Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

H2020 - 728018

D2.1: Manuscript: Effects of probiotics on symptoms of impulsivity, compulsivity and aggression

Dissemination level	Public
Contractual date of delivery	28.02.2023
Actual date of delivery	04.04.2023
Type	Report
Version	1.0
Workpackage	WP2 - Nutritional interventions
Workpackage leader	Nanda Rommelse, KARAKTER

This project has received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No 728018.

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Executive summary

(Add-on) treatment with the multispecies synbiotic resulted beneficial for the management of irritability and several frequently associated symptoms in at least, a subgroup of adults with ADHD and/or BPD. These symptoms are often considered as “difficult-to-treat” symptoms, defaulting prognosis and increasing mortality. In addition, a synbiotic-specific improvement in functioning and in perceived stress levels was also found. Taken together, our findings might therefore have important clinical and therapeutic implications. However, replication in larger samples, including longer follow-ups and inflammatory/immune marker measurements are warranted to confirm the findings and provide further insight.

1. DELIVERABLE REPORT

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Effectiveness of a synbiotic on irritability, impulsivity, inattentive/hyperactive and emotional symptoms, global and psychological functioning, and on perceived stress in adults with ADHD and/or Borderline Personality Disorder: results from a multicenter, randomized, placebo-controlled, basket trial

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ABSTRACT

Background: An increasing body of evidence suggests that intestinal dysbiosis may be involved in the pathophysiology of attention-deficit and hyperactivity disorder (ADHD), and borderline personality disorder (BPD). Manipulation of the gut microbiota might thus be beneficial for the management of ADHD and/or BPD symptoms.

Objective: To assess the efficacy of a multispecies synbiotic in the management of *irritability* in adults diagnosed with ADHD and/or BPD, and high levels of irritability. In addition, the effectiveness of this agent on *perceived stress levels*, *functioning*, and on i*nattentive, hyperactive, impulsive, obsessive-compulsive,* and *emotional* symptoms was also evaluated.

Methods: In a 10-week randomized, double-blind, placebo controlled study, 180 outpatient adults (age range:18-65 years) with an ADHD and/or BPD diagnosis, and high levels of irritability, were randomly allocated, in an 1:1 ratio, to a synbiotic, or to placebo. Clinical status was collected at baseline, and after 5, and 10 weeks of starting medication. The primary outcome measure was *response to treatment* (i.e., a reduction at least 30% in the ARI-S total score compared to baseline, together with a CGI-I score of 1 or 2, at week 10). Secondary outcome measures included the change in different psychological questionnnaires, as well as safety. A total of 134 patients completed study assessments, and were included in the final analyses.

Results: Complete response was achieved by 81% of patients allocated to the synbiotic group, and by 19% allocated to the placebo group (X^2=5.2, p=0.02). By week 10, patients allocated to the synbiotic showed a significantly higher improvement in *inattention* (p=0.001, np^2=0.08), *hyperactivity* (p=0.002, np^2=0.07), *impulsivity* (p=0.02, np^2=0.04), and *emotional* symptoms (p<0.001, np^2=0.09), than those allocated to placebo. The improvement in impulsivity was in particular evident for *the lack of perseverance* domain (p=0.03, np^2=0.04). A significantly higher improvement in *peer relationship problems* (p=0.04, np^2=0.07), on *global functioning* (p=0.03, np^2=0.04), and on *perceived stress* levels (p=0.04, np^2=0.03) was also found. Changes in BMI, nutritional intake, and/or physical activity during the study did not significantly correlate with treatment response. The synbiotic was well tolerated and no severe adverse events occurred; the frequency of side effects did not significantly differ between

1 Introduction

Attention-deficit and hyperactivity disorder (ADHD) and borderline personality disorder (BPD) are common psychiatric conditions, with a particularly high symptomatic overlap. Patients with ADHD and BPD often suffer from high levels of *irritability*, *impulsivity*, and/or *emotional dysregulation*, making the differential diagnosis in some cases, difficult. During the last decades, researchers have wondered about the existence of a common biological background (Weiner et al., 2019). An increasing body of evidence has suggested that intestinal dysbiosis (i.e., an inappropriate diversity in gut microbiota) may be involved in the pathophysiology of both conditions. For example, an increased Bacteroidetes-to-Firmicutes ratio, and abnormal levels of different bacterial species have been repeatedly described in individuals with ADHD and/or BPD (Rössler et al., 2020; Richarte et al., 2021; Wang et al., 2022). In line with this, manipulation of the gut microbiota has been suggested as a viable treatment option in individuals diagnosed with both conditions (Kalenik et al., 2021); this option becomes in particular appealing for the management of nowadays still considered as "difficult-to-treat" symptoms, such as *irritability* (Fernández de la Cruz, 2015; Dyce et al., 2020). Treatment options include fecal microbiota transplantation (Hooi et al., 2022), or (add-on) treatment with probiotics. A probiotic is considered as a live, non-pathological microorganism that, when ingested in adequate amounts, exerts a health benefit (Dinan and Quigley, 2011); psychobiotics are defined as a live organism that, when ingested at adequate amounts, produces health benefit in patients suffering from a psychiatric disease (Dinan et al., 2013). Prebiotics were originally defined as dietary ingredients selectively inducing the growth or activity of gastrointestinal bacteria, improving the microbial balance in the gut, thereby exerting beneficial effects on the host (Gibson, 2022); a synbiotic contains both, prebiotics, and probiotics/psychobiotics. Interestingly, in a previous report performed in children, adolescents, and adults with ADHD, a beneficial effect of a multispecies synbiotic (i.e., Synbiotic 2000) on restricted, repetitive, and/or stereotyped behaviors, as well as on emotion regulation was suggested (Skott et al., 2019). Irritability is often associated with the presence of such symptoms (e.g., impulsivity, emotion dysregulation) (Blair et al., 2020; Keluskar et al., 2021). Based on previous reports, it can be thus hypothesized that (add-on) treatment with a synbiotic would improve irritability in adults with ADHD and/or BPD. The aim of this study was to explore, for the first time, the effectiveness of a synbiotic on improving *irritability* in a cohort of adults with two different (but symptomatic overlapped) psychiatric conditions, i.e., ADHD and/or BPD, and high levels of irritability. In addition, the effectiveness of this agent on other symptoms (e.g., *inattention, hyperactivity, impulsivity, obsessive-compulsive, emotional dysregulation*), on *global and psychological functioning,* and on *perceived stress,* was also investigated.

2 Material and Methods

2.1. Trial design and study setting

This 10-week, randomized, parallel group, double-blind, placebo-controlled trial was carried out at three European clinical centers: the University Hospital Frankfurt-Goethe University (Frankfurt, Germany), the Semmelweis University (Budapest, Hungary), and the Vall d´Hebron University Hospital (Barcelona, Spain). The study protocol (Arteaga-Henríquez et al., 2020) was reviewed and approved by the local Ethic Committees at the three study centers (Frankurt:269/18; Budapest:44101-1/2018/EKU; Barcelona:311/2018) and registered at ClinicalTrials.gov (NCT03495375) before the start of the trial. Research procedures adhered to the relevant national and institutional Good Clinical Practice (GCP) guidelines on human experimentation, as well as to the Declaration of Helsinki of 1975, as reviewed in 2008. All study participants provided written informed consent before enrolment in the trial.

2.2. Study participants

Eligible participants were recruited between February 2019 and October 2020 via predefined psychiatric out-patient clinics at the three study centers. Included were adult males and females aged 18-65 years with a high level of multidimensional irritability based on an *Affective Reactivity Index Self-Report* (ARI-S) total score of at least 5, and a *Clinical Global Impression-Severity* (CGI-S) total score of at least, 4 (i.e., moderately ill). Patients had to meet Diagnostic and Statistical Manual of Mental Disorders, 5th edition (DSM-5) criteria for ADHD and/or BPD; diagnoses had to be confirmed by a structured diagnostic interview (i.e., the Diagnostic Interview for Adult ADHD (DIVA 2.0), and the Structured Clinical Interview for DSM-IV (SCID-II)). Participants should also deem compliant with the protocol, speak and comprehend the native language of the country in which the assessments took place, and be able to sign the informed consent in order to be included. Excluded were patients currently taking probiotics, or those who have been under antibiotic therapy within the last 6 weeks prior to study entry. Patients showing psychotic symptoms, diagnosed with any major psychiatric condition requiring hospitalization at baseline, with neurological disorders involving central functions, or suffering from a major physical illness of the cardiovascular, endocrine, pulmonal, immune, or gastrointestinal system were excluded, too. Patients with a history of clinically relevant somatic acute or chronic disorders that, in the opinion of the investigator, may confound results, tolerability/safety assessments, or prohibit the patient from completing the study were also not included. Individuals undergoing immunosuppression, or with an intelligence quotient < 70 (as assessed by the Wechsler Adult Intelligence Sale (WAIS)), were not included, as were participants with a history of documented allergy, hypersensitivity, and/or intolerance to any ingredients of the intervention. Patients that had taken another investigational product, or that had taken part in another clinical study within 30 days prior to entering this study were excluded, too. To avoid asking patients to stop taking any medications before entry, participants were allowed to continue their regular medications but without alteration in medication and dosage during the duration of the trial. At least a 30-day period of stable treatment was also required before study entry.

2.3. Study intervention

Eligible participants were randomly assigned to received either a synbiotic or placebo for 10 weeks. The active treatment, i.e., Synbiotic 2000 Forte (Synbiotics AB, Sweden), was a lyophilized composition of 400 billion lactic acid bacteria/dose (i.e., *Pediococcus pentosaceus* 5-33:3/16:1, *Lactobacillus casei ssp paracasei* F19, *Lactobacillus plantarum* 2362, and *Leuconostoc mesenteroides* 77:1), and 2.5 g/dose of each of the following fermentable fibers: β-glucan, inulin, pectin, and resistant starch. The placebo (Synbiotics AB, Sweden) was composed of the oligosaccharide maltodextrin. The synbiotic and placebo were designed to be identical regarding packaging (i.e., sachets), volume, weight, content color, texture, and favor; both were without smell. Participants were instructed to mix the powder in drinks or foods (< 40ºC) and take it once daily; sachets had to be stored at a refrigerated temperature (i.e., 4-6ºC) during the 10 weeks of study.

2.4. Randomization and Blinding

Randomization was performed in a 1:1 ratio using a computer-generated code. All participants, families, and study personnel (including clinicians, nurses, and those performing baseline and outcome assessments) were blinded to group assignment for the entire study.

2.5. Study assessments

Clinical examinations were conducted on four separate events: at baseline (t0) (i.e., -7 to -1 days before the start of the intervention), at week 5 (t1) and 10 (t2) after intervention start, and post-intervention (t3) (i.e., within 1 week after last treatment intake) (Arteaga-Henríquez et al., 2020).

2.5.1. Primary outcome measures

Response to treatment was considered as the primary outcome measure, and was defined by a reduction in the ARI-S total score of at least 30% (compared to baseline), together with a *Clinical Global Impression-Improvement* (CGI-I) score of 1 (very much improved) or 2 (much improved), at week 10.

(1) The ARI-S is a 7-item questionnaire aimed at assessing chronic and dimensional irritability (i.e., threshold for an angry reaction, frequency of angry feelings/behaviors, duration of such feelings/behaviors). Although initially developed for the children population (Stringaris et al., 2012), it has also demonstrated a strong validity for the assessment of irritability in adolescents and adults (Mulraney et al., 2014). Each item is composed of a three-level response category: not true, somewhat true, certainly true, which are scored as 0,1 or 2, respectively (range of possible scores:0-12). The total score is the sum of the first six items, with higher scores representing higher levels of multidimensional irritability.

(2) The CGI-I is a seven-point clinician-rated scale that has been widely utilized as an efficacy measure in clinical drug trials, informing about how much the patient´s illness/symptom has improved or worsened relative to a baseline state at the beginning of the intervention (Guy, 1976). The CGI-I is rated from 1 (very much improved) to 7 (very much worse).

2.5.2. Secondary outcome measures

Secondary outcome measures were selected to assess intervention effect on *inattentive*, *hyperactive*, *impulsive*, *obsessive-compulsive*, and *emotional* symptoms, on several aspects of *functioning*, and on *perceived stress*, via a series of selected scales and questionnaires:

(1) The *Attention-deficit and Hyperactivity Disorder Rating Scale-IV* (ADHD-RS-IV) is a clinician-administered 18-item scale based on DSM-IV-TR criteria for ADHD, that provides a rating of the severity of symptoms in adults (Zhang et al., 2005). Scoring is based on a 4-point likert-type severity scale where 0=none, 1=mild, 2=moderate, and 3=severe. Clinicians scored the highest score that was generated for the prompts of each item. The ADHD-RS-IV total score is defined as sum of all 18 item scale scores (range 0-54).

(2) The *UPPS-P Impulsive Behavior Scale* is a 59-item measure of factors that could lead to impulsive behaviors (Whiteside and Lynam, 2001). It consists of five subscales aimed at assessing the following aspects related to impulsivity: *positive urgency* (i.e., the tendency to act impulsively due to a positive effect), *negative urgency* (i.e., the tendency to act impulsively due to a negative effect), *lack of premeditation* (i.e., the tendency to act rashly without first reflecting upon the decision to act) , *lack of perseverance* (i.e., the tendency not to complete projects), and *sensation seeking* (i.e., motivation to experience novelty). Participants respond to each item using a 4-point Likert scale: 1=agree strongly, 2=agree some, 3=disagree some, and 4=disagree strongly; the mean is calculated for the items on each subscale, from 1 to 4, where 1 indicates that the respondent did not indorse impulsive answers, and 4 indicating a high level of self-reported impulsivity.

(3) The *Yale-Brown Obsessive Compulsive Scale* (Y-BOCS) is a clinician-administered 10-item scale designed to measure the severity and type of obsessive and/or compulsive symptoms over the past seven days (Goodman and Price, 1992; Storch et al., 2010). Total Y-BOCS scores range from 0-40, with higher scores aimed at evaluating indicating greater severity of obsessive and/or compulsive symptoms.

(4) The *Strengths and Difficulties Questionnaire* (SDQ) is a 25-item questionnaire evaluating *emotional* symptoms, *hyperactivity/inattention*, and also, several aspects of psychological functioning such as *conduct problems*, *peer relational problems*, and/or *prosocial behavior* (Goodman, 200). These 25 items are rated on a 3-point Likert scale (not true, somewhat true, certainly true), and are designed to be divided between five subscales (scored 0-10). Each of the five subscales comprises of five questions,

each; the total difficulties score ranges from 0-40, and is generated from the sum of the four subscales of emotion, hyperactivity/inattention, conduct, and peer problems (20 items), the items for prosocial behaviors are not included in the total difficulties score.

(5) The *Difficulties in Emotion Regulation Scale-16* (DERS-16) is a self-reported measure of emotion regulation difficulties (i.e., a measure of functioning in an emotionally upset state) (Bjureberg, 2016). It includes five subscales aimed at assessing: *lack of emotional clarity, difficulties engaging goal-directed behaviors*, *impulse control difficulties*, *limited access to effective emotion regulation strategies*, and *non-acceptance of emotional response*, with higher scores indicating, more difficulties.

(6) The *Functioning Assessment Short Test* (FAST) was developed for the clinical evaluation of functional impairment presented by patients suffering from a mental disorder. The scale consists of 24 items, which are divided among 6 specific areas of functioning: *autonomy*, *occupational functioning*, *cognitive functioning*, *financial issues*, *interpersonal relationships*, and *leisure time* (Rosa et al., 2007).

(7) The *Perceived Stress Scale* (PSS-10) is a 10-item self-reported questionnaire developed by Cohen and colleagues (1983) widely used to assess stress levels in adolescents and adults (Cohen et al., 1983; Kechter et al., 2019). Respondents are asked how often they felt a certain way of a five-point scale from “never” to “very often”; the PSS-10 total score is then obtained by summing across all items, with higher levels indicating higher levels of perceived stress over the previous month (i.e., the degree to which an individual has perceived life as unpredictable, uncontrollable, and overloading).

In addition, and with the aim to identify putative major alterations in nutrient intake and/or in physical activity between baseline and follow-up, the changes in both nutritional intake and in physical activity were also assessed. Participants were asked to complete at least three 24-h dietary recalls (i.e., baseline, between baseline and week 5, and between week 5 and week 10), including two weekdays, and one weekend day, on non-consecutive days, describing the type and amount (in grams) of all drinks and foods consumed during the previous days, starting with the first intake after waking up in the morning. Physical activity was assessed by the *International Physical Activity Questionnaire* (IPAQ) (Hagströmer et al., 2007). The IPAQ is a 27-item self-reported measure of physical activity in adults (i.e., high: at least 3000 Metabolic Equivalent of Task (MET)-min/week, moderate: at least 600 MET-min/week, low: not meeting either criteria).

Also, the effect of the intervention on intestinal function was evaluated by the change, from baseline to week 10, in the *Bristol Stool Scale* (BSS) (Lewis and Heaton, 1997). The BSS is composed of combined and standardized graphic and descriptive methods representing seven types of stools with different shapes and consistencies: type 1 (separate hard lumps, like nuts; severe constipation), type 2 (lumpy and sausage like; mild constipation), type 3 (a sausage shape with cracks in the surface; normal), type 4 (like a smooth, soft sausage or snake; normal), type 5 (soft blobs with clear-cut edges; lacking fibre), type 6 (mushy consistency with ragged edges; mild diarrhoea), type 7 (liquid consistency with no solid pieces; severe diarrhoea).

Participant´s compliance was assessed by the *Probabilistic Medication Adherence Scale* (ProMAS) (Kleppe et al., 2015) throughout the 10 weeks of the intervention. Moreover, participants were asked to return unused envelopes from the previous visit. To classify a person as adherent, a cut-off point of at least 80% was chosen, for partial adherence, a cut-off point of 60-79% was set, patients were classified as no adherent to the intervention at a cut-off point of 59%, or less.

2.6. Safety and tolerability

Side-effects were structurally assessed by a Body System Questionnaire (i.e., a checklist composed of 12 possible adverse events: heat, gastrointestinal, musculoskeletal, dermatological, cardiovascular, pulmonal, genitourinary, drug allergy, immunologic, hematologic, psychiatric, other), and recorded by the research team at baseline, and at each follow-up visit. In addition, all patients were evaluated with

complete physical examination, electrocardiogram, complete blood counts, and liver function tests before the commencement of trial.

2.7. Statistical analyses

The mean and standard deviation (SD) of continuous variables, and the frequency of categorical variables are reported. Significant differences between the synbiotic and probiotic groups in relation to their baseline/post-intervention clinical and socio-demographic characteristics were assessed for continuous variables using Kruskall Wallis tests for continuous variables, by Chi-Squared (X^2) tests for categorical variables.

Changes in symptoms and/or functioning from baseline to the end of the intervention phase, at week 10 were assessed using paired *t*-test (two-tailed), and the mean difference (MD), it´s 95% confidence interval (95%CI) values, *p*-values, and estimates of effect size are reported. The efficacy analysis was performed on the study completers, i.e., per-protocol (PP) population (n=134). Due to the randomized nature of our study, the differences between the treatment groups over the different questionnaires were tested by an analysis of covariance model (ANCOVA) (van Breukele, 2006; Wang et al., 2019), with relative change from baseline (t0) to week 10 (t2) as the dependent variable, treatment group (i.e., synbiotic vs. placebo) as fixed effect, and study center (Germany, Hungary, Spain), age, sex (female/male), BMI, medication status (naïve/medicated), primary psychiatric diagnosis (ADHD and/or BPD), and corresponding scale/questionnaire score at baseline, as covariates. The mean difference (MD), 95% CIs, *p*-values, and estimates of effect size are provided, with negative values representing more improvement of symptoms and/or functioning by the synbiotic, compared to placebo. Proportion of outcome variable explained were indicated by partial eta squared (np^2). In addition, an intention-to-treat (ITT) analysis using Last Value Brought Forward (LVBF) with at least one post-treatment evaluation was used to handle missing data (Shao ad Zhong, 2003), leading to similar conclusions (**Supplementary Table 1**). All tests were two-tailed, with *p*-values equal or less than 0.05 being considered as significant; *p*-values less than 0.1 were considered as of suggestive statistical significance. Because this was designed as an exploratory analysis, we did not adjust *p*-values for multiple testing, which is consistent with prior recommendations (Rothman, 1990; Thompson, 1998).

3 Results

3.1. Patient characteristics

Out of 231 patients screened for eligibility, 180 satisfied all eligibility criteria and underwent randomization, 90 were assigned to the synbiotic, and 90 to placebo. A total of 46 (26%) individuals discontinued the study before the end of the intervention, at week 10. All reasons for loss to follow-up and discontinuation are noted in **Figure 1**. Psychological data were thus analyzed for the 134 participants that completed outcome assessments, 73 (55%) belonged to the synbiotic group, and 61 (45%) to the placebo group. Compliance was reported to be perfect or nearly perfect for 111/134 (83%) analyzable patients, 57 (51%) in the synbiotic group, and 54 (49%) in the placebo group ($X^2(2) = 3.325$, $p=0.190$). At least 151 post-baseline clinical assessments (i.e., week 5) were completed; these subjects were included in the ITT analyses (see 2.9, **Supplementary Table 1**).

Table 1 summarize the baseline characteristics of the 134 study participants included for analyses. Age ranged from 20 to 62 years; the mean (SD) age was 38 (12) years, 89 (66%) were females, and 126 (94%), white. The mean (SD) body mass index (BMI) was 26 (6), indicating normal weight to overweight. Patients included were suffering from high levels of irritability (i.e., the mean (SD) ARI-S score was 7 (2)), and, according to clinical experience, were moderately to markedly ill (i.e., the mean (SD) CGI-S score was 5 (1)) (**Table 1**). Baseline characteristics of the treatment groups were similar except for sex (i.e., a significantly higher percentage of females were included in the subgroup of patients allocated

to placebo), and in the mean (SD) SDQ prosocial behavior subscale score (i.e., individuals allocated to placebo were characterized by a significantly higher impairment in prosocial behavior, compared to those allocated to the synbiotic) (**Table 1, Supplementary Table 2** and **3**).

3.2. Primary outcome measures: response to treatment

From baseline to the end of the placebo-controlled phase, at week 10, a total of 16 (12%) of patients were considered as responders (i.e., as defined by a reduction in the ARI-S total score of at least 30% compared to baseline, together with a CGI-I score of 1 or 2, at week 10). Of the total of responders, 13 (81%) belonged to the synbiotic group, while 3 (19%) belonged to the synbiotic group (X^2= (1, 134) =5.251, *p*=0.02).

3.3. Secondary outcome measures: change in inattentive, hyperactive, impulsive, obsessive-compulsive, and emotional symptoms, in global and/or psychological functioning, and in perceived stress

3.3.1. ADHD-RS score

The effectiveness of the synbiotic on *inattentive*, *hyperactive*, and *impulsive* symptoms was assessed by the change, from baseline to week 10, in the ADHD-RS total and respective subscale scores (**Table 2**). Overall (i.e., combining the synbiotic and placebo groups), there was significant reduction in ADHD symptoms in patients (*p*<0.001, np2=0.3), i.e., a significant improvement in inattentive (p<0.001, np2=0.2), hyperactive (*p*<0.001, np2=0.3), and impulsive (*p*<0.001, np2=0.2) symptoms was found at the end of the intervention, at week 10 (**Table 2**). The reduction in ADHD symptoms was in particular evident in the subgroup of patients allocated to the synbiotic agent (i.e., patients allocated to the synbiotic were characterized by a significantly higher improvement in *inattentive* (*p*=0.001, np^2=0.08), *hyperactive* (*p*=0.002, np2=0.07), and *impulsive* symptoms (*p*=0.02, np2=0.04) compared to those allocated to placebo (**Table 2, Figure 2**)). Treatment effect was not moderated by study center, age, sex, BMI, primary psychiatric diagnosis, and/or medication status (data not shown).

3.3.2. UPPS-P score

The effectiveness of the synbiotic on different impulsive domains (i.e., *positive urgency*, *negative urgency*, *lack of premeditation*, *lack of perseverance*, *sensation seeking*) was assessed by the change, from baseline to week 10, in the UPPS-P total and respective subscale scores (**Table 2**). Overall, there was a significant increase in the impulsive symptoms assessed (*p*<0.001, np2=0.1); this was in particular evident for the symptoms: *positive urgency* (p<0.001, np2=0.03), *negative urgency* (p<0.001, np2=0.2), and *sensation seeking* (p<0.001, np2=0.1). Significant differences were not found between patients allocated to the synbiotic, and those allocated to placebo (**Table 2**). On the contrary, a significant improvement in patient´s *lack of premeditation* (p=0.01, np2=0.05), and *lack of perseverance* (p<0.001, np2=0.1) was found at the end of the intervention phase, at week 10. This was in particular evident in the subgroup of patients allocated to the synbiotic (i.e., patients allocated to the synbiotic showed a significantly higher improvement in their *lack of perseverance* (*p*=0.03, np2=0.04), compared to those allocated to placebo (**Table 2, Figure 2**)).

3.3.3. 3.3.3 YBOC-S score

The effectiveness of the synbiotic on obsessive and/or compulsive symptoms was assessed by the change, from baseline to week 10, in the YBOCS score (**Table 2**). At the end of the intervention, a similar degree of reduction in total obsessive and/or compulsive symptoms was found for both treatment group; i.e., there was no difference in effect between the interventions (**Table 2**).

3.3.4. SDQ score

The effectiveness of the synbiotic on *hyperactivity*, *emotional symptoms*, and on psychological functioning (i.e., *conduct* and/or *peer relational problems*, *prosocial behavior*) was assessed by the

change, from baseline to week 10, in the SDQ total and subscale scores (**Table 2**). Overall, a significant improvement in the SDQ total score was found (p<0.001, np2=0.3), this was at the expense of an improvement in *hyperactivity/inattention* (*p*<0.001, np2=0.2), *emotional symptoms* (*p*<0.001, np2=0.2), *conduct* (*p*=0.006, np2=0.06), and/or *peer relationship problems* (p=0.01, np2=0.05) (**Table 2**). A treatment type-effect (i.e., synbiotic superior than placebo) was found for the improvement in the SDQ total score (*p*<0.001, np2=0.097), *hyperactivity/inattention* (p=0.04, np2=0.03), *emotional symptoms* (p<0.001, np2=0.09), and in *peer relationship problems* (*p*=0.004, np2=0.07) (**Table 2, Figure 2**). In this case, treatment effect on *emotional symptoms* was moderated by study center (*p*=0.03, np2=0.03) (data not shown).

3.3.5. DERS-16 score.

The effectiveness of the synbiotic on functioning while in an emotionally upset state was assessed by the change, from baseline to week 10, in the DERS-16 total and respective subscale scores (**Table 2**). Overall, a significant reduction in the DERS-16 total score (p<0.001, np2=0.2) was found at the end of the intervention phase, at week 10; the reduction in the DERS-16 total score was at the expense of a reduction in all subscale scores (**Table 2**). A treatment type effect was found for the reduction in goal-directed behavior, and emotional strategies, i.e., patients allocated to the synbiotic were characterized a by a trend of significantly higher reduction in *goal-directed behavior* (*p*=0.08, np2=0.02), and in emotional strategies (*p*=0.09, np2=0.02) (**Table 2**). Treatment effect was not moderated by study center, age, sex, BMI, primary psychiatric diagnosis, and/or medication status (data not shown).

3.3.6. FAST score

The effectiveness of the synbiotic on different aspects of functioning was assessed by the change, from baseline to week 10, in the FAST total and respective subscale scores (**Table 2**). Overall, a significant reduction in the FAST total score (p<0.001, np2=0.1) was found at the end of the intervention phase, at week 10; the reduction was at the expense of an improvement in autonomy (p<0.001, np2=0.08), financial (p=0.004, np2=0.06), and interpersonal (p<0.001, np2=0.08) functioning (Table 2). A treatment type specific effect on the improvement in the FAST total score (i.e., synbiotic superior than placebo) was found at the end of the intervention phase, at week 10 (p=0.03, np2=0.04) (Table 2). Interestingly, and while treatment type effect was not found in relation to the above-mentioned improvements in autonomy, financial and interpersonal functioning, a trend of synbiotic-specific improvement was however found for *occupational functioning* (p=0.051, np2=0.03) , and for *leisure* (p=0.08, np2=0.02). Again, treatment effect was not moderated by study center, age, sex, BMI, primary psychiatric diagnosis, and/or medication status (data not shown).

3.3.7. PSS score

The effectiveness of the synbiotic on patient´s level of perceived stress was assessed by the change, from baseline to week 10, in the PSS score (**Table 2**). Overall, a significant reduction on the PSS score was found in patients, at the end of the intervention phase (p=0.002, np2=0.07) (Table 2); this was in particular evident in the subgroup of patients allocated to the synbiotic (i.e., patients allocated to the synbiotic showed a significantly higher improvement in the PSS score compared to those allocated to placebo (p=0.04, np2=0.03) (**Table 2**). Findings were not moderated by study center, age, sex, BMI, medication status, and/or primary psychiatric diagnosis.

4 Effect of BMI, diet, and physical activity changes on intervention effect

Changes in BMI, diet, and/or physical activity might confound the response. Hence, we attempted to identify major differences in BMI, physical activity, and nutrient intake between baseline and week 10. We detected no significant change between baseline and follow-up in both BMI and physical activity (as measured by MET-min/week); on the contrary, patients were characterized by a lower total energy

intake, and by a lower intake of carbohydrates, proteins, fiber, zinc, iron, vitamin B6, and folic acid at the end of the intervention phase, at week 10 (**Supplementary Table 4**). However, the detected change in total energy intake, as well as in the above-mentioned nutrients did not influence the intervention effect; i.e., a significant difference between the synbiotic and placebo groups in relation to total energy intake, carbohydrates, proteins, fiber, zinc, iron, vitamin B6, and folic acid intake over time was not found (data not shown).

4.1. Effect on BSC

At the end of the intervention phase, at week 10, patients allocated to the synbiotic were significantly less constipated compared to baseline (p=0.002) (**Table 4**). Significant differences between in relation to stool appearance were not found in patients allocated to the synbiotic.

5 Safety and tolerability

The synbiotic was safe and well tolerated. No severe adverse events were observed and, thus, no study participant was excluded for this reason. Side-effects included headache/migraine, musculoskeletal (e.g., muscle pain), dermatological (e.g., rash), psychiatric (e.g., sleep disturbances, anxiety), fever, and/or others (e.g. sore throat, fatigue) (**Table 4**). No significant between-group differences were found in the frequency of side-effects between the two trial groups.

6 Discussion

To the best of our knowledge, this is the first randomized, placebo-controlled clinical trial with the primary research question being the effectiveness of a multispecies synbiotic (i.e., Synbiotic 2000 Forte) on improving *irritability* in individuals diagnosed with two different psychiatric conditions (i.e., ADHD and/or BPD), and high levels of multidimensional irritability. A large study group comprising, in total, 134 adults, was treated daily with either the synbiotic or with placebo for 10 consecutive weeks. At the end of the intervention, individuals allocated to the synbiotic showed a significantly higher response rate (as defined by a decrease in the ARI-S total score of at least 30% compared to baseline, and a CGI-I score of 1 (much improved) or 2 (very much improved), at week 10) compared to those allocated to placebo. This suggests that (add-on) treatment with the synbiotic may be superior to (add-on) treatment with placebo in the management of *irritability* in, at least, a subgroup of adults with ADHD and/or BPD. Irritability, which is defined as "the interindividual differences in proneness to anger" (Vial Ribas et al., 2016) is overrepresented in a considerable number of psychiatric conditions, including as ADHD and/or BPD, but also, in autism spectrum disorder (ASD) (Bartram LA, 2019), and/or bipolar disorder (BD) (Faurholt-Jepsen et al., 2019; Patino and DelBello, 2021), among others. High levels of irritability predispose to aggressive behaviors (Zik et al., 2022), and contribute to not only disease persistence, but also, to mortality (Karam et al., 2015). Importantly, irritability has been described as an independent factor for suicidal behavior (Stringaris and Vidal-Ribas, 2019; Jha et al., 2021). Despite this, currently available treatment options (which include antipsychotics, psychostimulants, and/or cognitive behavioral therapy (Sukhodolsky et al., 2016; van Schalkwyk et al., 2017; Stuckelman et al., 2017)) are still limited (Dyce et al., 2020; Bell et al., 2021). We believe our findings might thus have potential clinical implications, and constitute a first step in the development of new approaches for the management of this symptom. In addition, the effectiveness of the synbiotic on other ADHD and/or BPD symptoms, such as *inattention, hyperactivity, impulsivity, obsessive-compulsive*, and/or *emotional* symptoms was also investigated. Interestingly, a significantly higher improvement in *hyperactivity*, *inattention*, and *impulsivity* (as assessed by the change, from baseline to week 10, in the ADHD-RS total and respective subscale scores) was found in the subgroup of patients allocated to the synbiotic, compared to those allocated to placebo. When assessing the

change in *hyperactive/inattentive* symptoms by another questionnaire (i.e., the SDQ), again, a synbiotic-specific improvement was also found in patients. Findings were robust, and the intervention effect was not moderated by potential confounding factors, such as study center, age, sex, BMI, primary psychiatric diagnosis (i.e., ADHD, BPD, both), and/or medication status. Our findings are supported by previous reports performed on children and adolescents suffering from different non-psychiatric and psychiatric conditions. For example, in a study performed on, in total, 71 male children and adolescents (age range: 7-15 years) diagnosed with autism spectrum disorder (ASD), 35 patients were randomly allocated to a probiotic containing *Lactobacillus plantarum* (i.e*., Lactobacillus plantarum* PS128, $3x10^9$ CFU/capsule), and 35 patients were allocated to placebo, and followed over a 4-week period (Liu et al., 2019). At the end of the intervention phase at week 4, a significant improvement in *inattention* (as assessed by the change, from baseline to week 4, in the Swanson, Nolan, and Pelham-IV (SNAP-IV) total and respective subscale scores) was found in patients (Liu et al., 2019). In another study, 57 children (age range: 6- 12 years) diagnosed with Tourette syndrome were randomly assigned to either placebo (n=29), or to a probiotic agent containing, also, *Lactobacillus plantarum* PS128 ($3x10^{10}$ CFU/capsule), and followed over 8 weeks (Wu et al., 2021). Again, a probiotic-specific significant improvement in *inattention*, and in *hyperactivity/impulsivity* (as assessed by the change in the SNAP-IV respective subscale scores) was found at the end of the intervention phase, at week 8 (Wu et al., 2021). An open-label, single-arm study performed on 30 children and adolescents (age range:4-16 years) with, specifically, ADHD, participants were supplemented with a probiotic containing *Bifidobacterium bifidum* -688) ($5x10^9$ CFU/day) for 8 weeks (Wang et al., 2022). Clinical symptoms were again assessed using the SNAP-IV; at the end of the intervention period at week 8, a significant improvement in *inattention* and in *hyperactivity/impulsivity* was found (Wang et al., 2022). Our findings are however not supported by a previous report performed on in total, 182 children (n=68) and adults (n=114) with specifically, ADHD (Skott et al., 2019). In this study, the effectiveness of a synbiotic on ADHD symptoms was investigated; the synbiotic (i.e., Synbiotic 2000) was very similar to the one used in our study, it was composed of $4x10^{11}$ CFU/dose of three acid lactic bacteria *Pediococcus pentosaceus* 5-33:3/16:1, *Lactobacillus casei ssp paracasei* F19, *Lactobacillus plantarum* 2362, and 2.5 g of each of the fermentable fibres β-glucan, inulin, pectin, and resistant starch; the change of ADHD symptoms was assessed by the change, from baseline to week 9 in the SNAP-IV (children), or in the Adult ADHD-questionnaire: self-report scale (ASRS-v1.1) (adults) (Skott et al., 2019). At the end of the intervention phase at week 9, researchers found that neither the active treatment, nor placebo had any statistically significant effect on ADHD symptoms (Skott et al., 2019). The reasons for the divergent findings can be only speculated on; sample size and follow-up period were lower than in our study. In addition, and also contrary to our study, about 72% of adult individuals were under diet supplements (e.g., vitamins, omega-3, and/or probiotics) at baseline, something which might have influenced the findings. As mentioned before, a significant higher improvement in impulsivity was found in the subgroup of patients allocated to the synbiotic, compared to those allocated to placebo. We additionally explored which areas of impulsive behavior improved by the intervention; interestingly, we found that, individuals allocated to the synbiotic showed, after 10 weeks of continuous treatment, a significantly higher improvement in, specifically, their difficulty to remain focused on a task that may be boring or difficult (i.e., *lack of perseverance*). This was assessed by the change, from baseline to week 10, in the respective UPPS-P subscale score. Interestingly, an association between *lack of perseverance* and inattention has been repeatedly described, both in individuals with ADHD and/or BPD (Gay et al., 2008; DeShong and Kurtz, 2013; López et al., 2015), something which supports our findings. Interestingly, and also in line with previous reports (Chahwan et al., 2019; Lee et al., 2021), not only hyperactivity, inattention, and several aspects of impulsive behavior were in particular improved by (add-on) treatment with the synbiotic, a significant higher improvement in *emotional symptoms* (as assessed by the change, from baseline to week 10 in the respective SDQ score), was also found in the subgroup of patients belonging to the synbiotic group. Individuals with ADHD and/or BPD often suffer from emotional symptoms and, as happened with

irritability, management of these symptoms in this subject population is not easy, defaulting prognosis (Lenzi et al., 2018; Mirkovic et al., 2021).

Individuals with ADHD and/or BDP often suffer from functional impairments (Adamou et al., 2013; Thadani et al., 2022). Taken the above-mentioned findings into account, one would expect also an improvement in several aspects of functioning. In line with this idea, a significantly higher improvement on *global functioning* (as assessed by the change, from baseline to week 10 in the FAST total score) and, especially, on *occupational functioning*, and on the structuring of their *leisure time* was found in the subgroup of patients allocated to the synbiotic, when compared to those allocated to placebo. In addition, a significantly higher improvement in functioning, while in an emotionally upset state (as assessed by the change in the DERS-16 scale and subscale scores from baseline to week 10) was also found in patients allocated to the synbiotic. In particular, a trend of synbiotic-specific improvement was found in patient´s ability of engaging *goal-directed behavior*, and also, in *emotion regulation strategies*, which is in line with a previous study performed also on adults with ADHD (Skott et al., 2021; see above). The fact that goal-directed behavior hinges on the ability to focus on relevant information and ignore distracters (i.e., the capacity to evaluate the consequences of our actions so that we can choose an optimal plan), which is a function referred to as selective attention and/or interference suppression (Legrain et al., 2012), also supports the findings.

Considering all the above-mentioned improvements in symptoms and/or functioning, a significantly higher improvement in the levels of *perceived stress* in the subgroup of patients allocated to the synbiotic, compared to those allocated to placebo was expected, something we indeed, found. In our study, the improvement in the levels of *perceived stress* was assessed by the change, from baseline to week 10, in the PSS total score. Our findings are supported by previous animal and human studies performed on both healthy individuals, and subjects with psychiatric or non-psychiatric conditions. These studies have repeatedly suggested that (add-on) treatment with prebiotics and/or probiotics may be associated with an improvement in physical and psychological symptoms associated with stress, such as anxiety, and/or depressive symptoms (McKean et al., 2017; Vitellio et al., 2020). For example, in a study performed by Liu and colleagues (2016), long-term gavage of *Lactobacillus plantarum* PS128 to early-life stressed adult mice was associated with an amelioration of depression and anxiety-like behaviors (Liu et al., 2016). In another study performed by Venkataraman and colleagues (2020), a total of 74 healthy adults (age range:18-24 years) were randomly allocated to placebo (n=38), or to a multi-strain probiotic (i.e., *Bacillus coagulans* Unique IS2, *Lactobacillus rhamnosus* UBLR58, *Bifidobacterium lactis* UBBLa70, *Lactobacillus plantarum* UBLP40, *Bacillus breve* UBBr01, and *Bacillus infantis* UBBI01) and followed over 28 days (i.e., pre- and after their examinations) (Venkataraman et al., 2020). The change in stress and anxiety levels were evaluated by the change, from baseline to day 28, in the PSS, in the depression anxiety stress scale (DASS), and in the state-trait anxiety inventory (STAI) questionnaire scores. At the end of the intervention, individuals allocated to the probiotic showed, compared to those allocated to placebo, a significantly decrease in all questionnaires. Interestingly, the improvement was accompanied by a significantly higher reduction in cortisol serum levels (Venkataraman et al., 2020). This study is also in accordance with previous findings suggesting that daily supplementation with *Lactobacillus plantarum* 299v ($1x^{10}$ CFU) for 14 days may be associated with a reduction in cortisol levels in adults (age range: 18-30 years) under examination stress (Andersson et al., 2016). In another study performed by Chong and colleagues (2019), a total of 111 adults (age range:18-60 years) with moderate baseline stress levels were randomly allocated to placebo (n=55) or to a probiotic (n=56; *Lactobacillus plantarum* DR7, $1x10^{10}$ CFU/day) and followed over a 12-week period of time. After the intervention period, individuals allocated to the probiotic showed, compared to those allocated to placebo, a significantly higher improvement in stress levels, and in anxiety (as assessed by the change, from baseline to week 12 in the DASS respective subscale scores) (Chong et al., 2019). Again, the administration of the probiotic also significantly reduced plasma cortisol levels in subjects compared to the placebo group.

Interestingly, a probiotic-specific improvement in other cognitive and/or memory functions, such as basic attention, emotional cognition, and associate learning was also demonstrated (Chong et al., 2019). Far from not paying attention to these findings, we believe these are of key relevance, due to the well-known consequences of stress, not only mentally, but also, at the physical level. Stress is considered as a non-specific response of the body against threatening demands, resulting in anxiety, discomfort, emotional tension, and difficulties in adjustment (Fink, 2009). The physiological of stress responses include activation of the autonomic nervous system and the hypothalamic-pituitary-adrenal axis, leading to increased blood and tissue levels of catecholamines, and glucocorticoids. These hormones alter immune functions and impairs regulation of the inflammatory response system (Dhabhar, 2014). Therefore, chronic and/or acute stress have been related to the development and/or persistence of a number of non-psychiatric and psychiatric conditions, such as diabetes, cancer, cardiovascular diseases, and/or major depressive disorder (Salleh, 2008; Musazzi et al., 2017), implying significant costs at not only the personal, but also at the economic level. Importantly, the synbiotic used in this study was safe and well tolerated, and the frequency, severity, and/or the type of adverse event (e.g., gastrointestinal, dermatological, headache) did not statistically differ between the synbiotic and placebo.

Although encouraging, our findings should be considered in light of several limitations. Unfortunately, this trial was conducted during some of the hardest month of the COVID-19 pandemic in Europe, in 2020, and a national lockdown was implemented in Germany, Hungary, and Spain. As a consequence, many individuals were not able to complete the psychological assessments in a proper way, resulting in a loss of follow-up. Despite this, the sample size was large (n=134), and the baseline characteristics were similar in completers and drop-outs, suggesting that drop-out did not add an important bias (data not shown). Another limitation is the relatively short follow-up period of 10 weeks, which may not reveal the long-term benefits and side-effects of (add-on) treatment with the synbiotic agent under study. In addition, and while the effect of synbiotics may be potentially attributed to their anti-inflammatory, anti-oxidant, and/or immunoregulatory actions (Cristofori et al., 2021), intervention effects on anti-inflammatory, anti-oxidant, and/or immune markers were not analyzed, and the effect in gut microbiota composition was also not confirmed. This is unfortunate, since it prevents us from discovering if there´s any specific marker that can predict a better response to treatment (personalized medicine). For example, in the study performed by Skott and colleagues (2019), patients with higher plasma levels of VCAM-1 showed a significantly higher improvement with the synbiotic in several impulsive domains compared to those with normal/low baseline levels of VCAM-1. Despite this, we believe our findings are of relevance and represent an important first step into the management of "difficult-to-treat" symptoms, such as irritability, in at least, a subgroup of adults with ADHD and/or BPD.

7 Conclusions

Our study offers, for the first time, preliminary evidence suggesting (add-on) treatment with a synbiotic (i.e., Synbiotic 2000 Forte), as a reliable and safe therapeutic option for the management of *irritability, inattention, hyperactivity*, and of several impulsive domains (i.e., *lack of perseverance)* in, at least, a subgroup of adults with ADHD and/or BPD. In addition, a beneficial effect of the synbiotic over placebo on several aspects of functioning, and on perceives stress, was found. As there are currently only few treatments available for the management of the above-mentioned symptoms, we believe our findings may have potential clinical implications. However, longer longitudinal studies, including larger sample sizes are urgently needed in order to provide further insight into important aspects, such as the minimum intervention period to observer clinically meaningful results should be investigated. In addition, the identification of (immune/inflammatory) biological markers to guide such interventions is also mandatory (personalized medicine).

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2. TABLES AND OTHER SUPPORTING DOCUMENTS WHERE APPLICABLE AND NECESSARY

Table 1. Baseline characteristics of study participants at baseline (t0)

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
	Mean [SD]	Mean [SD]	Mean [SD]	*p*-value
Age (years)	38 (12)	37 [12]	39 [11]	0.32
BMI (kg/m^2)	26 [6]	26 [5]	26 [6]	0.76
	n [%]	n (%)	n (%)	*p*-value
Sex (females)	89 [66%]	43 (59%)	46 (75%)	**0.04**
Ethnicity				0.56
White	126 [94%]	68 (93%)	58 (95%)	0.64
Asian	4 [3%]	3 (4%)	1 (2%)	0.40
Hispanic/Latino	3 [2%]	1 (1%)	2 (3%)	0.46
Black/African American	1 [1%]	1 (1%)	0 (0%)	0.36
Primary Psychiatric Disorder				0.60
ADHD	86 [64%]	49 (67%)	37 (61%)	
BPD	34 [25%]	18 (25%)	16 (26%)	
ADHD + BPD	14 [10%]	6 (8%)	8 (13%)	
Comorbid Psychiatric Disorder (yes)	72 [54%]	40 (55%)	32 (52%)	0.79
Psychiatric medication (yes)	88 [66%]	47 (64%)	41 (67%)	0.73
Psychostimulants	53 [39%]	32 (44%)	20 (33%)	0.19
Antidepressants	50 [37%]	23 (31%)	27 (44%)	0.13
Anxiolytics	30 [22%]	13 (18%)	17 (28%)	0.16

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
Stool type				0.89
Type 1: separate hard lumps, like nuts	12 [9%]	7 [10%]	5 [8%]	0.8
Type 2: lumpy and sausage like	13 [10%]	9 [12%]	4 [6%]	0.3
Type 3: a sausage shape with cracks in the surface	38 [28%]	19 [26%]	19 [31%]	0.5
Type 4: like a smooth, soft sausage or snake	46 [34%]	25 [34%]	21 [34%]	0.98
Type 5: soft blobs with clear-cut edges	16 [12%]	8 [11%]	8 [13%]	0.7
Type 6: mushy consistency with ragged edges	9 [7%]	5 [7%]	5 [7%]	0.9
Type 7: liquid consistency with no solid pieces	0 [0%]	0 [0%]	0 [0%]	ND
Study center:				0.8
Germany	47 [35%]	24[33%]	23 [38%]	
Hungary	46 [34%]	26 [36%]	20 [33%]	
Spain	41 [31%]	23 [31%]	18 [29%]	
	Mean [SD]	Mean [SD]	Mean [SD]	
ARI-S score	7 [2]	7 [2]	7 [2]	0.7
CGI-S score	5 [1]	4 [1]	5 [1]	0.1

Abbreviations: BMI: body mass index; ARI-S: Affectivity Reactivity Index-Self Report, CGI-S: Clinical Global Impression-Severity Scale. Mean and standard deviation (SD) values are given for continuous variables, sample size (n) and percentage are given for categorical variables. Analyses were based on Kruskal-Wallis Test (continuous variables) and on Chi-squared (χ^2) test (categorical variables). Significant *p*-values are highlighted in bold.

Table 2. Treatment effects on symptoms, functioning, and perceived stress in patients

	Synbiotic n=73		**Placebo** n=61		Effect for time	Effect for treatment type
Questionnaire	t0	t2	t0	t2		
	Mean [SD]	Mean [SD]	Mean [SD]	Mean [SD]	MD [95% CI]	MD [95% CI]
ADHD-RS	29.2 [9.9]	**20.8 [10.3]*****	30.2 [9,5]	**26.3 [9.9]****	**6.3 [4.6-7.9]*****	**-5.3 [-8.2- -2.3]*****
• Inattention	14.8 [5.4]	**10.8 [5.8]*****	15.3 [5.7]	**13.7 [6.1]***	**2.8 [1.9-3.7]*****	**-2.8 [-4.5- -1.2]*****
• Hyperactivity	7.9 [3.6]	**5.5 [3.4]*****	7.8 [3.7]	**6.7 [3.4]****	**1.8 [1.3-2.3]*****	**-1.43 [-2.33- -0.5]****
• Impulsivity	6.5 [3.3]	**4.5 [5.3]*****	7.1 [3.2]	**5.9 [3.1]*****	**1.6 [1.1-2.2]*****	**-1.1 [-2.0- -0.2]***
SDQ	18.7 [4.9]	**14.4 [5.9]*****	18.8 [4.9]	**17.1 [5.4]****	**3.1 [2.3-3.98]*****	**-2.9 [-4.5- -1.3]*****
• Hyperactivity/Inattention	6.5 [2.1]	**5.2 [2.3]*****	6.8 [2.1]	**6.1 [2.2]****	**1.0 [0.7-1.3]*****	**-0.7 [-1.3- -0.03]***
• Conduct problems	2.9 [1.3]	2.6 [1.5]	2.9 [1.3]	**2.7 [1.3]***	**0.3 [0.1-0.5]****	-0.1 [-0.6- 0.3]
• Emotional symptoms	6.2 [2.5}	**4.2 [2.7]*****	5.6 [2.9]	4.9 [2.8]	**1.3 [0.9-1.7]*****	**-1.3 [-2.0- -0.5]*****
• Peer relationship problems	3.0 [1.9]	**2.4 [2.0]*****	3.4 [2.0]	3.4 [1.8]	**0.4 [0.1-0.6]***	**-0.8 [-1.3- -0.2]****
• Prosocial behavior	7.9 [1.99]	8.2 [1.9]	8.6 [1.6]	8.5 [1.9]	-0.1 [-0.3-0.2]	0.2 [-0.4- 0.7]
UPPS-P	140.0 [14.4]	**145.5 [17.2]*****	135.7 [14.9]	**140.3 [17.2]***	**-4.9 [-7.3- -2.5]*****	2.7 [-2.2- 7.5]
• Positive Urgency	35.5 [9.4]	**39.2 [10.1]*****	32.5 [9.0]	**35.2 [9.3]****	**-3.6 [-4.9- -2.3]*****	2.6 [-0.1- 5.2]
• Negative Urgency	25.2 [5.9]	**28.1 [6.7]*****	24.5 [5.2]	**26.8 [5.7]*****	**-2.6 [-3.5- -1.7]*****	0.9 [-0.8- 2.7]
• Lack of premeditation	25.7 [5.9]	24.7 [6.2]	26.8 [6.1]	25.7 [7.0]	**0.97 [0.2-1.7]***	-0.3 [-1.8- 1.1]
• Lack of perseverance	24.9 [3.9]	**23.4 [3.3]*****	25.2 [4.0]	24.4 [4.1]	**1.2 [0.7-1.7]*****	**-1.01 [-1.9- 0.1]***
• Sensation Seeking	28.5 [8.3]	**30.2 [8.7]*****	27.4 [7.6]	**28.4 [8.5]***	**-1.4 [-2.5- -0.8]*****	0.7 [-1.01- 2.4]
YBOCS	6.9 [9.3]	**4.6 [7.6]****	5.0 [6.9]	4.7 [7.4]	**1.5 [0.3-2.6]***	-1.2 [-3.2- 0.79]

	Synbiotic n=73		Placebo n=61		Effect for time	Effect for treatment type
FAST	26.5 [12.1]	**20.7 [12.9]*****	27.2 [12.9]	24.8 [12.9]	**3.6 [1.9-5.4]*****	**-3.7 [-7.0- -0.3]***
• Autonomy	2.9 [2.6]	**2.0 [2.3]*****	3.1 [2.7]	2.7 [2.7]	**0.7 [0.3-1.1]*****	-0.5 [-1.2-0.2]
• Occupational functioning	5.5 [4.1]	**4.3 [4.2]***	5.9 [4.1]	6.0 [4.5]	0.5 [-0.2-1.2]	-1.3 [-2.7-0.01][a]
• Cognitive functioning	6.9 [3.4]	**5.9 [3.2]*****	7.5 [3.2]	**6.8 [3.4]***	**0.8 [0.4-1.1]*****	-0.4 [-1.1-0.3]
• Financial issues	1.9 [1.9]	**1.5 [1.8]*****	1.9 [1.7]	1.7 [2.0]	**0.4 [0.1-0.6]****	-0.3 [-0.8- 0.1]
• Interpersonal relationships	6.5 [4.2]	**5.2 [4.1]****	6.6 [4.7]	5.6 [4.3]	**1.1 [0.5-1.7]*****	-0.4 [-1.5- 0.7]
• Leisure time	2.4 [1.8]	2.0 [1.7]	2.0 [1.7]	2.3 [1.9]	0.0 [-0.3-0.3]	-0.5 [-0.99-0.05][a]
DERS-16	51.7 [13.6]	**44.3 [13.9]*****	50.6 [13.5]	**46.4 [14.9]***	**5.9 [3.7-8.1]*****	-3.4 [-7.8- 0.7]
• Impulse control	8.9 [2.9]	**7.8 [3.2]*****	9.3 [3.4]	**8.2 [3.6]****	**1.2 [0.7-1.7]*****	-0.2 [-1.2- 0.8]
• Non-acceptance	9.4 [3.5]	**8.0 [3.3]****	9.5 [3.1]	**8.8 [3.8]***	**1.1 [0.5-1.6]*****	-0.6 [-1.7- 0.4]
• Goal-directed behavior	11.6 [2.5]	**10.1 [3.0]*****	10.9 [3.1]	10.4 [3.4]	**1.1 [0.6-1.6]*****	-0.8 [-1.8- 0.1][a]
• Emotional strategies	16.1 [5.5]	**13.6 [5.3]*****	15.7 [5.4]	14.7 [5.3]	**1.9 [1.1-2.7]*****	-1.3 [-2.8- 0.2][a]
• Lack of emotional clarity	5.6 [2.5]	**4.7 [1.9]*****	5.1 [2.3]	4.8 [2.1]	**0.7 [0.3-1.1]*****	-0.2 [-0.9- 0.4]
PSS-10	23.2 [3.6]	**20.7 [3.6]*****	22.5 [4.3]	22.1 [5.0]	**1.4 [0.5-2.4]****	**-1.6 [-3.1- -0.05]***

t0=baseline, t2=10 weeks. The overall time effect was assessed using (two-sided) paired *t*-tests; the effect of treatment type was tested using analysis of covariance (ANCOVA), with outcome variable being the follow-up score, and adjusting for center, age, sex, BMI, medication, and primary psychiatric diagnosis. Significant values are highlighted in bold and marked with an asterisk.

Abbreviations: MD=mean difference.

Table 3. Effect of the intervention on stool type

Stool type	Synbiotic n=73			Placebo n=61		
	t0	t2		t0	t2	
	n (%)	n (%)	*p*-value	n (%)	N (%)	*p*-value
Type 1: separate hard lumps, like nuts	7 (10%)	1 (1%)	**0.002**	5 (8%)	3 (5%)	NS
Type 2: lumpy and sausage like	9 (12%)	9 (12%)	NS	4 (6%)	6 (10%)	NS
Type 3: a sausage shape with cracks in the surface	19 (26%)	22 (30%)	NS	19 (31%)	19 (31%)	NS
Type 4: like a smooth, soft sausage or snake	25 (34%)	29 (40%)	NS	21 (34%)	19 (31%)	NS
Type 5: soft blobs with clear-cut edges	8 (11%)	5 (7%)	NS	8 (13%)	10 (16%)	NS
Type 6: mushy consistency with ragged edges	5 (7%)	6 (8%)	NS	5 (7%)	2 (3%)	NS
Type 7: liquid consistency with no solid pieces	0 (0%)	0 (0%)	NS	0 (0%)	1 (2%)	NS

Table 4. Frequency of side effects in study participants

Side-effect	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
	n (%)	n (%)	*p*-value
Gastrointestinal	18 (25%)	17 (28%)	0.7
Musculoskeletal	10 (14%)	10 (16%)	0.7
Dermatological	7 (10%)	10 (16%)	0.2
Immunological	2 (3%)	1 (2%)	0.7
Hematological	0 (0%)	1 (2%)	0.3
Headache/Migraine	10 (14%)	6 (10%)	0.5
Psychiatric	7 (10%)	3 (5%)	0.3
Other	9 (12%)	10 (16%)	0.5

Supplementary Table 1. Treatment effects on symptoms, functioning, and perceived stress in patients (ITT sample)
To be completed

Supplementary Table 2. Baseline clinical characteristics of patients

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
Questionnaire:	Mean [SD]	Mean [SD]	Mean [SD]	*p*-value
ADHD-RS	30 [10]	29 [10]	30 [9]	0.5
Inattention	15 [5]	15 [5]	15 [6]	0.6
Hyperactivity	8 [4]	8 [4]	8 [4]	0.9
Impulsivity	7 [3]	6 [3]	7 [3]	0.3
UPPS-P	138 [15]	140 [14]	136 [15]	0.098
Negative Urgency	25 [6]	25 [6]	24 [5]	0.4
Lack of Premeditation	26 [6]	26 [6]	27 [6]	0.3
Lack of Perseverance	25 [4]	25 [4]	25 [4]	0.8
Sensation Seeking	28 [8]	28 [8]	27 [8]	0.4
Positive Urgency	34 [9]	35 [9]	32 [9]	0.07
FAST	27 [12]	26 [12]	27 [13]	0.8
Autonomy	3 [3]	3 [3]	3 [3]	0.6
Occupational	6 [4]	5 [4]	6 [4]	0.5
Cognitive	7 [3]	7 [3]	7 [3]	0.3
Financial	2 [2]	2 [2]	2 [2]	0.9
Interpersonal	6 [5]	6 [4]	7 [5]	0.9
Leisure	2 [2]	2 [2]	2 [2]	0.2
SDQ	19 [5]	19 [5]	19 [5]	0.9

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
Hyperactivity/Inattention	6.6 [2.1]			
Emotional	6 [3]	6 [2]	6 [3]	0.2
Conduct	3 [1]	3 [1]	3 [1]	0.98
Relation	3 [2]	3 [2]	3 [2]	0.3
Social	8 [2]	7.97 [1.99]	8.6 [1.6]	**0.04**
YBOCS	6 [8]	7 [9]	5 [7]	0.2
PSS	23 [4]	23 [4]	22 [4]	0.3
DERS-16	51 [13]	52 [13]	51 [13]	0.7
Clarity	5 [2]	6 [2]	5 [2]	0.2
Goals	11 [3]	12 [2]	11 [3]	0.2
Impulse	9 [3]	9 [3]	9 [3]	0.5
Strategies	16 [5]	16 [5]	16 [5]	0.7
Non-acceptance	9 [3]	9 [3]	9 [3]	0.8

Abbreviations: BMI: body mass index.

Mean and standard deviation (SD) values are given, analyses were based on Kruskal-Wallis Test. Significant *p*-values are highlighted in bold.

Supplementary Table 3. Baseline nutritional intake and physical activity of patients

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
Nutritional intake	Mean [SD]	Mean [SD]	Mean [SD]	*p*-value
Total energy intake [kcal/day]	1972.7 [675.6]	1993.3 [737.9]	1946.6 [593.3]	0.7
Carbohydrates [g/day]	218.8 [72.0]	221.1 [81.5]	215.96 [58.7]	0.7
Proteins [g/day]	81.2 [38.2]	85.1 [44.8]	76.4 [27.5]	0.2
Fat [g/day]	81.8 [35.2]	80.8 [35.8]	83.0 [34.7]	0.7
MUFA [g/day]	24.4 [15.2]	23.5 [14.2]	25.5 [16.4]	0.5
PUFA [g/day]	14.1 [9.7]	14.4 [9.4]	13.7 [10.1]	0.7
SFA [g/day]	29.3 [16.2]	28.9 [16.4]	29.8 [16.2]	0.8
ALA [g/day]	0.7 [0.6]	0.7 [0,5]	0.7 [0.6]	0.98
EPA [g/day]	0.1 [0.1]	0.05 [0.1]	0.1 [0.2]	0.4
DHA [g/day]	1.1 [0.2]	0.1 [0.2]	0.1 [0.3]	0.8
Omega-3 [g/day]	1.1 [0.9]	1.1 [0.98]	1.1 [0.8]	0.8
Omega-6 [g/day]	10.2 [7.3]	10.4 [7.4]	9.9 [7.2]	0.7
Fiber [g/day]	19.2 [10.2]	20.2 [10.5]	17.9 [9.6]	0.2
Cholesterol [mg/day]	262.9 [164.5]	272.2 [185.4]	251.2 [134.2]	0.5
Mg [mg/day]	278.1 [153.97]	288.3 [165.7]	265.4 [138.6]	0.4
Zinc [mg/day]	8.6 [5.6]	8.8 [6.2]	8.3 [4.8]	0.6
Iron [mg/day]	11.5 [6.5]	12.3 [7.8]	10.6 [4.2]	0.2
Copper [ug/day]	970.6 [1057.1]	911.5 [932.8]	1045.6 [1201.9]	0.5
Na [mg/day]	3223.6 [1782.7]	3338.3 [2052.6]	3080.2 [1379.8]	0.4
K [mg/day]	2302.4 [1057.0]	2284.98 [1123.6]	2324.5 [976.5]	0.8
Vitamin D [mg/day]	2.5 [2.0]	2.5 [2.1]	2.5 [1.8]	0.9
Vitamin E [mg/day]	11.8 [9.7]	11.4 [9.2]	12.4 [10.3]	0.6

	Analyzed sample n=134	Synbiotic n=73	Placebo n=61	Synbiotic vs. Placebo:
Vitamin K [mg/day]	72.5 [57.7]	74.4 [59.6]	70.3 [55.7]	0.7
Vitamin B6 [mg/day]	1.6 [1.3]	1.7 [1.5]	1.4 [1.0]	0.2
Vitamin B12 [mg/day]	2.9 [2.4]	3.1 [2.9]	2.8 [1.5]	0.5
Vitamin A [mg/day]	597.4 [636.5]	596.4 [663.5]	598.7 [607.0]	0.98
Folic acid [mg/day]	168.1 [142.7]	178.2 [155.3]	155.3 [125.3]	0.4
Tryptophan [mg/day]	563.3 [539.9]	576.1 [575.0]	547.4 [497.6]	0.8
OH [g/day]	2.7 [5.9]	2.7 [5.9]	2.7 [5.9]	0.998
Physical activity	Mean [SD]	Mean [SD]	Mean [SD]	*p*-value
MET-min/week	4630.8 [8605.9]	4176.9[5994.9]	5173.9 [10976.8]	0.5

Abbreviations: MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Mg: magnesium; Na: sodium; K: potassium; OH: alcohol, MET-min/week: metabolic equivalent of task-min/week.

Mean and standard deviation (SD) values are given, analyses were based on Kruskal-Wallis Test. Significant *p*-values are highlighted in bold.

Supplementary Table 4.

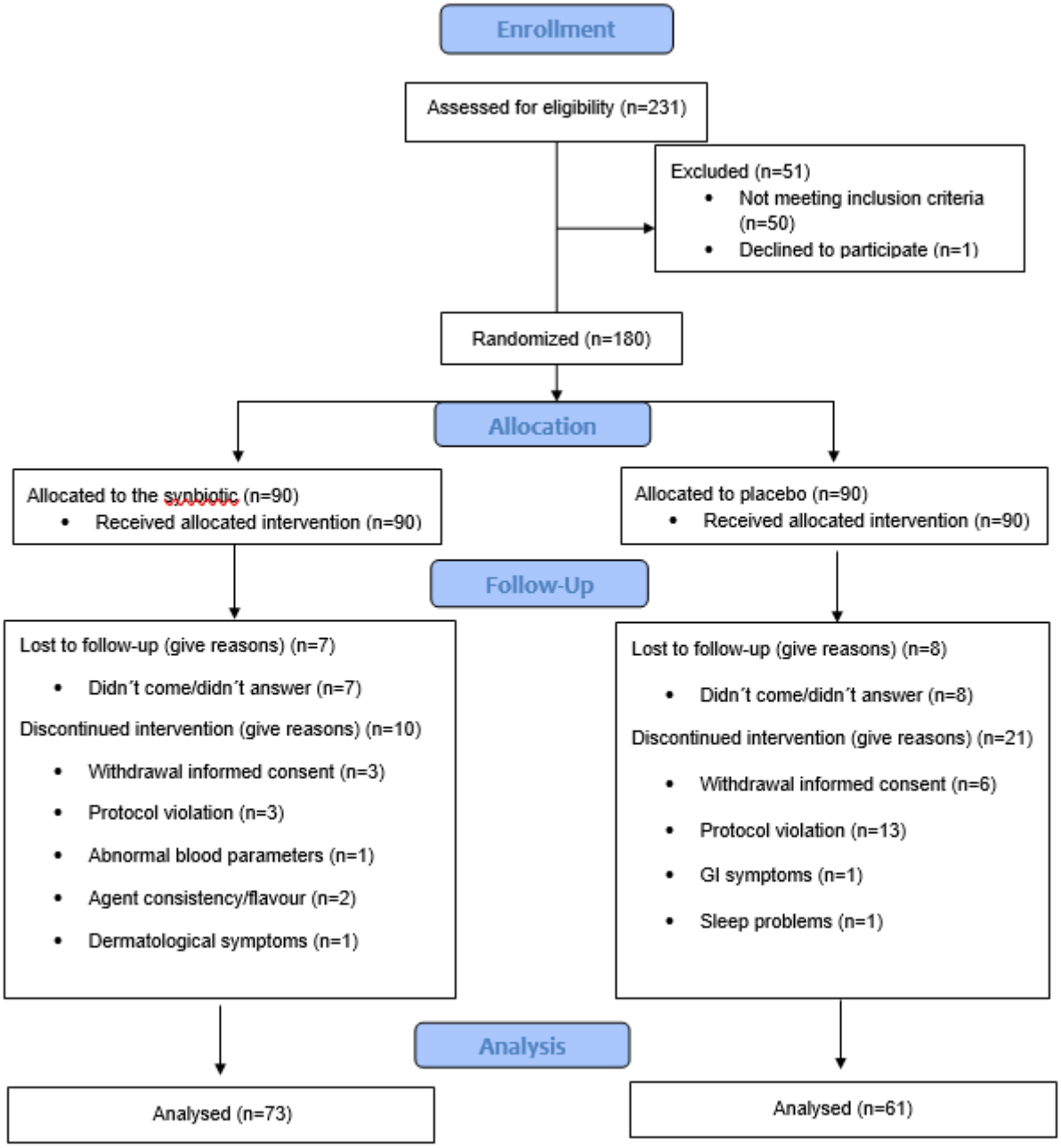
	t0 n=134	t2 n=73	Synbiotic vs. Placebo:
	Mean [SD]	Mean [SD]	*p*-value
BMI	26 [6]	26 [6]	0.2
Nutritional intake	Mean [SD]	Mean [SD]	*p*-value
Total energy intake [kcal/day]	1972.7 [675.6]	1782.5 [605.0]	**0.02**
Carbohydrates [g/day]	218.8 [72.0]	198.0 [78.7]	**0.02**
Proteins [g/day]	81.2 [38.2]	71.1 [26.7]	**0.03**
Fat [g/day]	81.8 [35.2]	74.8 [29.5]	0.2
MUFA [g/day]	24.4 [15.2]	22.5 [12.8]	0.7
PUFA [g/day]	14.1 [9.7]	13.2 [8.9]	0.6
SFA [g/day]	29.3 [16.2]	26.2 [13.7]	0.09
ALA [g/day]	0.7 [0.6]	0.7 [0.5]	0.7
EPA [g/day]	0.1 [0.1]	0.1 [0.2]	0.2
DHA [g/day]	1.1 [0.2]	0.1 [0.2]	0.4
Omega-3 [g/day]	1.1 [0.9]	1.2 [0.9]	0.9
Omega-6 [g/day]	10.2 [7.3]	10.0 [7.4]	0.7
Fiber [g/day]	19.2 [10.2]	17.1 [8.3]	**0.05**
Cholesterol [mg/day]	262.9 [164.5]	234.5 [135.6]	0.7
Mg [mg/day]	278.1 [153.97]	249.2 [132.4]	0.08
Zinc [mg/day]	8.6 [5.6]	6.9 [3.6]	**0.01**
Iron [mg/day]	11.5 [6.5]	9.8 [4.0]	**0.03**
Copper [ug/day]	970.6 [1057.1]	850.2 [813.4]	0.3
Na [mg/day]	3223.6 [1782.7]	2865.9 [1236.45]	0.3
K [mg/day]	2302.4 [1057.0]	2163.9 [997.5]	0.3
Vitamin D [mg/day]	2.5 [2.0]	2.8 [2.3]	0.4

	t0 n=134	t2 n=73	Synbiotic vs. Placebo:
Vitamin E [mg/day]	11.8 [9.7]	10.9 [9.3]	0.4
Vitamin K [mg/day]	72.5 [57.7]	76.4 [40.4]	0.6
Vitamin B6 [mg/day]	1.6 [1.3]	1.3 [0.8]	**0.01**
Vitamin B12 [mg/day]	2.9 [2.4]	2.3 [1.5]	0.1
Vitamin A [mg/day]	597.4 [636.5]	525.9 [508.8]	0.7
Folic acid [mg/day]	168.1 [142.7]	135.6 [121.2]	**0.03**
Tryptophan [mg/day]	563.3 [539.9]	521.1 [479.97]	0.8
OH [g/day]	2.7 [5.9]	2.5 [5.3]	0.7
Physical activity	Mean [SD]	Mean [SD]	*p*-value
MET-min/week	4630.8 [8605.9]	3461.7 [4556.6]	0.1

Abbreviations: MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; ALA: alpha-linolenic acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; Mg: magnesium; Na: sodium; K: potassium; OH: alcohol, MET-min/week: metabolic equivalent of task-min/week.

Mean and standard deviation (SD) values are given, analyses were based on Kruskal-Wallis Test. Significant *p*-values are highlighted in bold.

Figure 1. CONSORT 2010 Flow Diagram



3. ACKNOWLEDGEMENT AND DISCLAIMER

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 728018.