TITLE: Prebiotic supplementation does not affect reading and cognitive performance in children: a randomised placebo-controlled study.

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ABSTRACT

Based on the emerging interest in the effects of gut microbiota on cognition, this proof-of-concept study assessed how children aged 7-9 with low reading scores responded to the ingestion of a 3-month prebiotic supplement versus placebo. As a secondary aim, the effects of the prebiotic on cognition, sleep, behaviour, mood, anxiety, and cortisol were assessed. In this sample, the prebiotic did not affect any of the outcome measures.

INTRODUCTION

A significant proportion of children attending primary school demonstrate difficulties in reading, which may impair their academic performance and increase the risk of behavioural and emotional problems (Montgomery, 2014). Although the influence of nutritional supplements such as omega-3 (fish oil) on reading, behaviour and sleep function in children aged 7 to 9 years old are equivocal (e.g., Montgomery et al., 2014, 2018), the effects of prebiotic supplements on reading and cognition in children remain unexplored.

Prebiotics are non-digestible dietary fibres that grow beneficial gut bacteria, and there is evidence showing that prebiotics improve some components of emotional, cognitive and sleep processes in both rodents and humans (for a review see Desmedt et al., 2018). For instance, in a study by Smith and colleagues (2015), the prebiotic inulin improved memory recall in healthy volunteers, but had no effects on spatial memory or sustained attention. In a separate studies, galacto-oligosaccharides (Bimuno, B-GOS) improved cognitive flexibility in rats (Gronier et al., 2018), and attentional vigilance to positive emotional stimuli in healthy volunteers (Schmidt et al, 2015).

Psychotropic actions of prebiotics have also been observed in early-life. We have demonstrated elevated central brain-derived neurotrophic factor (BDNF) and glutamate receptor proteins that are key for the maintenance of healthy cognitive function, in adult rats that were fed B-GOS from birth until weaning age (Williams et al., 2016). Similarly, the early-life feeding of rats with prebiotics has been shown to impart long-term reductions in anxious behaviour and improved spatial memory (Oliveros et al., 2016). These data suggest, therefore, that prebiotic administration may enrich the developing brain with emotional and cognitive functions.

The aim of this study was to investigate whether the daily intake of the B-GOS prebiotic by primary school children influenced their reading and cognitive abilities. Reading was the primary outcome in this investigation because underperformance in this ability can impact on cognition and behaviour. This assessment was therefore coupled with a range of secondary measures to assess the effects of supplementation on functions important for a healthy child development including cognition, sleep, subjective symptoms of mood and anxiety and the stress hormone cortisol.

METHODS

Children from year groups 3-5 (typically aged 7–9 years) from Oxfordshire, Swindon, Milton Keynes and London (UK) mainstream primary schools, who had below-average literacy skills (scored ≤34th percentile on the British Ability Scales word reading test, BAS-III, Elliott & Smith, 2011), were recruited into the study. Children were excluded if they required special educational support, had significant medical problems, and/or exposure to antibiotics or prebiotics/probiotics in the 2 months preceding the start of the study, or any recent changes in diet.

A total of 88 children from 16 schools were screened to take part in the study, and 56 were deemed eligible. Of these, 15 children withdrew (due to difficulties from parents in committing to giving the supplement or uncertainty about the prebiotic), 2 children interrupted the treatment because of side-effects and 4 children completed the post-intervention session outside the expected timeframe. Therefore, the final analysis was conducted on data from 35 children. Parents and teachers provided reports on core outcomes. Table 1 reports the demographic characteristics of the sample.

Table 1. Demographic characteristics

		Whole sample (N = 35)		Active (N = 17)		Placebo (N = 18)	
		Mean	SD	Mean	SD	Mean	SD
Age	(Years, months)	8.84	0.95	8.54	0.79	9.12	1.02
		N	%	N	%	N	%
Sex							
	Male	24	68.6	12	70.6	12	66.7
	Female	11	31.4	5	29.4	6	33.3
Ethnicity							
	White	26	74.3	12	70.6	14	77.8
	Mixed	6	17.1	2	11.8	4	22.2
	Asian	1	2.9	2	5.9		
	Other	2	5.7	2	11.8		

A power calculation was performed on data from our previous study showing that the daily intake of a prebiotic for 3 weeks by adult healthy volunteers (N=15 in each group) increased attention to positive versus negative stimuli with an effect size of 0.78 (Schmidt et al., 2015). A similar sample size was therefore targeted, with a minimum of 14 participants per group.

Before screening, written informed consent was obtained from parents and teachers, and verbal assent from the children. The study was approved by the University of Oxford Central University Research Ethics Committee (ref: R44843/RE001), and registered with ClinicalTrials.gov (NCT02926508).

Participants received a daily, 12-week administration of a prebiotic (B-GOS) or a matched placebo (maltodextrin), in a randomised, double-blind, between-subjects, placebo-controlled design. The supplement, which had previously been used in a study of healthy adults (Schmidt et al., 2015), was given in sachets, and parents were asked to mix the content in the children's breakfast. A randomisation programme from sealed envelope (https://www.sealedenvelope.com/) was used, with a minimization algorithm to ensure balanced allocation of participants across the treatment groups for school and sex.

Children's reading and working memory were assessed using the British Ability Scales (BAS-III, Elliott & Smith, 2011), specifically the sub-tests Word Reading Achievement and Recall of Digits (Forward and Backward), respectively. Cognition more broadly was also evaluated using the CogTrack™ System (www.wesnes.com), an online set of automated cognitive tests, which provided composite measures of Attentional Intensity Index, Sustained Attention Index, Attentional Fluctuation Index, Cognitive Reaction Time, Working Memory Capacity Index, Memory Retrieval Speed and Response Variability. This is a well-validated battery that is sensitive to detect changes in cognitive performance in children as young as five years old.

Symptoms of anxiety and mood were assessed using the State-Trait Anxiety Inventory for children (STAIC, Spielberger, 1973) and the Children Mood and Feelings Questionnaire – child short version (SMFQ, Angold and Costello, 1987), respectively. The Conners' Scale (Conners, 1997) for both parents (CPRS-S) and teachers (CTRS-L) were used to assess behavioural problems. Sleep was measured subjectively using the Child Sleep Habits Questionnaire (CSHQ) and sleep diaries (see Owens et al. 2000 for details), and objectively using actigraphy (MotionWatch8, CamNTech, Ltd, Cambridgeshire, UK). Actigraphy measured sleep duration, latency and immobile minutes, which were recorded for 5 consecutive nights immediately pre- and post- the 12-week treatment. Each child also provided four saliva samples pre- and post-intervention for the measurement of cortisol: before the two main visits, three of these samples were collected by the families in the morning, over a period of 30 minutes; a fourth sample was provided by the child during the visit. Demographic information and medical history were collected from parents.

Side-effects were recorded using the Barkley's Side Effects Rating Scale (Barkley et al., 1990),

and compliance assessed by counting empty sachets and checking the daily calendar/checklist returned by the parents at the end of the study. Since there was a concern with over-burdening children, families and schools, only pre- and post-intervention measures were made which was in keeping with our prebiotic study in adults (Schmidt et al., 2015), and a previous dietary intervention trial in children (Montgomery et al., 2014). Intermediary measures were therefore avoided.

Item-missing data on the STAIC, SMFQ, CPRS, CTRS and CSHQ were imputed using the mean value of the respective subscale, if ≥80% of the total questionnaire had been completed. The MotionWare Software was used to collect and score the actigraphy data (CamnTech).

All data were expressed as mean + SEM. In order to investigate group, time-point and group x time-point interaction effects, mixed-design (split-plot) analyses of variance (ANOVAs) were conducted for the outcomes of interest. All analyses were performed using IBM SPSS Statistics for Windows (version 24.0; IBM Corp., Armonk, NY).

RESULTS

Baseline characteristics and compliance

Groups were comparable at baseline for reading level, objective and subjective sleep, most questionnaires and cognitive measures and for cortisol levels. However, for the measures of Cognitive Reaction Time (as measured using CogTrack) and the child's self-report of mood (SMFQ), the placebo group displayed significantly lower scores at baseline ($t_{(31)}$ = -2.280, p =.030 and $t_{(33)}$ = -2.657, p =.012, respectively). Compliance rates were high and did not differ significantly between groups (82.69% empty sachets; 91.73% for calendar days/checklist ticked by parents, p >.28).

Effects of treatment (prebiotic vs. placebo)

a) Reading and cognition

Figure 1 shows that reading improved significantly over time in both treatment groups ($F_{(1,33)}$ = 19.810, p <.001; pre-intervention = 19.80 $\pm$ 1.61, post-intervention = 30.54 $\pm$ 3.07), but in the absence of group differences ($F_{(1,33)}$ = .392, p = .536), or time x treatment interactions ($F_{(1,33)}$ = 1.395, p = .246).

Figure 1>>>>>

Digit forward and *digit backward* BAS-III subtests did not show a significant change over time, and scores were not significantly different between the two treatment groups.

In the CogTrack test battery, *memory retrieval speed* was significantly faster (increased reaction time) in both treatment groups, ($F_{(1,30)}$ = 7.684, p =.009; pre-intervention = 3713.73 $\pm$ 117.09; post-intervention = 3496.91 $\pm$ 101.62 (Fig 1B). No effect of treatment ($F_{(1,30)}$ = 2.206, p = .148) or time-point x treatment interaction were found ($F_{(1,30)}$ = .010, p = .922). Data were not significantly different between groups for all other composite measures (data not shown).

b) Sleep

Overall, the variables *actual sleep time* ($F_{(1,29)}$ = 10.336, p =.003, see Figure 1C) and *immobile minutes* ($F_{(1,29)}$ = 10.868, p =.003) decreased (worsened) over time for both treatment groups. There was no significant main effect of treatment ($F_{(1,29)}$ = .030, p = .864 for actual sleep time; $F_{(1,29)}$ = .000, p = .984 for immobile minutes) or time-point x treatment interaction ($F_{(1,29)}$ = .345, p = .562 for actual sleep time; $F_{(1,29)}$ = .075, p = .786 for immobile minutes). There were no effects of treatment on the other objective sleep variables, including sleep latency.

For *subjective sleep*, the variables from the CSHQ questionnaire *bedtime resistance* ($F_{(1,\ 27)}$ = 27.313, p <.001), *sleep onset delay* ($F_{(1,27)}$ = 33.216, p <.001), *sleep disorder breathing* ($F_{(1,27)}$ = 4.596, p =.041), *sleep anxiety* ($F_{(1,27)}$ = 5.258, p =.030) and *total sleep disturbance* ($F_{(1,27)}$ = 6.666, p =.016) showed lower scores at baseline than at post-intervention in both groups, suggesting a significant worsening in sleep quality in both groups over time. A significant time-point x treatment interaction for *Daytime Sleepiness* ($F_{(1,27)}$ = 7.831, p =.009) was also found. Pairwise comparisons showed that this result was driven by higher scores for B-GOS at baseline, which showed a trend of increased sleepiness, ($F_{(1,29)}$ = 2.996., p = .094). No other significant effects were found.

c) Psychological symptoms and cortisol levels

Parent's reports of *child's anxiety (*STAIC), *mood (*SMFQ) *and behavioural problems (*CPRS-L) did not significantly change over time, and there was no significant effect of treatment ($F_{(1,27)}$ = <

0.5, p > 0.05). Differences in child's self-reported anxiety and mood (STAIC and SMFQ, respectively) were also not significant. A similar lack of treatment effects was seen for the teacher's report of behavioural problems *(*CTRS-L), though an effect of time was found ($F_{(1, 25)}$ = 5.979, p =.022), with children from both groups showing lower scores (fewer behavioural problems) at post-intervention than at pre-intervention.

Salivary cortisol concentrations were not significantly different between groups following the 12-week treatment period.

d) Side-effects

Two participants in the placebo group withdrew due to side-effects. The analysis of the Barkley scale showed that there was no significant time x treatment interaction, indicating that the groups did not differ in the type of symptoms experienced as result of participating in the study (p >0.5).

Repeating the analyses above with simple mean imputation for those children who completed the baseline session but had missing data at post-intervention generated a similar pattern of results regarding treatment and interaction effects. The only new significant treatment x time-point interaction effect found was for *digit forward* BAS-III ($F_{(1, 45)}$ = 6.444, p =.015), which was driven by higher, albeit non-significant, scores for the prebiotic group at baseline ($F_{(1,45)}$ = 2.985, p = .091)

DISCUSSION

To our knowledge, this is the first study investigating the effects of a prebiotic on reading and cognition in children. The daily intake of B-GOS for 12 weeks did not affect reading, our main outcome measure, sleep, cortisol levels or any other parameters compared to placebo. Both treatment groups demonstrated improved reading and memory retrieval speed over the 12 weeks, but a decline in actual sleep time and immobile minutes. These current data suggest that B-GOS does not influence the developing brain in the age-range assessed here. It is unlikely that these null effects reflected a lack of sensitivity in the tasks/questionnaires administered, since they have been successfully used in previous nutritional interventions in children. However, limited sampling times (pre- versus post-, rather than daily/weekly assessments) may have contributed to the null effects reported since changes during the supplementation period may have occurred.

Gut microbial communities in children reach an adult-like pattern around the age of three, but their stability and responsiveness to prebiotics may be influenced by variable diets and high sugar

intake. Although the children were consuming B-GOS during term-time, over the vacations their diets would have varied, particularly over the festive periods (e.g., Christmas, Easter) when sweet foods would have been ingested in greater quantities. The absence of food diaries is therefore one limitation of our study, but these were not included because they are considered less reliable when implemented in children, and an additional burden to them and their parents. Another limitation of our study was the lack of information on the bacterial communities in each subject. The prebiotic used proliferates Bifidobacteria in the gut (Gronier et al, 2018), and so their enumeration in faecal samples would have informed on whether B-GOS intake caused the expected changes. Some children may have already had optimal pre-existing levels of Bifidobacteria which do not respond to B-GOS. Moreover, the dietary patterns mentioned above may have favoured the growth of other bacteria which hindered the normal fermentation of the prebiotic by the beneficial bacteria. To understand if the composition of the pre-adolescent microbiome fluctuates more than that of adults, and thence influences the fundamental actions of B-GOS and downstream psychotropic effects, it is necessary to compare the populations of gut bacteria in children and adults through the collection of faecal samples and sequencing approaches.

The decrease in sleep over time in both B-GOS and placebo groups is consistent with a previous meta-analysis, which reported a negative correlation between age and sleep in school-aged children older than five years (Ohayon et al., 2004). Interestingly, this decrease was only seen in studies in which sleep was measured during school days (compared to weekends and holidays). Hence future research with children should take into consideration the periods in which sleep is assessed (term time vs. weekends/holidays), as school attendance seems to reduce sleep duration.

Finally, we performed an a-priori calculation based on previous prebiotic data with adults (Schmidt et al, 2015). Given the lack of research investigating the effects of prebiotics on reading and cognition in children, it is possible that the power calculation was not adequate for this sample and therefore we cannot exclude the possibility that the study was underpowered. Future studies with larger samples and varying age groups are needed.

To conclude, the current study demonstrates that the prebiotic B-GOS does not lead to improved reading or cognitive performance in elementary school children aged 7 to 9 years. The reasons for this are unknown, but a simple interpretation is that the influence of gut bacteria on the brain is not fully established in early-life. In support of this notion is a study showing that the pro-cognitive effect of post-natal prebiotic administration was not observed in young rats, but manifested only

in later-life (Oliveros et al., 2014). Future studies should further explore the effects of prebiotics across development, to understand the differential effects of age in the relationship between the gut microbiome and cognition.

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Declaration of conflicting interests

The study was financially supported by the company supplying the test compound (see above). The authors declare no other conflicts of interest with respect to the research, authorship, and/or publication of this study.

Disclaimer

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health.

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Legend to Figure

Figure 1. Reading (percentile), memory retrieval speed (msec) and actual sleep time results (sec). The three outcomes significantly changed across time for both groups (reading and memory retrieval speed improved; actual sleep time declined), without a significant difference between them. Error bars are standard error of the means. ** p <.01; *** p <.001