (SPANE NE), only in the High *Coprococcus* group (Fig. 4 and Supplementary Table 3).

Since mood and cognition, especially executive function, are often related (Driessen and Hollon, 2010; Gotlib and Joormann, 2010; Joormann and Gotlib, 2010), we tested whether the *Coprococcus*-based stratification was also associated with changes in cognition. As shown in Supplementary Table 4, the reaction time during flexibility task decreased in all groups except placebo-treated High *Coprococcus* subjects while an effect of prebiotic was observed only in Low *Coprococcus* strata for the Flexibility and Working-memory Z-score.

4. Discussion

This study reveals that 3-month inulin supplementation in obese patients exerts selective moderate beneficial effects on psychological parameters, namely improved emotional competence (PEC) and cognitive flexibility. Stratifying patients according to their baseline gut microbiota composition reveals further beneficial effect of the inulin intervention. In an exploratory analysis higher *Coprococcus* level allows to distinguish obese subjects prone to positively respond to inulin supplementation. IL-8, insulin resistance and mood alterations were also

Table 2
Baseline characteristics of the participants.[1]

		Low Coprococcus	High Coprococcus	P-value
Anthropometry, metabolic health, blood pressure, liver function	BMI, kg/m^2	36.5 ± 6.1	36.0 ± 4.1	0.68
	Fat mass, %	41.1 ± 13.3	41.1 ± 8.9	0.75
	Fat Free mass, %	63.1 ± 14.6	60.9 ± 14.6	0.28
	Waist/hip ratio	0.96 ± 0.10	0.94 ± 0.08	0.56
	Visceral fat	238.6 ± 104.8	231.7 ± 102.0	0.73
	Subcutaneous fat	339.0 ± 139.2	379.2 ± 124.5	0.15
	SBP, mm Hg	138.2 ± 14.1	136.9 ± 16.7	0.35
	DBP, mm Hg	85.6 ± 10.2	84.1 ± 12.4	0.46
	Total cholesterol, mg/dl	191.2 ± 44.0	197.5 ± 47.1	0.52
	LDL cholesterol, mg/dl	116.6 ± 42.4	118.6 ± 42.8	0.79
	HDL cholesterol, mg/dl	45.4 ± 9.9	49.5 ± 11.2	0.08
	Triglycerides, mg/dl	162.9 ± 78.1	166.4 ± 141.3	0.46
	Elasticity, kPa	6.9 ± 3.0	6.4 ± 4.8	0.09
	AST, U/l	25.9 ± 11.4	26.0 ± 9.3	0.51
	ALT, U/l	36.8 ± 24.9	35.0 ± 19.9	0.84
	γgt, U/l	40.5 ± 31.3	41.6 ± 31.5	0.89
	DPP-IV, mUI/ml	17.3 ± 6.9	18.3 ± 4.8	0.30
	C-reactive protein, mg/l	4448 ± 5656	3603 ± 3378	0.93
Inflammation	Il-17a, pg/ml	8.2 ± 8.9	9.9 ± 8.6	0.12
	Il-1β, pg/ml	8.9 ± 9.7	9.9 ± 8.4	0.16
	MCP1, pg/ml	207.8 ± 45.1	202.4 ± 54.7	0.61
	IFNg, pg/ml	21.1 ± 22.7	22.0 ± 18.7	0.30
	Il-10, pg/ml	14.6 ± 19.1	16.7 ± 18.5	0.28
	Il-12p70, pg/ml	18.3 ± 19.0	20.4 ± 16.7	0.19
	Il-8, pg/ml	5.2 ± 3.6	5.2 ± 3.1	0.74
	TNFα, pg/ml	23.6 ± 11.5	25.6 ± 12.7	0.38
Nutrition	Energy, kcal/day	2101 ± 635	1964 ± 440	0.56
	Protein, g/day	89.9 ± 23.2	85.2 ± 17.1	0.60
	Lipid, g/day	86.7 ± 44.8	78.4 ± 22.3	0.96
	Carbohydrates, g/day	216.2 ± 60.2	212.5 ± 61.5	0.88
	Fiber, g/day	22.2 ± 8.1	21.2 ± 8.0	0.48
Glucose Homeostasis	HbA1c, %	6.10 ± 0.97	6.07 ± 1.37	0.58
	Glycemia, mg/dl	114.1 ± 33.7	106.9 ± 26.7	0.40
	Insulin, mU/L	15.2 ± 9.2	16.7 ± 10.8	0.50
	HOMA IR	4.6 ± 4.1	4.7 ± 4.4	0.56

[1] Values are means ± SD (n = 49 in each group). Baseline data were analyzed by unpaired *t*-test or Mann Whitney. BMI, body mass index ; SBP-DBP, systolic and diastolic blood pressure; LDL-HDL, low and high density lipoprotein; AST-ALT, aspartate and alanine transaminase; γgt, gamma-glutamyltranspeptidase; DPP-IV, dipeptidyl peptidase-4; IL, interleukins; IFN, interferons; TNF, tumor necrosis factor; HbA1c, Glycated hemoglobin; HOMA IR, Homeostasic model assessment of insulin resistance.

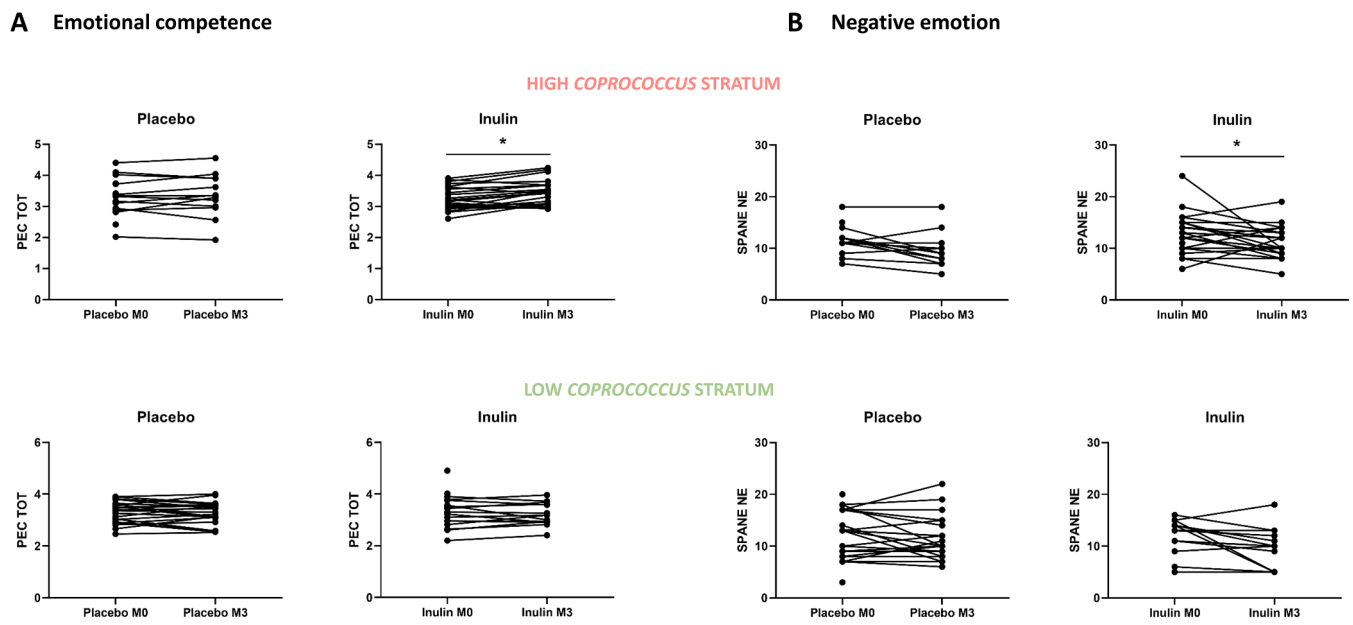

Fig. 4. Graphical representation of the evolution of psychological parameters in placebo and inulin treated-subjects: Emotional competence (A) and Negative Emotion (B). Extended data and statistical analysis are presented in Supplementary Tables 3 and 4. Comparisons between strata (High and Low *Coprococcus*) was made using mixed models adjusted for center, gender, age, alcohol intake, tobacco, marital status and educational level. Here are represented the post-hoc comparisons of the mixed models (HSD Tukey test) performed in each stratum.

higher at baseline in these responders.

Regarding the effect of inulin in the whole cohort, one study showed improvements of memory tasks and mood 4 h after a single inulin ingestion in healthy subjects (Smith et al., 2015), an effect unlikely to be attributable to gut microbiota changes. As shown upon inulin supplementation in our study, the Fructo-oligosaccharides (FOS) led to an increased level of *Bifidobacterium* in the IBS patients (Azpiroz et al., 2017). Interestingly, as discussed later, the patients who best responded in terms of improvement of mood were also the ones with the highest increase in Bifidobacteria.

The gut microbiota is frequently proposed as a biological factor driving response towards nutritional interventions. Here, we show that baseline *Coprococcus* levels predict the response to inulin in terms of improved mood but not cognition in obese individuals. As mood disturbances are associated with cognitive impairments (executive function, memory) and cognitive therapy is efficient to treat depression (Driessen and Hollon, 2010; Gotlib and Joormann, 2010), we examined whether the High *Coprococcus* subjects also had improved cognition, but this was not the case. While brain structures (i.e., hippocampus, prefrontal cortex) and neurobiological processes (i.e., synaptic plasticity, neurogenesis) involved in emotion and cognition overlap, the lack of a larger prebiotic effect on cognition in High *Coprococcus* group could be due to specificity in gut-to-brain communication (i.e., difference in the sensitivity of specific structure or cell population towards gut-derived messengers) (Okon-Singer et al., 2015). We tested an important domain of cognition by investigating executive functions, but a weakness of our study is that we did not investigate other domains like long-term memory or attention. Sad mood specifically affects emotion-related cognitive processes (Chepenik et al., 2007). The absence of depressed patients with more diverse cognitive alterations in this cohort did not allow to test for effects of inulin in this condition specifically. The beneficial effect on mood might occur earlier than that on cognition and longer follow-up might be needed to detect changes in the latter. Our cohort was also rather young (<60 years old). The selective effect on mood points to a possible pivotal role of *Coprococcus* in mediating mood-related neurobiological processes. Preclinical studies targeting this genus will help clarify this.

Interestingly, we observed that positive responders with high *Coprococcus* levels also displayed higher baseline IL-8 levels, which further increased upon prebiotic use. The latter observation was quite surprising, since IL-8 is classically described as a proinflammatory cytokine. Bruun and colleagues observed an increase in IL-8 was also observed in obese subjects during a weight-loss intervention in spite of reduced fat mass, IL-6, TNFα and insulin resistance and proposed that the release of organic compounds from adipose tissue could in turn trigger IL-8 release (Bruun et al., 2003, 2002). More studies on the role of IL-8 in obesity are needed to interpret this intriguing result.

The positive responder subgroup had greater baseline insulin resistance and a larger decreased of the DPP-IV activity (known to degrade GLP-1) with inulin. Insulin resistance has been associated with altered mood and cognition in several studies (Arnold et al., 2018). Changes in hormonal status and modification of brain insulin signaling may mediate the neurobiological alterations. Metformin or GLP-1 agonists also have beneficial effects on neurobiological process and behaviour (Athauda et al., 2018; Zemdegs et al., 2019). Metformin exerts its metabolic effects by modulating the gut microbiota, among other mechanisms (Rodriguez et al., 2018, p. 201). Inulin improves metabolic health and glucose homeostasis in obese patients (Chambers et al., 2019; Dewulf et al., 2013; Hiel et al., 2020; van der Beek et al., 2018). Thus, the beneficial effect of inulin on mood in insulin resistant patients suggests that inulin, like glucose-lowering drugs, could have beneficial effects on mood through improved metabolic health. Of note, as previously described, all participants were submitted to caloric restriction advices. We have previously reported that most subjects achieved a substantial decrease in energy intake upon intervention in both groups, the decrease reaching 16.3% of the initial energy intake in the placebo group, and 20.6% in the prebiotic group (Hiel et al., 2020). Even if there was no significant difference between groups, we cannot rule out the fact that decreased energy intake was beneficial for subjects with a worst metabolic profile (i.e: the positive responders displayed alters glucose homeostasis). Indeed, it has been postulated that caloric restriction could be beneficial for mood based on several preclinical and clinical reports (Manchishi et al., 2018).

Positive responders exhibited higher baseline *Coprococcus* levels and a larger increase in *Bifidobacterium* due to the 3-month inulin supplementation. Other studies pointed out the fact that dietary fiber intake as

well as bifidobacteria levels are drivers of the response towards prebiotic intervention regarding gut microbiota composition (de Preter et al., 2008; Healey et al., 2018; Kolida et al., 2007). We recently showed, in mice transferred with the fecal material of obese subjects, that some bacteria are essential to trigger the response to inulin in terms of metabolic health (Rodriguez et al., 2020). The present work shows that *Coprococcus* levels change the response of obese subjects in terms of behaviour. A possibility is that *Coprococcus* and co-occurring bacteria enable subjects to fully benefit from the inulin intervention. The High and Low *Coprococcus* groups displayed differences in microbiome at baseline that concerns four genera: *Clostridium XIVa, Dorea, Collinsella* or *Fusobacterium*. Among *Clostridium XIVa* genus, some species are known to use inulin as a substrate (Moens and De Vuyst, 2017). Some *Bifidobacterium* species' promoted by inulin supplementation are known to produce acetate, that can be further used by other bacteria *(*Hiel et al., 2020; Rossi et al., 2005*)*. It is the case of *Coprococcus catus* that produces butyrate from acetate released by other bacteria including bifidobacteria (Belenguer, 2006; Duncan, 2004). Therefore, the profile of SCFA production can be influenced by the cooperation of bacteria that are concomitantly influenced by inulin. The production of SCFA like butyrate, known to exert beneficial effects on the brain, could be one of the mechanisms involved in the beneficial effects of inulin on mood (Cryan et al., 2019; Stilling et al., 2016). Overall, our work showed that the behavioural response upon inulin supplementation in obese subjects differs between individuals depending on the level of bacteria -such as *Coprococcus*- prior intervention. Further studies are needed to understand if it relies on differences in the ability to produce SCFA or other gut metabolites, and to determine if *Coprococcus* is solely a biomarker or a central player in behavioural response.

In order to have more insights regarding the possible differences in gut microbiota functions, we conducted PICRUSt2 analysis. It revealed that after being exposed to inulin supplementation several pathways related to fatty acids and amino acids metabolism were downregulated in positive responders while it was not the case in negative responders. It is interesting to note that these pathways were not related to the metabolism of inulin itself but rather to the metabolism of other nutrients (lipids and amino acids). The beneficial effects of unsaturated fatty-acids on behaviour have been largely described (Bazinet and Layé, 2014; Chianese et al., 2018). Amino-acids and fatty acids metabolism by the gut microbiota has recently been associated with cognition in obese patients (Arnoriaga-Rodríguez et al., 2020). The authors elegantly showed that the gut microbiome is involved in behaviour and neurobiological alterations seen in obesity. They found that aromatic amino acids and some of their gut-derived metabolites are associated with such effects (Arnoriaga-Rodríguez et al., 2020). Further metabolomics studies could help to clarify whether prebiotic fiber intake can exerts part of its beneficial effect on the host by modulating the ability of the gut microbiome to metabolize other important nutrients.

A recent study showed that depression is associated with lower *Coprococcus* levels (Valles-Colomer et al., 2019). These commensal bacteria, belonging to the *Lachnospiraceae,* are also decreased in patients with Parkinson disease (Keshavarzian et al., 2015). Authors postulated that they are involved in dopamine metabolism which might modulate mood (Valles-Colomer et al., 2019). At baseline the High *Coprococcus* group displayed a slightly lower positive affect score. Our results could appear as contradictory with these previous findings (Valles-Colomer et al., 2019). However, a recent systematic review showed that obese subjects exhibit higher *Coprococcus* levels than leaner people (Castaner et al., 2018). SCFA production is also higher in obese subjects (Kim et al., 2019). These differences between lean and obese subjects could explain the difference in the relationship between mood and *Coprococcus* levels in our study. The causal role between *Coprococcus* levels and mood is still unproven. Future functional studies measuring the profile of microbiota-derived metabolites, hormonal or inflammatory mediators would help to better understand the role of *Coprococcus* in the gut-brain crosstalk.

The fact that the High *Coprococcus* subjects tended to have worse psychological profiles (decreased positive affect scores) may explain why improvements by inulin were visible in this group. Further studies designed to specifically examine treatment response according to baseline microbiota composition will define which individuals may benefit most. Studies are also needed to investigate the collaboration of bacteria in the response to prebiotics.

Among the limitations of this study, we did not assess depression and anxiety with the most appropriate tools (even if PANAS is known to be correlated with standard depression tests). The analysis of gut microbiota composition without functional characterization (i.e., measurements of bacterial metabolites) does not allow to speculate on the mechanism by which *Coprococcus* impact the response toward the prebiotic intervention.

Overall, the present study supports the fact that gut microbiota composition could be used to predict the effect of a prebiotic approach on mood of obese subjects. Defining pivotal gut bacteria in the response toward food-based therapy will help to personalize these approaches. This work points to potential neuroactive properties of *Coprococcus* and its use as a gut microbiota biomarker for a beneficial response to prebiotics.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported *i*n this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.01.014.

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