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Effects of a synbiotic on symptoms, and daily functioning in attention deficit hyperactivity disorder – A double-blind randomized controlled trial

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ABSTRACT

Some prebiotics and probiotics have been proposed to improve psychiatric symptoms in children with autism. However, few studies were placebo-controlled, and there is no study on persons with an attention deficit hyperactivity disorder (ADHD) diagnosis. Our aim was to study effects of Synbiotic 2000 on psychiatric symptoms and functioning in children and adults with ADHD without an autism diagnosis. Children and adults (n = 182) with an ADHD diagnosis completed the nine weeks randomized double-blind parallel placebo-controlled trial examining effects of Synbiotic 2000 on the primary endpoints ADHD symptoms, autism symptoms and daily functioning, and the secondary endpoint emotion regulation, measured using the questionnaires SNAP-IV, ASRS, WFIRS, SCQ, AQ and DERS-16. Levels at baseline of plasma C-reactive protein and soluble vascular cell adhesion molecule-1 (sVCAM-1), central to leukocyte-endothelial cell adhesion facilitating inflammatory responses in tissues, were measured using Meso Scale Discovery. Synbiotic 2000 and placebo improved ADHD symptoms equally well, and neither active treatment nor placebo had any statistically significant effect on functioning or sub-diagnostic autism symptoms. However, Synbiotic 2000, specifically, reduced sub-diagnostic autism symptoms in the domain restricted, repetitive and stereotyped behaviors in children, and improved emotion regulation in the domain of goal-directed behavior in adults. In children with elevated sVCAM-1 levels at baseline as well as in children without ADHD medication, Synbiotic 2000 reduced both the total score of autism symptoms, and the restricted, repetitive and stereotyped behaviors. In adults with elevated sVCAM-1 at baseline, Synbiotic 2000 significantly improved emotion regulation, both the total score and four of the five subdomains. To conclude, while no definite Synbiotic 2000-specific effect was detected, the analysis of those with elevated plasma sVCAM-1 levels proposed a reduction of autism symptoms in children and an improvement of emotion regulation in adults with ADHD.

Trial registration number: ISRCTN57795429.

1. Introduction

Attention deficit hyperactivity disorder (ADHD) is characterized by persistent features of inattention and/or hyperactivity-impulsivity leading to functioning impairments in areas such as work/school, social relations and family settings (Austerman, 2015), and is often associated with emotion dysregulation. ADHD has high comorbidity with other neurodevelopmental disorders, among them autism (Jensen and Steinhausen, 2015). Most patients with ADHD are treated with pharmacological agents for long periods, a treatment directed only against symptoms and frequently associated with negative side effects (Storebø, 2015; Castells et al., 2018). Nutritional deficiencies of fatty acids, zinc and iron have been observed in children with ADHD, and correlations with ADHD symptom severity were reported (Curtis and Patel, 2008). Dietary supplementation has been proposed as a possible option to ameliorate some ADHD symptoms (Heilskov Rytter, 2015). Omega-3 fatty acids had a stable, but small, positive effect on ADHD symptoms (Chang, 2018).

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Gastro-intestinal (GI) symptoms are overrepresented in patients with ADHD and autism, who often show increased psychiatric symptoms along with increased GI symptoms (McKeown, 2013; Kang et al., 2014). The GI tract has a bidirectional communication with the central nervous system, the gut-brain axis, via e.g. the vagus nerve, hormones, immune activity mediators and gut bacterial metabolites transferred through the blood. Also, increased immune activation is overrepresented in autism, located peripherally but for a subgroup also in the cerebrospinal fluid, and is proposed to have a neuropathological role (Osokine and Erlebacher, 2017; Meltzer and Van de Water, 2017). There is some, but less, evidence of involvement of immune activation in ADHD (Mitchell and Goldstein, 2014). C-reactive protein (CRP) is an acute phase protein produced in the liver during infection and inflammation. Vascular cell adhesion molecule-1 (VCAM-1) is expressed predominantly by endothelial cells and is central to leukocyte–integrin-endothelial cell adhesion and leukocyte extravasation into the surrounding tissue facilitating inflammatory responses (Kong, 2018; Cook-Mills, 2002). sVCAM-1 is the soluble isoform and an indirect plasma measure of the transmembrane VCAM-1 expression. Elevated sVCAM-1 levels are commonly seen in inflammatory disorders such as inflammatory bowel disorder (IBD) and metabolic disorder. Drugs targeting VCAM-1-binding integrins have been approved for treatment of IBD (Park and Jeen, 2018). Growing evidence show that the gut microbiota moderates the gut-brain axis pathways, affecting systemic low-grade inflammation, permeability of the blood–brain-barrier, maturation and activation of microglia cells, synaptogenesis and motor control (Fung et al., 2017). In autism patients, there is some evidence for symptom improvements by fecal transplantation (Kang, 2017), and also in ADHD an altered microbiota has been reported (Aarts, 2017; Prehn-Kristensen, 2018). Likewise, feces from patients with autism, depression or schizophrenia transferred to the rodent intestine produced disorder-related behaviors and biochemical modulations (Kelly, 2016; Zheng, 2019; Sharon, 2019).

A probiotic is a live organism that, when ingested in adequate amounts, exerts a health benefit (Dinan and Quigley, 2011). Prebiotics were originally defined as dietary ingredients selectively inducing the growth or activity of GI bacteria improving the microbial balance in the colon with beneficial effects on the host (Gibson, 2010). A synbiotic is a mixture of both prebiotics and probiotics. Several studies have shown that some pre- and probiotics have positive effects on the immune system and vitamin synthesis and modulate brain activities (Frei et al., 2015; LeBlanc, 2017). Two randomized controlled trials (RCTs) of probiotics in metabolic syndrome reported a decrease in plasma sVCAM-1 levels (Tenorio-Jiménez, 2020; Rezazadeh, 2019; Tripolt, 2013). Several open-label prebiotic and/or probiotic dietary interventions in children with autism have indicated reductions in GI and psychiatric symptoms, but there are only few randomized placebo-controlled trials and the results should therefore be interpreted with caution (Ng, 2019). Of randomized placebo-controlled trials, there were suggestive positive effects of 4 weeks with *Lactobacillus plantarum* PS128 on opposition/defiance behavior (n_{total} = 71, (Liu, 2019), 6 weeks exclusion diet in combination with Bimuno galactooligosaccharide (B-GOS) on anti-social behavior (n_{total} = 26, (Grimaldi, 2018), and no effect of 3 weeks with *L. plantarum* WCSF1 on autism symptoms (n_{total} = 39, (Parracho, 2010). Notably, oral administration of *L. rhamnosus* GG the first 6 postnatal months reduced the prevalence of ADHD and Asperger syndrome at the age of 13 years (Partty, 2015). However, no protective effect on offspring neurocognitive outcomes up to 11 years of age was found of *L. rhamnosus* HN001 and *Bifidobacterium animalis* subsp. *lactis* HN019 treatment prenatally or during the first two postnatal years (n_{total} = 342, (Slykerman, 2018). It is elusive which strains, prebiotic compounds and dosages have the strongest candidacy for a putative effect. And, there is no result yet reported from intervention with prebiotics and/or probiotics in ADHD.

The aim of this study was to determine if Synbiotic 2000 composed of three anti-inflammatory lactic acid bacteria and four anti-inflammatory fibers (Bengmark, 2017) has an effect on ADHD, comorbid autistic symptoms and daily functioning in patients with an ADHD diagnosis using a placebo-controlled randomized trial.

2. Method

2.1. Study design

We conducted a multicenter double-blind parallel group RCT to assess the effectiveness of Synbiotic 2000, a mixture of probiotics and fibers, in patients with ADHD. The primary outcomes were ADHD symptoms, autism symptoms and daily functioning measured with questionnaires. Each intervention lasted for nine weeks with assessments at baseline (the day before treatment start) and post-treatment (within 2 weeks after last treatment intake). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. All procedures involving human subjects/patients were approved by the Stockholm Ethics Review Board (2015/884–31, 2017/771-32). Written informed consent was obtained from all participants. The trial registration number is ISRCTN57795429.

2.2. Patient recruitment

Participants were recruited between January 2016 and June 2018 via predefined psychiatric out-patient clinics in Stockholm County Sweden, and through advertisement in a local newspaper. Eligibility criteria for participation were: a confirmed ADHD-diagnosis (International statistical Classification of Disease 10th revision (ICD-10) or DSM-V), age 5–55 years, stable pharmacological treatment (no change during the last four weeks) and an ability to read Swedish (due to the questionnaires being in Swedish) (Wolraich, 2011; Pettersson, 2017). Child and adolescent participants are referred to as child/children in the following text. Exclusion criteria were: autism diagnosis, GI-disorder diagnosis other than irritable bowel syndrome (IBS), antibiotic treatment during the last six weeks, diabetes and celiac disease.

Patients were informed about the study by a primary care provider and thereafter referred to a research nurse who conducted a more detailed eligibility interview controlling for inclusion and exclusion criteria. The three research nurses, responsible for patient recruitments, baseline and follow-up assessments and treatment allocation, were experienced psychiatric care nurses and had received necessary training to conduct the steps according to protocol and good clinical praxis. Patients who provided informed consent were allocated to one of the two parallel treatments: Synbiotic 2000 or placebo, allocation ratio 1:1, via computerized randomization by the independent Karolinska Trial Alliance, not involved in the study. Allocation sequence, block size and individual allocations were unknown to the participants, research nurses, researchers, outcome assessors and data analysts until the all analyses were completed.

In total, 248 participants met the inclusion criteria participated in the treatment (Fig. 1). The drop-out rate between baseline and the follow-up was 66/248. The baseline clinical characteristics were similar between drop-outs and completers (Supplemental Table S1).

2.3. Participant characteristics

Baseline characteristics in the completers (n = 182) are shown in Table 1. A majority of the children (up to 18 years of age) were male (73.5%), whereas most of the adult participants were female (71.1%). A majority of the participants used pharmacological ADHD treatment (children: 58.8%; adults: 70.2%). The median age among children was 12 years, and among adults 36 years. The most common ADHD diagnosis was F90.0B (children: 60.3%; adults: 56.1%).

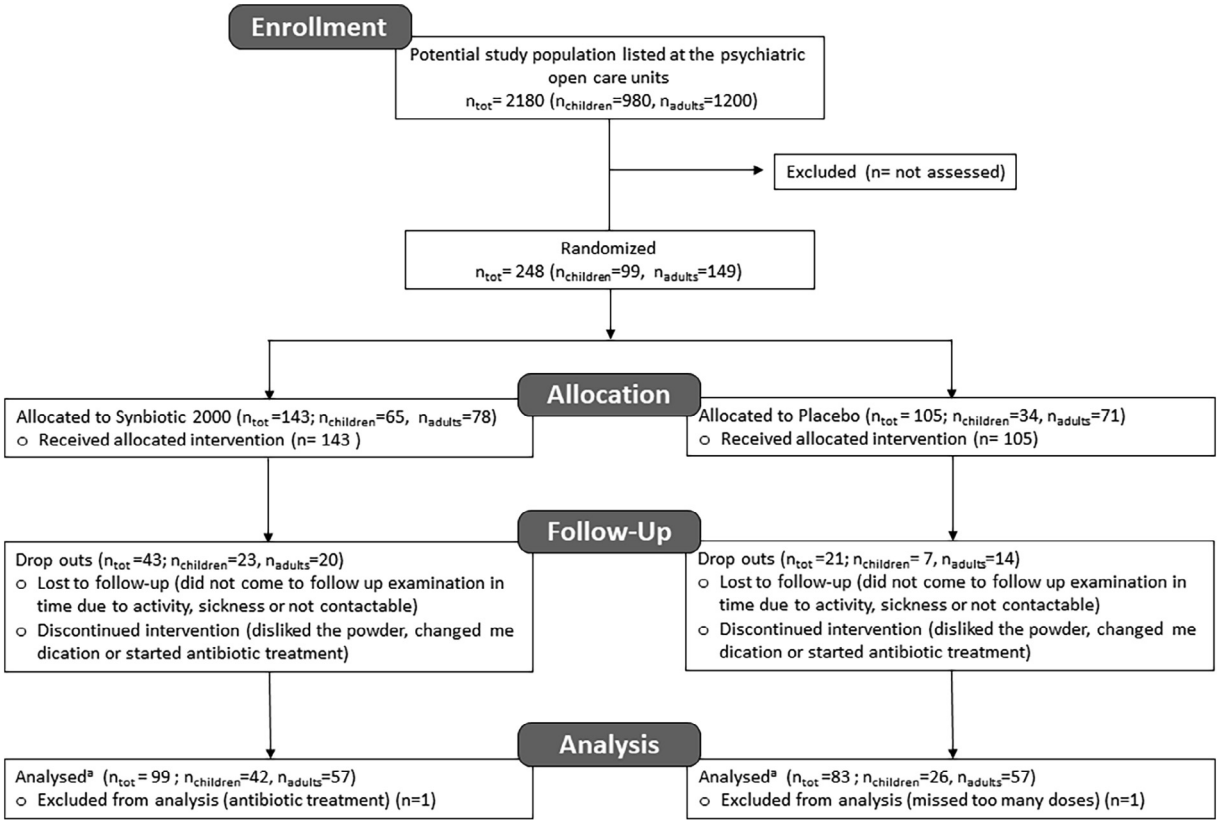

Fig 1. Flow chart of participants' progress. The exact number assessed for eligibility is unknown. [a]Criteria for 'per-protocol' analysis: Follow-up assessment within 14 days after the last dose (the median was 3 days, and 25th-75th percentiles were 0–7.25 days), and without treatment less than 21 days and less than 5 days in a row.

2.4. Measures

2.4.1. Interview at baseline

The participants were interviewed regarding delivery route (vaginal, cesarean section (CS), acute CS, don't know), breastfeeding (not at all, less than 3 months, 3–6 months, > 6 months, don't know), body mass index (BMI) and pharmacological treatment (Table 1) where melatonin, and some antipsychotic and antidepressant medication have been reported to influence the gut microbiota (Flowers, 2017; Zhu, 2018; Serrano-Contreras, 2016).

2.4.2. Questionnaires at baseline and post-treatment

Self-reported psychiatric scales reflecting the last seven days and a diet questionnaire reflecting the last four weeks were administered at baseline and post-treatment (Swanson, 2001; Kessler, 2005; Alliance., C.A.R. CADDRA ADHD ASSESSMENT TOOLKIT (CAAT) FORMS., 2011; Rutter et al., 2003; Baron-Cohen, 2001; Woodbury-Smith, 2005; Bjureberg, 2016; Kautto, 2014). The questionnaires and their scoring are described in Table 2. For children up to 12 years of age, the questionnaires were filled in by, or with support from, parents.

For measuring ADHD symptoms the following validated questionnaires were used: for children Swanson, Nolan and Pelham-IV scale (SNAP-IV) – parent rating scale 18 item, and for adults Adult ADHD Self-Report Scale (ASRS) – self-reported (ASRS 1.1). In each scale, nine items assess inattention and nine assess hyperactivity/impulsivity, higher scores indicating more ADHD symptoms. In SNAP-IV, the scores are calculated as mean score per item and the 95th percentiles in the population is for inattention 1.78, and for hyperactivity/impulsivity 1.44 (Swanson, 2001). In ASRS the maximum sum-score is 72. For each subscale, a sum-score > 24, of maximum 36, is interpreted as very probable ADHD diagnosis, while > 17 is interpreted as likely probable ADHD diagnosis, corresponding to mean scores of 1.33 and 0.94 per item, respectively (Kessler, 2005).

The Weiss Functional Impairment Rating Scale (WFIRS) is a validated instrument to measure functional impairment in individuals with ADHD. For children, parents rated functioning using WFIRS-parent-reported for child (PC), whereas for adults WFIRS-self-reported (SA) was used. Both scales include six subscales: family, work/school, lifeskills, self-concept, social activities and risky activities. Higher scores indicates more impaired functioning. A mean score > 1.5 is considered clinically impaired (Alliance, 2011).

Autistic symptoms were assessed using the Social Communication Questionnaire (SCQ, current) for children and the Autism Spectrum Quotient (AQ) for adults, higher scores indicating more autistic symptoms. In SCQ, the items are divided into three subscales: communication, reciprocal social interactions and restricted, repetitive behaviors and interests. A sum-score > 15 indicates strong probability of autism corresponding to 0.38 per scale question (Rutter et al., 2003). In AQ the items are divided into the five subscales social skills, attention switching, attention to detail, communication and imagination. An AQ sum score > 32 indicates strong probability of autism (Baron-Cohen, 2001), while an AQ sum score of > 25 was proposed for suspected Asperger syndrome or high functioning autism (Woodbury-Smith, 2005). In clinical practice a sum score > 19 is interpreted as possible autism. Sum scores > 32, > 25 and > 19 corresponds to > 0.64, > 0.50 and > 0.38 per scale question.

Difficulties in Emotion Regulation Scale-16 (DERS-16) is a validated and brief self-reported scale for the assessment of overall emotion regulation difficulties, i.e. functioning when being emotionally upset, in adults (Bjureberg, 2016). DERS-16 includes the five subscales clarity (lack of emotional clarity), goals (difficulties engaging in goal-directed behavior), impulse (impulse control difficulties), strategies (limited access to effective emotion regulation strategies) and nonacceptance (nonacceptance of emotional responses), higher scores indicating more

Table 1
Baseline clinical characteristics of the study participants with ADHD.

		Children (n = 68) Median (IQR)/N (%)	Adults (n = 114) Median (IQR)/ N (%)
Age [years]		12 (10–14)	36 (29–42)
Sex	Female	18 (26.5%)	81 (71.1%)
	Male	50 (73.5%)	33 (28.9%)
Body Mass Index [kg/m^2]		NA	23.9 (22.3–26.9)
Delivery route*	Acute C-section	1 (1.5%)	2 (1.8%)
	C-section	9 (13.4%)	12 (10.5%)
	Vaginal	55 (82.1%)	100 (87.7%)
	Unknown	2 (3.0%)	0 (0.0%)
Breast fed*	less than3 months	8 (11.9%)	18 (15.9%)
	3–6 months	6 (9.0%)	23 (20.4%)
	> 6 months	50 (74.6%)	56 (49.6%)
	None	0 (0.0%)	3 (2.7%)
	Unknown	3 (4.5%)	13 (11.5%)
ICD-10	F90.0	15 (22.1%)	20 (17.5%)
	F90.0B	41 (60.3%)	64 (56.1%)
	F90.0C	9 (13.2%)	23 (20.2%)
	F90.0X	0 (0.0%)	4 (3.5%)
	F90.1	1 (1.5%)	0 (0.0%)
	F90.8	0 (0.0%)	1 (0.9%)
	F90.9	1 (1.5%)	0 (0.0%)
	F98.8	1 (1.5%)	2 (1.8%)
Autism traits[a]		6.0 (3.0–9.0)	18.0 (13.0–23.0)
Treatment	Placebo	26 (38.2%)	57 (50.0%)
	Synbiotic	42 (61.8%)	57 (50.0%)
Antibiotic drugs[b]	> 3 times	1 (1.5%)	4 (3.5%)
	1–3 times	17 (25.0%)	30 (26.3%)
	None	49 (72.1%)	75 (65.8%)
	Unknown	1 (1.5%)	5 (4.4%)
ADHD medication[c]	Methylphenidate	21 (30.9%)	39 (34.2%)
	Lisdexamfetamine	12 (17.6%)	36 (31.6%)
	Atomoxetine	7 (10.3%)	4 (3.5%)
	Dexamfetamine†		13 (11.4%)
	Guanfacine†		0 (0.0%)
	Any of above	40 (58.8%)	80 (70.2%)
Other prescribed drugs[c]	Melatonin	23 (33.8%)	27 (23.7%)
	Antidepressant†		40 (35.1%)
	Antipsychotic†		3 (2.6%)
	Anxiolytic†		12 (10.5%)
	Sleeping pill†		25 (21.9%)
	Laxative†		2 (1.8%)
	Protonpump inhibitor†		5 (4.4%)
	Statin†		1 (0.9%)
	Other prescribed drug	1 (1.5%)	21 (18.4%)
Dietary supplements[d]		28 (41.2%)	82 (71.9%)
Plasma CRP level[e]	> 2 mg/L	7 (14.3%)	25 (24.5%)
	≤2 mg/L	42 (85.7%)	77 (75.5%)

Results are given as median (25th–75th percentile [IQR]) or as number (%) of subjects.
a. autism symptoms measured as mean per scale question, using the Social Communication Questionnaire (SCQ) for children and the Autism Spectrum Quotient (ASQ) for adults.
b. proportion of participants on categories of antibiotic drug use in the last two years.
c. proportion of participants on medication in the last 3 months.
d. supplements (e.g. vitamins, omega-3, probiotics) taken in the last 4 weeks. The probiotics used were *L. plantarum* 299v (8% of participants), Synbiotic 15 (similar constituents as Synbiotic 2000 but 15*10^9 CFU instead of the 4*10^{11} CFU in Synbiotic 2000, 3 adults) and other (6 adults).
e. 19 children and 9 adults did not provide blood sample.
* The participant's birth/infancy.
† Medication prescribed for only adults.

difficulties.

A food-frequency questionnaire, covering 4 weeks retrospectively, was used to obtain information about the participant's food intake. The aim was to identify putative major alterations in nutrient intake between baseline and follow-up to adjust for confounding by diet. The food questionnaire used in this study was based on the ETICS diet study questionnaire and consisted of 57 items representing common food units or common food groups (Kautto, 2014). The options range from "2 times or more per day" to "never in the last four weeks". The frequency intake of the food items were converted into nutrient intake per participant based on the Swedish national food agency's nutrition content database's portion sizes and nutrient compositions adjusted for age and sex (Livsmedelsverket, 2015).

2.4.3. Plasma CRP and sVCAM-1 levels at baseline

Blood was collected in EDTA tubes at baseline and stored at –80°C for one-two years with two freeze-thaw cycles. CRP and sVCAM-1 levels were measured in the plasma using sandwich immunoassay with Meso Scale Discovery (MSD) V-PLEX Vascular Injury Panel 2 Human Kit (Cat. #K15198D, Meso Scale Diagnostics, Maryland, USA). Experiments were conducted according to the manufacturer's instructions applying standard curves and two internal controls in duplicate on each of the five 96-well plates. All standard curves had a robust correlation ($R^2 > 0.999$). The inter-plate coefficient of variation (CV) from inter-plate controls was for CRP 4.6% and for sVCAM-1 6.6%, and the intra-plate CV from calibrators for the analytes was for CRP 2.4%, and for sVCAM-1 1.6%. Baseline and follow-up samples from each patient were run within the same plate. The calculated lower limit of detection was 2.39–3.35 μg/L for CRP and 5.43–8.43 μg/L for sVCAM-1 with the samples diluted 1000x. None of the participants had level indicating acute infection (CRP level ≥15 mg/L, (Yousuf, 2013).

2.4.4. Primary and secondary outcomes

The primary outcomes were change in ADHD symptoms (assessed using SNAP-IV (Swanson, 2001) for children and ASRS (Kessler, 2005) for adults), autism symptoms (children: SCQ (Rutter et al., 2003), adults: AQ (Baron-Cohen, 2001)) and functioning (children: WFIRS-PC, adults: WFIRS-SA (Alliance, 2011)). Secondary outcome analysed so far was change in emotion regulation assessed in adults with DERS-16 (Bjureberg, 2016).

2.5. Intervention

The treatment was administered orally once daily for nine weeks. Active treatment and placebo were designed to be similar regarding packaging (sachet), volume, weight, content color, texture, flavour and were without smell. All the participants received the same information, were asked not to change their diet during the study, and were provided a questionnaire to record any missed treatment day. The participants were instructed to mix the powder in drinks or food (not above 40 °C). They were provided with sachets for 2–3 weeks at a time, and returned the empty sachets and any unused sachets when collecting new ones. The record of missed treatment was returned to the nurse post-treatment.

2.5.1. Active treatment

The active treatment, Synbiotic 2000 (Synbiotics AB, Sweden), was a lyophilized composition of 4 × 10^{11} CFU per dose of three lactic acid bacteria *Pediococcus pentosaceus* 5–33:3/16:1 (Strain deposit number: LMG P20608), *Lactobacillus casei ssp paracasei* F19 (LMG P-17806), *Lactobacillus plantarum* 2362 (LMG P-20606), and 2.5 g of each of the fermentable fibers betaglucan, inulin, pectin and resistant starch. The composition has a documented anti-infectious and anti-inflammatory effect (Bengmark, 2004; Rayes, 2007; Rayes, 2002; Olah, 2007; Plaudis, 2012; Kotzampassi, 2006; Giamarellos-Bourboulis, 2009; Koutelidakis, 2010; Vidot, 2019), and was reported to prevent leaky gut (Spindler-Vesel, 2007). The sachets were stored at −20 °C, and the viability of the bacteria throughout the study was ensured by culturing.

Table 2
Psychiatric scales.

Scale	Target	Main area	Items, n	Min-max per item	Scoring	Subscales, n	Reference
SNAP-IV	Child	ADHD symptoms	18	0–3	Mean†	2	(Swanson, 2001)
ASRS	Adult	ADHD symptoms	18	0–4	Mean†	2	(Kessler, 2005)
WFIRS-PC	Child	Functioning	60	0–3	Mean†	7	(Alliance., C.A.R. CADDRA ADHD ASSESSMENT TOOLKIT (CAAT) FORMS., 2011)
WFIRS-SA	Adult	Functioning	69	0–3	Mean†	7	(Alliance., C.A.R. CADDRA ADHD ASSESSMENT TOOLKIT (CAAT) FORMS., 2011)
SCQ	Child	Autism symptoms	40	0–1	Mean†	3	(Rutter et al., 2003)
AQ	Adult	Autism symptoms	50	0–3	Mean†	5	(Baron-Cohen, 2001)
DERS-16	Adult	Emotion reactivity	16	0–4	Sum-score	5	(Bjureberg, 2016)
Food questionnaire	All	Diet	25	NA	Sum-score	0	(Kautto, 2014)

SNAP-IV parent report, Swanson, Nolan and Pelham scale; ASRS, Adult ADHD Self-Report Scale; WFIRS-PC/SA, The Weiss Functional Impairment Rating Scale (PC, parent-reported for children; SA, self-reported for adults); SCQ, Social Communication Questionnaire; AQ, Autism Spectrum Quotient; DERS-16, Difficulties in Emotion Regulation Scale; Sum score: sum score of all items (questions), † Sum score divided by number of items.

2.5.2. Placebo

Placebo was maltodextrin, an oligosaccharides without prebiotic effect, commonly used as placebo in controlled trails (Linetzky Waitzberg, 2012; Kolida et al., 2007).

2.6. Statistical analysis

Differences in baseline CRP and sVCAM-1 levels between ADHD patients and healthy controls were tested using analysis of covariance (ANCOVA) adjusting for age and sex. Mean and interquartile range (IQR i.e. 25th and 75th percentiles) values of symptoms and functioning in the treatment groups over time (t_1 to t_2) are reported. The changes of symptoms, functioning or emotion regulation from baseline (t_1) to 9-week follow-up (t_2) irrespective of treatment group (combining active and placebo groups, i.e. overall) were tested using paired *t* test, and estimated (t_1-t_2) mean and 95% confidence interval (CI) values are reported. Differences between treatment types (Synbiotic 2000 compared to placebo) in scale score changes over time (baseline to 9-week follow-up) were tested using ANCOVA with outcome variable being the respective follow-up score adjusting for the corresponding baseline score, age and sex. The estimate, and its 95% CI, of the treatment type effect are reported, negative values representing more improvement of symptoms or functioning by Synbiotic 2000 than by placebo. Likewise, the estimate and 95% CI values of the effect of sex and age group were determined. ADHD symptoms are known to change during lifespan (Philipp-Wiegmann, 2016), and age groups were arbitrarily defined as follows: for children (5–12 years and 13–18 years) and for adults (19–25 years and 26–55 years). For the ANCOVA models, suggestive statistical significance was defined as $\alpha = 0.10$ and statistical significance as $\alpha = 0.050$. Proportion of outcome variance explained is indicated by partial eta squared (η^2). Sensitivity analyses of treatment type effects on psychiatric symptoms were performed in subgroups: (i) plasma sVCAM-1 level > 519519.7 mg/L versus sVCAM-1 level ≤ 519519.7 mg/L, and (ii) ADHD medication [yes/no]. The sVCAM-1 level cut-off (519519.7 mg/L) corresponded to the median level in the ADHD patient group, which was equal to the 84th percentile in a healthy control group with baseline data available (n = 61, aged 5–55 years with no ADHD diagnosis and fulfilling the exclusion criteria). In the sVCAM-1 level-subgroups, the outcomes assessed were the total scales, and the corresponding subdomains for the total scales with statistical significance of treatment type. In the ADHD-medication-subgroups (Table 1), only outcomes with suggestive statistical significance of treatment type in the original analysis were assessed (i.e. SCQ total and repetitive, restricted and stereotyped behavior, and DERS-16 goal-directed behavior). Changes in food intake between t_1 and t_2 were tested with paired *t*-tests comparing each nutrient, where statistical significance was defined as $\alpha = 0.050$. All statistical analyses were performed using R programming language.

3. Results

3.1. Primary outcome – ADHD symptoms, functioning and autism symptoms

The changes of ADHD symptoms (scored by SNAP-IV for children and ASRS for adults), functional impairments (children: WFIRS-PC, adults: WFIRS-SA) and autism symptoms (children: SCQ, adults: AQ) over the 9 weeks intervention were assessed in children and adults. The pre-post changes overall (combining Synbiotic 2000 and placebo groups), and the differences between type of treatment (Synbiotic 2000 versus placebo) at follow-up adjusted for corresponding baseline scores, sex and age, are shown for children in Table 3 and for adults in Supplemental Table S2. The ADHD symptoms were significantly reduced from baseline (t_1) to post-treatment (t_2) for both children and adults (95% $CI_{children}$: 0.065, 0.268; 95% CI_{adults}: 0.111, 0.243). There was a similar degree of reduction in total ADHD symptoms for both treatment groups: Synbiotic 2000 (mean of t_1-t_2: 0.142 for children, 0.180 for adults) and placebo (mean of t_1-t_2: 0.211 for children, 0.174 for adults), i.e. there was no difference in effect between the interventions (95% $CI_{children}$: −0.153, 0.263; 95% CI_{adults}: −0.133, 0.110). Similar findings were obtained in the subscale inattention: reduction in inattention over time for all participants (95% $CI_{children}$: 0.033, 0.259; 95% CI_{adults}: 0.141, 0.287) and no difference between Synbiotic 2000 and placebo (95% $CI_{children}$: −0.123, 0.352; 95% CI_{adults}: −0.111, 0.164), likewise, reduction in hyperactivity/impulsivity symptoms were found over time for all participants (95% $CI_{children}$: 0.068, 0.305; 95% CI_{adults}: 0.060, 0.221) and there was no difference in reduction over time between Synbiotic 2000 and placebo (95% $CI_{children}$: −0.262, 0.211; 95% CI_{adults}: −0.195, 0.092).

The interventions had no effect on change in total score of functioning for children (WFIRS-PC, 95% $CI_{children}$: −0.077, 0.068), but functioning was improved for adults (WFIRS-SA, 95% CI_{adults}: 0.066, 0.191) but there was no difference in change in functioning between active treatment and placebo (95% $CI_{children}$: −0.163, 0.125; 95% CI_{adults}: −0.149, 0.085) (Table 3 and Supplemental Table S2).

Autism symptoms were assessed using SCQ in children and AQ in adults. Among children, there was a tendency for a Synbiotic 2000-specific reduction of autism symptoms in total SCQ scale (95% CI: −0.072, 0.003, $\eta^2 = 0.081$). Moreover, the Synbiotic 2000 treatment reduced the restricted, repetitive and stereotyped behaviors significantly more than placebo did (95% CI: −0.166, −0.013, $\eta^2 = 0.052$; mean of t_1-t_2: 0.052 for Synbiotic 2000, −0.034 for placebo), suggesting that Synbiotic 2000 had specific benefits in reducing autistic symptoms in children. In adults, there was no measurable effect of intervention type with regard to AQ scores neither on the total scale nor on the five subscales (Supplemental Table S2). However, in the AQ scale the assessment of restricted, repetitive and stereotyped behavior is

Table 3
Treatment effects on ADHD symptoms (SNAP-18), daily functioning (WFIRS-PC) and autism symptoms (SCQ) in children (n = 68). Treatment type confidence interval (CI) below 0 means Synbiotic 2000 specific improvement of psychiatric symptoms and functioning.

Scale[a]	Placebo			Synbiotic 2000			Overall	Effect for treatment type
	t1	t2	t1-t2	t1	t2	t1-t2	t1-t2	
	Mean (IQR)			*Mean (IQR)*			*Estimated mean change (95%CI)*	*Estimate (95% CI)*
SNAP-18	1.55 (1.39,1.83)	1.33 (1.03,1.58)	0.211 (0.000,0.438)	1.54 (1.07,1.94)	1.40 (1.00,1.81)	0.142 (−0.116,0.375)	**0.166 (0.065,0.268)***	0.055 (−0.153,0.263)
Inattention	1.63 (1.41,1.94)	1.43 (1.17,1.78)	0.202 (0.000,0.417)	1.77 (1.44,2.22)	1.65 (1.14,2.17)	0.115 (−0.174,0.333)	**0.146 (0.033,0.259)***	0.115 (−0.123,0.352)
Hyperactivity/ Impulsitivity	1.45 (1.17,1.83)	1.24 (0.833,1.67)	0.213 (−0.062,0.507)	1.32 (0.694,2.00)	1.15 (0.556,1.64)	0.172 (−0.111,0.444)	**0.186 (0.068,0.305)***	−0.025 (−0.262,0.211)
WFIRS-PC	0.875 (0.625,1.08)	0.887 (0.58,1.16)	−0.012 (−0.194,0.219)	0.893 (0.585,1.19)	0.893 (0.572,1.12)	0.000 (−0.193,0.196)	−0.004 (−0.077,0.068)	−0.019 (−0.163,0.125)
Family	1.06 (0.500,1.54)	0.965 (0.400,1.48)	0.091 (−0.100,0.275)	1.1 (0.425,1.65)	1.04 (0.500,1.48)	0.066 (−0.200,0.383)	0.075 (−0.022,0.172)	0.021 (−0.171,0.214)
School	1.14 (0.600,1.48)	1.21 (0.625,1.38)	−0.068 (−0.200,0.300)	0.934 (0.500,1.20)	1.04 (0.600,1.40)	−0.098 (−0.362,0.100)	−0.086 (−0.238,0.066)	−0.013 (−0.323,0.297)
Life skills	0.937 (0.700,1.11)	0.945 (0.700,1.2)	−0.063 (−0.300,0.100)	1.09 (0.889,1.30)	0.981 (0.700,1.32)	0.102 (−0.200,0.300)	0.040 (−0.056,0.135)	−0.081 (−0.260,0.098)
Self-concept	0.994 (0.667,1.33)	0.853 (0.333,1.00)	0.060 (−0.333,0.333)	1.07 (0.333,1.67)	0.976 (0.333,1.33)	0.081 (−0.333,0.667)	0.073 (−0.073,0.219)	0.035 (−0.248,0.317)
Social activities	0.721 (0.429,0.857)	0.686 (0.286,1.00)	−0.010 (−0.143,0.286)	0.704 (0.143,1.00)	0.668 (0.298,1.00)	0.036 (−0.143,0.143)	0.019 (−0.079,0.117)	−0.063 (−0.234,0.108)[b]
Risky activities	0.396 (0.100,0.500)	0.408 (0.100,0.400)	−0.008 (−0.125,0.200)	0.573 (0.100,0.800)	0.665 (0.100,0.500)	−0.020 (−0.100,0.100)	−0.016 (−0.167,0.136)	0.052 (−0.269,0.374)
SCQ	0.170 (0.103,0.235)	0.185 (0.103,0.231)	−0.015 (−0.051,0.045)	0.164 (0.077,0.231)	0.146 (0.053,0.189)	0.018 (−0.026,0.061)	0.005 (−0.014,0.024)	**−0.035 (−0.072,0.003)†**
Reciprocal social interaction	0.080 (0.000,0.117)	0.077 (0.000,0.133)	0.003 (−0.064,0.067)	0.089 (0.000,0.091)	0.098 (0.000,0.133)	−0.009 (−0.067,0.067)	−0.005 (−0.032,0.023)	0.016 (−0.037,0.069)
Communication	0.296 (0.077,0.519)	0.323 (0.173,0.519)	−0.027 (−0.077,0.058)	0.28 (0.125,0.385)	0.262 (0.154,0.385)	0.018 (−0.077,0.077)	0.001 (−0.040,0.041)	−0.051 (−0.121,0.02)
Restricted repetitive stereotyped behavior	0.154 (0.000,0.219)	0.188 (0.000,0.250)	−0.034 (−0.125,0.000)	0.141 (0.000,0.250)	0.088 (0.000,0.125)	0.052 (0.000,0.125)	0.019 (−0.022,0.059)	**−0.089 (−0.166,-0.013)***

ADHD symptoms were assessed using SNAP-IV (SNAP-IV-18-Item Parent Rating Scale). Functioning was assessed using WFIRS-PC (Weiss Functional Impairment Rating Scale Parent-reported). Autism symptoms and traits were assessed using for children SCQ (Social Communication Questionnaire). ADHD symptoms and WFIRS: Values are mean scores per question (item) (min 0, max 3), while SCQ is mean score per question (item) (min 0, max 1).
t_1 = baseline, t_2 = 9 weeks.
The overall time effect was assessed using paired *t*-test and the effect of treatment type was tested using analysis of covariance (ANCOVA), outcome variable being follow-up score and adjusting for baseline score, age and sex.
a. lower scores indicate less symptoms or less functional impairment.
b. statistical significance for covariate age.
Statistical significance: * (α = 0.05); suggestive significance: † (α = 0.10).

subdivided between subscales. Restricted behavior is reflected in the subscales Attention to details and Attention switching, where there was no indication of treatment effect. For assessing repetitive behavior, we arbitrarily used the single question existing (item 2). There was no difference in change over time for item 2 between the two treatment groups (95% CI: −0.246, 0.292, data not shown). Stereotyped behavior, however, was not assessed in AQ. Age and sex did not moderate the treatment type effect for childhood autism symptoms (Table 3, Supplemental Table S2).

3.2. Primary outcome – stratification for vascular inflammation marker sVCAM-1 and ADHD medication

Two sensitivity analyses were performed. First, the baseline sVCAM-1 levels in the ADHD patients were elevated compared to healthy controls (95% CI of difference: 26648.2, 107603.2 pg/mL), while CRP levels were not (95% CI of difference: −555042.2, 153810.0 pg/mL). As the Synbiotic 2000-constituents have known anti-inflammatory properties, the analysis of effects on total scales (for ADHD symptoms, functioning and autism symptoms) was stratified for baseline plasma sVCAM-1 level: above versus below the median level in the ADHD patient group (median level = 519519.7 pg/mL) (Table 4). While among those with sVCAM-1 ≤ median there was no difference in treatment effect between Synbiotic 2000 and placebo for any total scale, the aforementioned suggestive Synbiotic 2000-specific improvement in total autism symptom score (95% CI: −0.072, 0.003, η^2 = 0.081, Table 3) was driven by children with elevated sVCAM-1 levels (95% CI: −0.083, −0.001, η^2 = 0.117, Fig. 2A). Thus, also autism symptom subdomains were studied, again detecting a Synbiotic 2000-specific improvement on restricted, repetitive and stereotyped behaviors (95% CI: −0.199, −0.006, η^2 = 0.123, Table 4, Fig. 2B). In adults with elevated sVCAM-1, Synbiotic 2000 only suggestively improved total autism score better than placebo did (95% CI:-0.165, 0.015, η^2 = 0.088, Table 4), and hence the AQ subdomains were not studied. Second, as ADHD medication might have influenced suggested treatment effects, the analyses of scale scores with significant treatment type effect in the original analysis (Table 3) were stratified for ADHD-medication [yes/no]. The aforementioned tendencies of Synbiotic 2000-specific improvement in total autism score of all children (95% CI: −0.072, 0.003, η^2 = 0.081 Table 3) was driven by those with no ADHD medication (total autism score: 95% CI: −0.115, −0.011; η^2 = 0.205, Fig. 2C). Likewise, the Synbiotic 2000-specific reduction in the restricted, repetitive and stereotyped behavior (95% CI:-0.166, −0.013, η^2 = 0.052, Table 3) was driven by those without ADHD medication (95% CI: −0.265, −0.010, η^2 = 0.170, Fig. 2D).

Table 4
Treatment effects in children (n = 39) and adults (n = 37) with vascular inflammation (plasma sVCAM-1 levels > median level) on ADHD symptoms, daily functioning, autism symptoms and emotion regulation. Treatment type confidence interval (CI) below 0 means Synbiotic 2000 specific improvement of psychiatric symptoms and functioning.

Scale[a]	Placebo			Synbiotic 2000			Overall	Effect for treatment type
	t_1	t_2	t_1-t_2	t_1	t_2	t_1-t_2	t_1-t_2	
	Mean(IQR)			*Mean(IQR)*			*Estimated mean change (95%CI)*	*Estimate (95%CI)*
SNAP-18	1.57 (1.39,1.83)	1.40 (1.14,1.69)	0.168 (−0.028,0.382)	1.52 (1.06,2.01)	1.37 (0.986,1.74)	0.142 (−0.111,0.375)	**0.155 (0.027,0.283)***	−0.002 (−0.265,0.260)
ASRS	1.80 (1.53,2.00)	1.63 (1.49,1.72)	0.167 (−0.146,0.281)	1.99 (1.74,2.20)	1.76 (1.58,1.93)	0.235 (0.002,0.375)	**0.205 (0.051,0.360)***	0.047 (−0.216,0.310)
WFIRS-PC	0.92 (0.65,1.12)	0.938 (0.610,1.13)	−0.018 (−0.192,0.213)	0.887 (0.640,1.22)	0.880 (0.564,1.12)	0.008 (−0.180,0.220)	−0.005 (−0.110,0.010)	−0.064 (−0.274,0.147)
WFIRS-SA	0.939 (0.556,1.21)	0.893 (0.499,1.18)	0.046 (−0.118,0.211)	0.873 (0.514,1.09)	0.693 (0.509,0.888)	0.180 (−0.065,0.309)	**0.004 (−0.039,0.046)**†	**−0.217 (−0.473,0.040)**†
SCQ	0.164 (0.0897,0.231)	0.174 (0.103,0.218)	−0.011 (−0.051,0.026)	0.166 (0.064,0.218)	0.133 (0.052,0.195)	0.034 (0.001,0.062)	0.012 (−0.011,0.034)	**−0.042 (−0.083,-0.001)***
Reciprocal social interaction	0.060 (0.000,0.067)	0.067 (0.000,0.133)	−0.007 (−0.062,0.033)	0.118 (0.000,0.133)	0.098 (0.000,0.167)	0.020 (−0.055,0.067)	0.007 (−0.024,0.037)	0.004 (−0.045,0.053)
Communication	0.279 (0.077,0.423)	0.292 (0.154,0.423)	−0.013 (−0.077,0.038)	0.272 (0.119,0.423)	0.240 (0.154,0.346)	0.032 (0.000,0.077)	0.010 (−0.041,0.060)	−0.037 (−0.126,0.051)
Restricted repetitive stereotyped behavior	0.178 (0.000,0.188)	0.204 (0.063,0.250)	−0.026 (−0.125,0.000)	0.099 (0.000,0.188)	0.066 (0.000,0.125)	0.033 (0.000,0.063)	0.003 (−0.048,0.054)	**−0.102 (−0.199,-0.006)***
AQ	0.428 (0.300,0.495)	0.430 (0.350,0.475)	−0.002 (−0.010,0.065)	0.321 (0.212,0.430)	0.312 (0.202,0.380)	0.009 (−0.060,0.102)	0.004 (−0.039,0.046)	**−0.075 (−0.165,0.015)**†
DERS-16	47.8 (36.0,62.0)	47.5 (30.0,64.5)	0.286 (−4.00,6.75)	43.6 (28.0,58.5)	38.0 (27.5,46.0)	5.63 (−2.50,11.5)	**3.36 (−0.456,7.18)**†	**−9.70 (−17.0,-2.40)*[c]**
Clarity	5.00 (4.00,5.75)	5.07 (3.00,7.50)	−0.071 (0.000,1.00)	4.63 (3.00,6.00)	3.68 (2.00,4.50)	0.947 (0.000,2.00)	0.515 (−0.125,1.16)	**−1.32 (−2.56,-0.088)***
Goals	11.2 (9.50,13.8)	11.3 (8.50,14.0)	−0.071 (−1.75,1.00)	10.9 (8.50,14.5)	9.42 (6.50,13.0)	1.47 (−0.500,4.00)	0.818 (−0.24,1.88)	**−2.33 (−4.37,-0.291)***
Impluse	8.21 (6.00,11.2)	8.07 (5.00,11.8)	0.143 (−1.50,1.00)	7.37 (4.00,10.5)	6.37 (4.00,8.00)	1.00 (0.000,2.00)	0.636 (−0.146,1.42)	−1.33 (−3.00,0.343)
Strategies	14.5 (9.25,20.2)	14.2 (9.00,20.0)	0.286 (−2.00,2.75)	12.7 (7.50,17.5)	11.2 (6.50,15.0)	1.53 (−1.00,3.00)	1.00 (−0.339,2.34)	**−2.99 (−5.51,-0.457)*[bc]**
Nonacceptance	8.86 (6.00,11.0)	8.86 (5.00,12.2)	0.000 (−1.50,1.00)	8.05 (4.00,11.5)	7.37 (5.00,8.50)	0.684 (−1.50,2.50)	0.394 (−0.531,1.32)	**−1.76 (−3.39,-0.125)*[c]**

sVCAM-1: soluble Vascular Cell Adhesion Molecule-1; ADHD symptoms were assessed using SNAP-IV for children (SNAP-IV-18-Item Parent Rating Scale), and ASRS for adults (ADHD Self-Report Scale). Functioning was assessed using WFIRS-PC (Weiss Functional Impairment Rating Scale Parent-reported) for children and WFIRS-SA (WFIRS self-reported) for adults. Autism symptoms and traits were assessed using for children SCQ (Social Communication Questionnaire), and for adults AQ (Adult Spectrum Quotient). ADHD symptoms, WFIRS and AQ: Values are mean scores per question (item) (min 0, max 3), while SCQ is mean score per question (item) (min 0, max 1). For DERS-16 (Difficulties in Emotion Regulation Scale-16 item version) values are sum scores (total scale: min 16, max 80; Clarity: min 2, max 10; Goals: min 3, max 15; Impulse: min 3, max 15; Strategies: min 5, max 25; Non-acceptance: min 3, max 15).
t_1 = baseline, t_2 = 9 weeks,
The overall time effect was assessed using paired *t*-test and the effect of treatment type was tested using analysis of covariance (ANCOVA), outcome variable being follow-up score and adjusting for baseline score, age and sex.
a. lower scores indicate less symptoms or less functional impairment,
b. statistical significance for covariate age,
c. statistical significance for covariate sex,
Statistical significance: * (α = 0.050); suggestive significance: † (α = 0.10).

3.3. Secondary outcome – emotion regulation

Supplemental Table S2 shows the levels of emotion regulation, ie functioning when being emotionally upset, measured by the questionnaire DERS-16 in the adult participants, after adjustment for sex and age. While pre-post changes overall showed improved total (95% CI: 1.95, 5.56) and subdomain scores, there was a treatment type effect where Synbiotic 2000 improved only the difficulties in engaging in goal-directed behavior (95% CI: −2.07, −0.014, η^2 = 0.040). In the sensitivity analysis (Table 4), however, for those with elevated sVCAM-1 levels at baseline there was a Synbiotic 2000-specific improvement in emotion regulation total scale (95% CI: −17.0, −2.40, η^2 = 0.209, Fig. 2E), and hence the subdomains were studied. The total score finding for those with elevated sVCAM-1 levels was supported by significantly larger improvement in four out of the five subdomains. Thus, those treated with Synbiotic 2000 improved more than those treated with placebo in the subscales clarity (95% CI: −2.56, −0.088, η^2 = 0.147), goals (95% CI: −4.37, −0.291, η^2 = 0.164), strategies (95% CI: −5.51, −0.457, η^2 = 0.173), and nonacceptance (95% CI: −3.39, −0.125, η^2 = 0.148) (Fig. 2F-2I). In the second sensitivity analysis, we explored the influence of ADHD medication on the effect of Synbiotic 2000 on DERS-16 goal-directed behavior. No significant intervention effect was detected in any of the groups with ADHD medication, and without ADHD medication.

4. Discussion

4.1. Key findings and interpretation

This is the first randomized placebo-controlled trial exploring the effects of a probiotic or synbiotic on symptoms and functioning in individuals with ADHD. A large study group, 182 children and adults, was

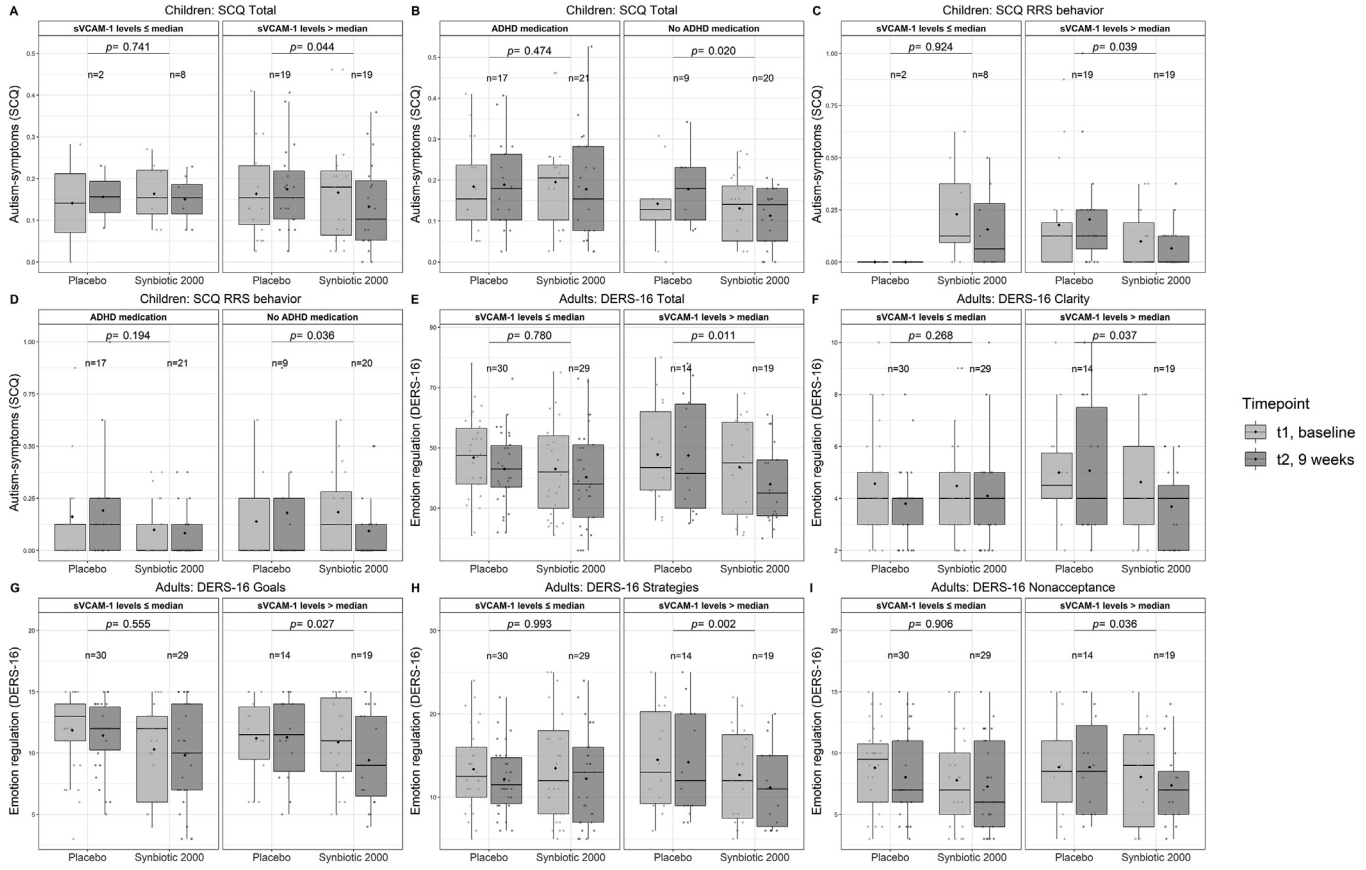

Fig 2. Sensitivity analyses. **A**) The suggestive Synbiotic 2000-specific improvement over 9 weeks treatment (t_1 to t_2) in total autism symptom score among children (95% CI: −0.072, 0.003, Table 3) was driven by those with plasma sVCAM-1 level above median at baseline ($p = 0.044$, [95% CI: −0.083, −0.001], Table 4) and by **B**) those without ADHD medication treatment ($p = 0.020$, [−0.115, −0.011]). **C**) Synbiotic 2000 treatment reduced the restricted, repetitive and stereotyped (RRS) behavior significantly more than placebo did (95% CI: −0.166, −0.013, Table 3), driven by those with elevated sVCAM-1 levels ($p = 0.039$, [−0.199, −0.006]) and by **D**) those without ADHD medication ($p = 0.036$, [−0.265, −0.010]). **E**) In adults with elevated plasma sVCAM-1 levels at baseline there was a Synbiotic 2000-specific improvement in emotion regulation (DERS-16) total scale ($p = 0.011$, [−17.0, −2.40]). **F-I**) The total DERS-16 score finding was supported by a significantly larger improvement in four out of the five subdomains for those with elevated sVCAM-1 levels treated with Synbiotic 2000 compared to those treated with placebo (Clarity: $p = 0.037$, [−2.56, −0.088]; Goals: $p = 0.027$, [−4.37, −0.291]; Strategies: $p = 0.002$, [−5.51, −0.457]; Nonacceptance: $p = 0.036$, [−3.39, −0.125]). Median values are indicated with a horizontal line, mean values with a strong black dot. Grey dots represent individual participant scores. t_1 = baseline; t_2 = after 9 weeks treatment; sVCAM-1: soluble Vascular Cell Adhesion Molecule-1; SCQ: Autism Social Communication Questionnaire; DERS-16: Difficulties in Emotion Regulation Scale-16 item version, DERS-16 Clarity: DERS-16 subscale lack of emotional clarity, DERS-16 Goals: engagement in goal-directed behavior. DERS-16 Strategies: limited access to effective emotion regulation strategies, DERS-16 Nonacceptance: nonacceptance of emotional responses. Lower scores indicate less symptoms or less difficulties in emotional regulation. SCQ scores are mean per question (min 0, max 1) in the subscale restricted, repetitive and stereotyped behavior. DERS-16 values are sum scores (total score: min 18, max 80; clarity score: min 2, max 10; goals score: min 3, max 15; strategies score: min 5, max 25; nonacceptance score: min 3, max 15).

treated daily with either Synbiotic 2000 or placebo for 9 consecutive weeks. On group level, our study showed that Synbiotic 2000 and placebo interventions improved the ADHD symptoms, inattention and hyperactivity/impulsivity. However, there was no difference in ADHD symptom improvement between the two arms. Likewise, functioning scored by WFIRS was not affected by the treatment.

Autism is a common comorbidity with ADHD and previous studies have suggested that prebiotics and probiotics may have positive effects on autism symptoms, although most studies were open-label (Ng, 2019; Liu, 2019; Grimaldi, 2018). In our study, no study participant had an autism diagnosis at recruitment, but as expected, autistic traits were common. The child cohort with ADHD showed a tendency towards greater reduction in autistic symptoms after treatment with Synbiotic 2000 compared to placebo (95% CI: −0.072, 0.003, $\eta^2 = 0.081$), and a statistically significant reduction was found for the autistic symptom subscale restricted, repetitive and stereotyped behavior (95% CI: −0.166, −0.013, $\eta^2 = 0.052$). In accordance with that the Synbiotic 2000 has known anti-inflammatory properties (Zhu, 2018; Serrano-Contreras, 2016; Swanson, 2001; Kessler, 2005; Alliance, 2011; Rutter et al., 2003; Baron-Cohen, 2001; Woodbury-Smith, 2005; Bjureberg, 2016; Kautto, 2014), an effect on total autism symptoms and on the restricted, repetitive and stereotyped behaviors was seen in children with elevated baseline sVCAM-1 levels (total scale: 95% CI: −0.083, −0.001, $\eta^2 = 0.117$; restricted, repetitive and stereotyped subscale: 95% CI: −0.199, −0.006, $\eta^2 = 0.123$), and not in the children with sVCAM-1 levels below median levels of the study cohort. This putative effect of Synbiotic 2000 on autism symptoms is supported by the previously suggested positive effects of prebiotics, probiotics and clinical fecal transplantation on autistic symptoms, and the extensive animal model studies demonstrating a role of the gut-brain axis on rodent repetitive, stereotyped and antisocial behavior (Kang, 2017; Sharon, 2019; Hsiao, 2013). In fact, fecal transplantation from autism patients into mice caused autism-like behaviors (increased repetitive behavior and reduced sociability) in their adult offspring, that had been exposed from fetal stage, with a particular dose–response for repetitive behavior. The effect was suggested to be driven by neuroactive bacterial metabolites (Sharon, 2019).

In our study, the adult participants showed only a tendency for

Synbiotic 2000-specific improvement of autism traits, namely the total AQ score and that in those with elevated sVCAM-1 levels (95% CI:-0.165, 0.015, $\eta^2 = 0.088$). In the AQ, assessment of restricted, repetitive and stereotyped behavior is subdivided so that restricted behaviors are reflected in the Attention to details and Attention switching subscales. For repetitive behavior we used the only one question existing (item 2), whereas stereotyped behavior was not assessed in AQ. There was no effect of treatment on the aforementioned restricted (95% $CI_{Attention\ to\ details}$: −0.095, 0.032 and 95% $CI_{Attention\ switching}$: −0.062, 0.078) or repetitive behavior (95% CI: −0.246, 0.292). The fact that we detected only a tendency for association between intervention and autistic traits in the adult participants might reflect the limitation in phenotyping, a lack of statistical power or the possibility that the window of treatment opportunity for autistic traits is restricted to young age as demonstrated in rodent models (Hsiao, 2013).

The secondary outcome emotion regulation is a measure of functioning in an emotionally upset state and was assessed only in adults. We found that Synbiotic 2000 improved difficulties in engaging in goal-directed behavior (95% CI:-2.07, −0.014, $\eta^2 = 0.040$). Moreover, among those with elevated sVCAM-1 levels at baseline, there was a Synbiotic 2000-specific improvement in emotion regulation, for both the total scale (95% CI:-17.0, −2.40, $\eta^2 = 0.209$) and four of the five subdomains, namely clarity, goals, strategies and nonacceptance (η^2: 0.147–0.173). As emotion dysregulation is a symptom also in e.g. personality disorders and mood disorders, this finding might be relevant to explore further not only in ADHD.

The majority of participants had an ongoing ADHD pharmacotherapy during this intervention study. This medication might have influenced the detected suggestive treatment effects. To explore this we stratified the analyses with suggestive treatment type effect in the original analysis for ADHD-medication [yes/no], that is, two autism symptom scores in children and the DERS-16-goals score in adults. We found that the Synbiotic 2000-specific improvements in autistic symptoms were driven by those children without ADHD-medication (total SCQ score: 95% CI: −0.115, −0.011, $\eta^2 = 0.205$; repetitive, restricted and stereotyped behaviors score: 95% CI: −0.265, −0.010, $\eta^2 = 0.170$). As the children without ADHD medication had a lower total SCQ score at baseline than those on ADHD medication, the Synbiotic 2000-specific effect on SCQ in those without ADHD medication might reflect a milder pathophysiology easier to improve, or there might be an influence of ADHD pharmacotherapy on the gut microbiome. However, the detected Synbiotic 2000-specific improvement of total SCQ score in those without ADHD medication was because of a worsening of the total SCQ score in the placebo-treated group. The Synbiotic 2000 effect on DERS-16 goal-directed behavior was not large enough to be detected after stratifying the participants in those with and those without ADHD medication.

Both Synbiotic 2000 and placebo were tolerated well. Individuals with neuropsychiatric disorders often have sensory issues and restrictive eating patterns. The drop-out rate obtained in this study was similar to RCTs of Omega-3 in children (van der Wurff et al., 2017).

Changes of diet during the intervention might confound the responses (Heilskov Rytter, 2015), hence we attempted to identify major differences in nutrient intake between baseline and follow-up.

We detected no significant change between baseline and follow-up in the macronutrient intake like proteins, fatty acids or carbohydrates. However, the intake of provitamin A carotenoid, β-carotene, was significantly lower at follow-up compared to at baseline (Supplemental Table S3). Lowered levels of β-carotene at the follow-up could counteract an improvement in the microbiome (Lyu, 2018). However, the detected change in β-carotene did not influence the intervention effect, as there was no difference in β-carotene change over time between the placebo and Synbiotic 2000 group (95% CI: −1489.5, 2076.5).

5. Strengths and limitations

The main strength of this study is the placebo-controlled design. The total sample size was large and treatment time long for being a probiotic intervention trial in neuropsychiatry. The baseline clinical characteristics were similar in completers and drop-outs, suggesting that drop-out did not add important bias (Supplemental Table S1). However, the number of drop-outs were among children greater in the Synbiotic 2000 group (Fig. 1). Nevertheless, the study has limitations. First, our findings suggest that Synbiotic 2000 improves autistic symptoms and emotion regulation in ADHD patients with elevated plasma sVCAM-1 levels. However, we did not explore intervention effects on markers of inflammation, such as CRP and sVCAM-1, in this study. Second, when stratifying for sVCAM-1-levels or ADHD medication in the sensitivity analyses, the sample sizes were small. In an attempt to identify preliminary putative indications for effects we arbitrarily defined suggestive statistical significance as $\alpha = 0.10$ and statistical significance as $\alpha = 0.050$. We did not correct for multiple testing in this study. We provide 95% CIs and partial eta squared for effect size estimates. Our preliminary suggestive findings need to be confirmed by larger cohorts. Third, 34% of the children and 49% of the adult participants used melatonin, antipsychotic or antidepressant medication, some of which have been reported to influence the gut microbiota, consequently possibly influencing the intervention with Synbiotic 2000. Also, an intervention effect in this study might have been concealed by the use of diet supplements at baseline, such as vitamins, omega-3 and probiotics (41% of children, 72% of adults). The probiotics used before the start of the trial were *L. plantarum* 299v (fruit juice, 8% of participants), Synbiotic 15 (similar constituents as in Synbiotic 2000 but $15*10^9$ CFU instead of the $4*10^{11}$ CFU in Synbiotic 2000, 3 adults) and other probiotics (6 adults). These limitations may however increase the external validity of the findings. Fourth, the diet questionnaire was retrospective and unlikely to allow detection of minor or dynamic changes over the 9 weeks. Fifth, we did not explore intervention effects on symptoms of depression or anxiety which are common comorbidities of ADHD (Chen, 2018).

5.1. Conclusion, potential clinical implications and future research

Our exploratory study detected no definite Synbiotic 2000-specific effect on ADHD symptoms, functionality or comorbid autistic symptoms. However, the results of those with elevated sVCAM-1 levels at start of intervention suggest a Synbiotic 2000-specific reduction of autism symptoms in children and an improvement of emotion regulation in adults with ADHD. Further, an improvement of autism traits in children by Synbiotic 2000 was driven by those without ADHD medication. There are only few treatments specific for autism symptoms. If synbiotic dietary supplements could ameliorate symptoms and improve functioning in persons with ADHD that would potentially benefit many patients. However, our findings are preliminary and need replication in larger samples to explore also possible sex and age influences.

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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.2020.05.056.

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