



Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study

F. Azpiroz, C. Dubray, Annick Bernalier Donadille, M. Cardot, A. Accarino,
J. Serra, A. Wagner, F. Respondek, M. Dapoigny

► To cite this version:

F. Azpiroz, C. Dubray, Annick Bernalier Donadille, M. Cardot, A. Accarino, et al.. Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study. *Neurogastroenterology & Motility*, 2017, 29 (2), pp.1-8. 10.1111/nmo.12911 . hal-01658456

HAL Id: hal-01658456

<https://hal.science/hal-01658456>

Submitted on 26 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution - NonCommercial 4.0 International License

Effects of scFOS on the composition of fecal microbiota and anxiety in patients with irritable bowel syndrome: a randomized, double blind, placebo controlled study

F. Azpiroz^{1,2} | C. Dubray³ | A. Bernalier-Donadille⁴ | J.-M. Cardot³ | A. Accarino^{1,2} |
J. Serra^{1,2} | A. Wagner⁵ | F. Respondek⁵ | M. Dapoigny⁶

¹Digestive System Research Unit, University Hospital Vall d'Hebron, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (Ciberehd), Barcelona, Spain

²Departament de Medicina, Universitat Autònoma de Barcelona, Bellaterra (Cerdanyola del Vallès), Spain

³Inserm CIC-501, Clinical Pharmacology Centre, Clermont-Ferrand Hospital, Clermont-Ferrand, France

⁴UR454 Microbiology Unit, Clermont-Ferrand Research Centre, Saint Gènes-Champanelle, France

⁵Tereos, ZI et portuaire, Marckolsheim, France

⁶CHU Clermont-Ferrand, Université d'Auvergne, INSERM UMR 1107, Neuro-Dol, Clermont-Ferrand, France

Correspondence

Dr Frédérique Respondek, Tereos, ZI et portuaire, Marckolsheim, France.
Email: frédérique.respondek@tereos.com

Abstract

Background: Short-chain fructooligosaccharides (scFOS) have beneficial effects in subjects with minor digestive complaints, but the potential mechanisms involved have not been elucidated. The aim of the study was to evaluate changes in rectal sensitivity related to the clinical effects of scFOS in a selected group of patients with irritable bowel syndrome (IBS) and rectal hypersensitivity.

Methods: In 79 IBS patients (defined by Rome III criteria) with rectal hypersensitivity (defined as discomfort threshold ≤ 44 g) a parallel, placebo-controlled, randomized, and double-blind study was performed to assess the effects of dietary supplementation (5 g d^{-1}) with scFOS vs placebo for 4 weeks on rectal sensitivity (primary outcome: tolerance to increasing wall tension applied by a tensostat), clinical outcomes (IBS, anxiety/depression and quality of life scores) and composition of fecal microbiota.

Key Results: Rectal discomfort threshold, and IBS and quality of life scores, significantly improved during treatment, but in a similar manner in both scFOS and placebo groups; a post-hoc analysis showed that the effect of scFOS on rectal sensitivity was more pronounced in constipation-predominant-IBS patients ($P=.051$ vs placebo). Contrary with placebo, scFOS significantly reduced anxiety scores and increased fecal Bifidobacteria ($P<.05$ for both) without modifying other bacterial groups.

Conclusions & Inferences: The effect of scFOS on anxiety may be related to modulation of the gut microbiota; demonstration of effects of scFOS on rectal sensitivity may require higher doses and may depend on the IBS subgroup.

KEY WORDS

anxiety, fibers, fructooligosaccharides, irritable bowel syndrome, microbiota

1 | INTRODUCTION

Irritable bowel symptoms have been related to a combination of sensory and motor abnormalities of the gut.^{1,2} The cause of these abnormalities is not known, but several predisposing and triggering factors have been investigated. In particular, recent studies suggest that intestinal microbiota influence gut sensitivity, and may therefore

play a part in this context. Indeed, there is evidence from several studies that the intestinal microbiota of IBS patients is altered when compared to healthy controls^{3,4} and differences in microbiota may be related to their sensory dysfunction.⁵

Short-chain fructo-oligosaccharides (scFOS) naturally present in onions, bananas or Jerusalem artichokes, are considered to be prebiotic ingredients known to selectively modulate the composition

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes

and activity of the intestinal microbiota.^{6–8} In humans, increasing doses of scFOS supplementation of between 2.5 and 10 g d⁻¹ is associated with an increase in fecal bifidobacteria⁷ which is generally greater when the initial concentration of bifidobacteria is relatively low.^{9,10}

The increase in fecal butyrate following scFOS supplementation is particularly efficient when the initial concentration of butyrate is low.¹¹ A randomized, double-blind, placebo controlled study performed on healthy subjects presenting mild functional bowel symptoms demonstrated that regular consumption of 5 g d⁻¹ of scFOS reduced the frequency and intensity of digestive symptoms and improved intestinal discomfort and quality of life.¹²

We hypothesized that the effect of scFOS on functional IBS-type symptoms is mediated, at least in part, by changes in microbiota and reduce rectal hypersensitivity in patients with IBS. To test this hypothesis, we designed a double-blind, placebo-controlled, parallel and well-powered study of selected IBS patients with rectal hypersensitivity. The primary outcome was a reduction in rectal sensitivity; secondary outcomes were improvement of clinical symptoms and changes in fecal microbiota pattern.

2 | MATERIAL AND METHODS

2.1 | Patients

IBS patients (18–60 years age) fulfilling Rome III criteria¹³ were recruited in gastroenterology clinics at the Hotel Dieu Hospital, Clermont-Ferrand, France, and University Hospital Vall d'Hebron, Barcelona, Spain. Patients were not included if they had taken antibiotics in the last 2 months, were currently under treatment for depression, presented known psychiatric pathology, had a history of organic intestinal disease, gastrointestinal surgery, family history of colon cancer, inflammatory bowel disease, thyroid dysfunction, Hirschprung disease, diabetes, anorexia, scleroderma, pregnancy, known allergy, alcohol or tobacco abuse (more than 30 g alcohol or 20 cigarettes per day) or were included in another clinical study. Treatments with potential effects on gastrointestinal motor function were discontinued at least 8 days before inclusion. Patients were required to have a customary consumption of <20 g of dietary fibers as evaluated by a pre entry dietary questionnaire. Furthermore, only patients with rectal hypersensitivity demonstrated by a screening test (see Experimental design and procedures below) were included in the treatment phase.

The study protocol was applied in accordance with International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use guidelines and the principles laid down in the current version of the Declaration of Helsinki. The protocol was approved by ethical committees in Clermont-Ferrand (France, AU 753) and the University Hospital Vall d'Hebron and was registered in the ClinicalTrials.gov (NCT00806104) database. All participants gave written informed consent prior to inclusion in the study.

Key Points

- Short-chain fructooligosaccharides (scFOS) improve functional digestive complaints. Our aim was to evaluate the effects of scFOS on rectal sensitivity, fecal microbiota and symptoms in patients with irritable bowel syndrome.¹¹
- Rectal sensitivity improved with scFOS and placebo alike; however, scFOS, but not placebo, significantly increased fecal Bifidobacteria and reduced anxiety scores.
- Changes in microbiota induced by scFOS may be particularly beneficial in patients with digestive symptoms and anxiety.

2.2 | Test products

The scFOS used were produced from sucrose and contained 5% of simple sugars (sucrose, fructose and glucose) and 95% of pure scFOS divided into 37 ± 6% 1-kestose (GF2), 53 ± 6% nystose (GF3), and 10 ± 6% 1F-β-fructofuranosyl nystose (GF4) (Actilight 950P; Beghin-Meiji, Marckolsheim, France). The placebo consisted in completely digestible maltodextrins containing the same proportion (5%) of simple sugars (sucrose, fructose, and glucose) (Maldex 120; Tereos Syral, Marckolsheim, France). Both products were administered in identical 2.5 g sachets. The patients were instructed to take two sachets (5 g) per day diluted in a drink or mixed with solid food over a 28-day period.

2.3 | Experimental design and procedures

The parallel, placebo-controlled, randomized, and double-blind study assessed the effects of dietary supplementation (5 g d⁻¹) with scFOS vs placebo for 4 weeks on rectal sensitivity (primary outcome), clinical outcomes and composition of fecal microbiota in IBS patients with rectal hypersensitivity (defined as a discomfort threshold ≤44 g; see Rectal sensitivity below). A computer-generated randomization list was balanced between the two treatments in groups of four subjects. In each group, patient outcomes were measured immediately before and at the end of the treatment period.

2.3.1 | Rectal sensitivity

Rectal sensitivity was assessed by measuring perceived rectal distension. Rectal distension was produced by inflating a rectal bag by means of a computerized air pump (Tensostat, Sicie, Barcelona, Spain) that applied fixed tension levels to the rectal wall.^{14–16} Stepwise rectal distension was produced in 4-g steps every 2 minutes until participants reported discomfort. At the end of each distending step participants were asked to report the sensations perceived during the preceding minute on a scale of 0–6.¹⁷ The discomfort threshold was defined as the lowest wall tension that induced discomfort (perception score ≥5 on the 0–6 scale). This test was performed before (day 0) and at the end of the intervention period (day 28).

2.3.2 | Clinical outcomes

Stool form was evaluated at inclusion using the validated Bristol stool chart.¹⁸ Patients were instructed to fill in at home, validated versions in French and Spanish of the Hospital Anxiety Depression (HAD) scale¹⁹ and Severity Scoring System of IBS.²⁰

2.3.3 | Fecal microbiota analysis

Fecal samples from IBS patients were collected at home within the 15 hours preceding each rectal sensitivity test. The patients were instructed to place the sample in a closed plastic box, to add an Anaerocult A® sachet (Merck, Darmstadt, Germany) to maintain anaerobic conditions, and to store the box below +15°C until the visit. After opening the Anaerocult A® sachet, chemical reactions release CO₂ and absorb O₂ to maintain anaerobic conditions but with no contact of the content of the sachet with the fecal sample.

Fecal samples were immediately homogenized and separated into five tubes stored at -80°C in liquid nitrogen for further processing. The population levels of taxonomic groups of the fecal microbiota previously shown to be affected in IBS gut microbiota^{21,22} or to be modified by FOS administration,²³ were quantified in fecal samples using real-time PCR (Eppendorf Master Cycler ep RealPlex™ 2.0). Total DNA from fecal materials (200 mg) was extracted using the QIAamp DNA Stool Mini kit (Qiagen Inc., Mississauga, ON, Canada) after mechanical disruption of cells obtained by adding glass beads (3 mm) to the fecal mixture and homogenisation in a bead beater for 5 minutes at 1500 rpm (Retsch MM200; Retscg GmbH, Haan, Germany) before DNA extraction. The DNA extracted was then used to amplify regions of 16S rDNA genes with specific primers. Real-time quantitative PCR was performed using the Brilliant SYBR green system (Roche, Manheim, Germany) with specific primers and under the conditions previously described for Eubacteria,²⁴ Bifidobacteria,²⁵ Lactobacilli,²⁶ Enterobacteriaceae,²⁷ and Roseburia - *E. rectale* and *Faecalibacterium prausnitzii*²⁸ (see supplementary table for details). Standard curves, threshold cycle (TC) vs the number of target gene copies, were carried out for each test. To determine the number of copies of the target gene present in the fecal stool sample, the DNA was diluted and three assays per dilution performed. Calculation of the number of copies of the target gene present in the sample was based on the threshold cycle value obtained during the quantitative PCR reaction with respect to the corresponding standard curve.

2.4 | Statistical analysis

Sample size was calculated anticipating a difference in rectal discomfort threshold between scFOS and saline of 4 g; 30 patients per group were estimated necessary to demonstrate statistical significance with a power of 80% and a significance level of 5%. Considering a possible exclusion of 20% of patients between the intention to treat (ITT) and the per protocol (PP) populations, 36 subjects per group were needed at the start of the study. Statistical analysis was performed on PP population and not on ITT population because there was only

a difference of two subjects between the two (one from the scFOS group and one from the placebo group). The chi-square test was used to analyze qualitative data. Analysis of variance, including periods, subjects, treatment and center as factors, was used to analyze quantitative data. Verification of normal distribution was performed on the residual values. Post-hoc analyses were performed by the Tukey test. Analyses were made using SAS software (v 9.2 for Windows, Carry, NC, USA) and differences were considered significant with a P value=.05.

3 | RESULTS

3.1 | Demographic data

Nine patients were screened but finally not randomized because they presented normal rectal sensitivity according to the inclusion criteria (see Participants and Experimental design and Procedures above). The ITT population consisted of 41 subjects in the scFOS group and 38 subjects in the placebo group. Two subjects were excluded from the PP population because they received unauthorized medical treatment (colonic lavage prior to the rectal sensitivity test and antibiotic treatment). The patients were randomly distributed in a harmonious manner (Fig. 1) between the two centers with slightly more men in Spain than in France ($P=.03$) but the distribution of patients according to IBS clinical sub-groups was identical between the two treatments groups (Table 1) and the two centers.

3.2 | Adverse effects

Intake of scFOS was well-tolerated and the number of adverse events was similar in the scFOS (n=18) and placebo (n=21) groups.

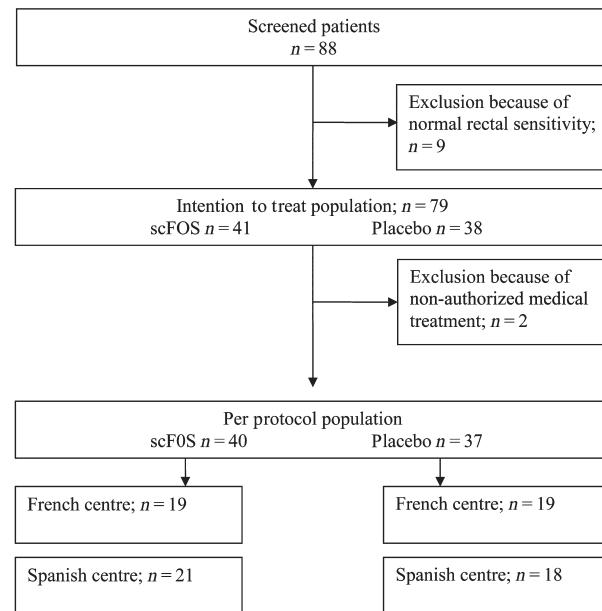


FIGURE 1 Distribution of subjects among intention to treat and per protocol populations

TABLE 1 Characteristics of patients in the intention-to treat groups at baseline

	scFOS group (n=41)		Placebo group (n=38)		P values
	Mean	SD	Mean	SD	
Age (years)	41.0	11.1	42.4	10.6	.582
Height (m)	1.66	0.09	1.67	0.09	.693
Weight (kg)	64.1	11.9	61.8	10.5	.314
Gender M/F (%)	22/78		26/74		.798
IBS type					
Constipation	10		11		.349 ^a
Diarrhea	11		12		
Unclassified	19		12		
Mixed	0		2		
Rectal distension (g)	28.6	11.9	28.7	11.8	.868
Bristol total score	6.3	4.1	6.8	3.9	.642
Abdominal pain score	52.1	21.9	53.0	22.6	.791
Number of days of abdominal pain	5.87	2.75	6.03	2.71	.760
Total IBS ^a score	296.1	90.1	284.4	79.7	.577
Total HAD	15.4	5.5	14.0	7.1	.250
Anxiety score	10.0	3.1	9.3	4.1	.129
Depression score	5.4	3.3	4.6	4.1	.686

^aAn chi-squared test was carried out to determine the difference in IBS subgroup distribution between the groups.

TABLE 2 Maximum tension (g) applied during the rectal sensitivity evaluation at D0 and D28 by IBS sub-type

	scFOS group	Placebo group	P values
Constipation	n=10	n=10	
D0	32.8 ± 13.2	27.7 ± 29.6	.215
D28	38.0 ± 14.1	29.6 ± 17.3	.051
Diarrhea	n=11	n=12	
D0	26.2 ± 9.4	29.0 ± 12.8	.507
D28	36.5 ± 18.7	38.7 ± 14.0	.616
Unclassified	n=19	n=12	
D0	27.8 ± 12.6	28.3 ± 12	.977
D28	33.8 ± 17.2	34.7 ± 17.1	.804

3.3 | Rectal sensitivity

Rectal sensitivity (discomfort threshold measured as the lowest wall tension that induced discomfort) before treatment was identical in the two treatment groups (Table 2). After 28 days of treatment, there was a significant improvement in rectal sensitivity both in the scFOS group (discomfort threshold increased by $+7.0 \pm 13.8$ g; $P=.003$ vs basal) and in the placebo group (by $+5.9 \pm 13.8$ g; $P=.013$ vs basal), and the difference between groups was not statistically significant ($P=.565$). However, post hoc analysis showed that the effect of scFOS was more pronounced in constipation-predominant IBS patients (IBS-C) than in other subgroups (i.e. those with diarrhea or alternating bowel habit): in IBS-C the threshold of discomfort at the end of the study tended to be higher in those receiving scFOS than those receiving placebo ($P=.051$) (Fig. 2).

3.4 | Clinical outcomes

The IBS severity composite score was identical in the two groups prior to treatment (Table 1). This score significantly improved after 28 days ($P=.001$) but with no difference between the scFOS and placebo groups (-68.7 ± 124.4 and -76.2 ± 123.5 , respectively; $P=.721$). In the sub-group of constipated IBS patients, the reduction in severity score was greater for the scFOS than the placebo group, but the difference was not statistically significant (-122.3 ± 157.2 and -38.1 ± 120.7 , respectively; $P=.1258$). However, the proportion of subjects feeling abdominal distension at the end of treatment in the scFOS group (72%) was significantly lower ($P=.001$) than at the start of the study, whereas the effect was not significant in the placebo group where 78% still experienced abdominal distension ($P=.110$) (Fig. 3).

Before treatment, anxiety and depression scores were similar in the scFOS and placebo groups (Table 1). These scores improved after treatment, but the improvement of both the global score and the anxiety score was significantly greater in the scFOS than in the placebo group (Fig. 4).

3.5 | Fecal microbiota

The composition of the subjects' fecal bacterial populations was similar in both groups at inclusion (Table 3). By the end of the treatment period a significant increase in Bifidobacteria in the scFOS-supplemented group was observed ($P<.05$) while total anaerobes and most of the other bacterial groups studied were not modified (Table 3). The change in Bifidobacteria in the scFOS group was not

FIGURE 2 Change in threshold of discomfort between D28 and D0 with placebo and short-chain fructooligosaccharides (scFOS) in the sub-group of constipated irritable bowel syndrome patients

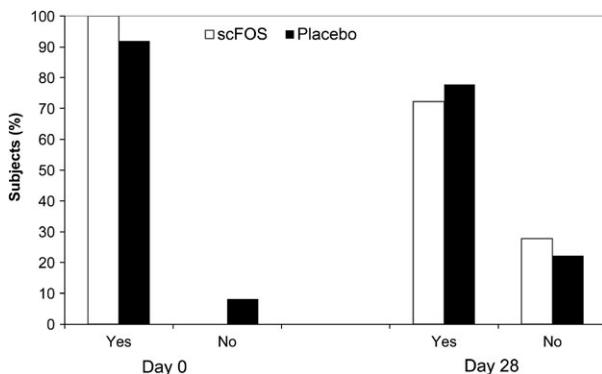
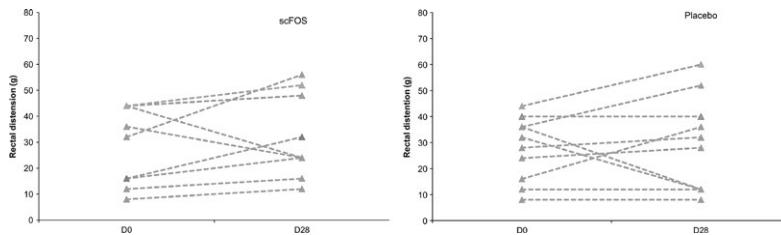


FIGURE 3 Distribution of subjects experiencing abdominal pain (irritable bowel syndrome questionnaire) before and after 4 weeks of short-chain fructooligosaccharides (scFOS) or placebo consumption in the per protocol population. A chi-squared test was carried out to determine the difference between day 0 and day 28 within each group: scFOS: $P<.05$, placebo: $P=.11$

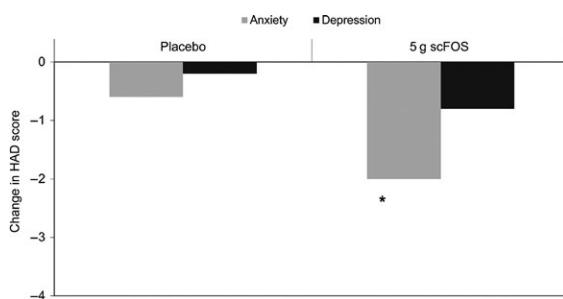


FIGURE 4 Change in Hospital Anxiety Depression (HAD) subscore of anxiety and depression between d28 and d0 for placebo and scFOS groups. *Total score: $P=.045$; Anxiety score: $P=.037$; Depression score: $P=.468$

statistically different than that in the placebo group. The change of Bifidobacteria was $+0.6\log$ with scFOS and $-0.04\log$ with placebo in the IBS-C subgroup and the difference did not reach statistically significance.

4 | DISCUSSION

This study confirms that scFOS intake stimulates the growth of Bifidobacteria and further shows a significant improvement in anxiety scores in IBS patients as compared with placebo. However, their effect on rectal sensitivity depended on clinical subgroup, with a tendency for improvement with respect to placebo only in the subgroup with constipation.

Many studies indicate that the composition of fecal microbiota in IBS patients differs from that of healthy people^{21,29} and appears to be less stable over time.^{30,31} In particular, a reduction in the fecal bifidobacteria population and *Bifidobacterium catenulatum* was observed in six of eight studies in a review including European and Asian populations.^{21,32} In addition, differences in the fermentation activities of fecal microbiota have been also described in IBS patients, with a shift toward acetate and propionate rather than butyrate production,³³ and this may be pathophysiologically important, because butyrate may be able to lower visceral perception.³⁴ A functional dysbiosis in IBS-C gut microbiota was recently reported,²¹ leading to higher levels of sulfides and less butyrate production compared with healthy subjects. Furthermore, altered microbiota of IBS-C patients was also shown to play a role in genesis of visceral hypersensitivity observed in IBS-gut microbiota associated rats, probably through a specific bacterial metabolite.²² In our study, the gut microbiota composition of IBS was investigated through molecular quantification of the main bacterial taxa of the population levels. We acknowledge that Next Generation Sequencing technology, such as mRNA sequencing, could have provided additional information. As previously observed,⁷ the intake of 5 g of scFOS per day for 4 weeks in the present study significantly increased the concentration of fecal Bifidobacteria without significant changes in the other microbial populations that were analyzed, and without increasing the total number of anaerobes. It has been shown that the stimulating effect of scFOS on Bifidobacteria is more prominent when the initial level is low¹⁰ but the dose of scFOS used in our study could have not been enough to induce detectable effects.

The symptom severity score in our IBS pool was in the range of moderate to severe, and was identical in both groups of patients prior to treatment.²⁰ Symptom severity significantly improved after 4 weeks of treatment but with no difference between the scFOS and placebo groups; note that the placebo effect was very strong and this could explain these negative findings. In a previous study on the effect of scFOS on digestive symptoms in healthy subjects, scFOS reduced the frequency of abdominal distension after 4 weeks of supplementation, and this was not observed with placebo, even if the placebo effect was very strong (more than 70% of subjects felt improved intestinal discomfort).¹²

For the first time, our study shows that scFOS improves anxiety-depression scores in IBS patients, specifically due to a reduction in anxiety. Whereas the depression score evaluated at the beginning of the study was within the normal range (what is considered as "non case"), anxiety scores reached a level of borderline clinical significance (anxiety set above 11).¹⁹ These pretreatment levels could explain the differential effects of scFOS on anxiety (improved) vs depression (no

TABLE 3 QPCR quantification (\log_{10} copies of 16S rRNA gene g^{-1}) of bacterial communities in fecal samples from IBS patients before and after a 28-day period of scFOS (5 g d^{-1}) or placebo administration

	scFOS group (n=34)		Placebo group (n=34)		<i>P</i> values Treatment × Time
	D0	D28	D0	D28	
Eubacteria	10.78 ± 0.73	10.82 ± 0.63	10.94 ± 0.49	11.14 ± 0.52	.090
Bifidobacterium	8.25 ± 0.91	8.96 ± 0.74 ^a	8.59 ± 0.88	8.62 ± 0.87	.001
Lactobacillus	6.99 ± 0.82	7.24 ± 0.96	6.75 ± 0.66	6.84 ± 0.57	.388
Enterobacteriaceae	7.28 ± 1.15	7.58 ± 1.21	7.94 ± 0.95	7.88 ± 1.14	.510
Roseburia spp/ <i>Eubacterium rectale</i> group	9.08 ± 0.91	8.84 ± 1.06	8.92 ± 0.77	9.22 ± 0.93 ^a	.032
<i>Faecalibacterium prausnitzii</i>	9.57 ± 0.92	9.34 ± 0.80	9.85 ± 0.72	9.50 ± 0.90	.723

^aSignificant change from D0 within the same group.

change). The link between modulation of the gut microbiota and a reduction of HAD score has already been observed in a few studies evaluation pre- or probiotic supplementation.^{35,36} And more generally more and more studies acknowledge the link between gut microbiota and anxiety especially in the case of patients with irritable bowel syndrome.⁵ Authors suggest an interaction among gut microbiota, stress level, and higher perception of visceral pain. One study in healthy adults demonstrated a strong inverse correlation between the level of fecal Bifidobacteria and abdominal pain. Subjects who experienced pain had two times less fecal Bifidobacteria (as evaluated by qPCR) than subjects without recorded pain.³⁷ The changes of metabolites (e.g. SCFA) produced by the bacterial inhabitants of the gut may partly explain changes in symptomatology and especially visceral pain.⁵

Rectal hypersensitivity has been proposed as an IBS marker.^{38,39} Abdominal pain could be related to hypersensitivity, and indeed, some studies indicate that hypersensitivity to rectal distension in IBS is predicted by symptom severity.^{40,41} In-vitro and animal studies using human fecal microbiota have also revealed an effect of scFOS on other microbial groups, in particular the *Eubacterium rectale* - *Clostridium coccoides* group,^{23,42} that included some of the major butyrate-producing species from the human gut. Accordingly, scFOS are known to stimulate the production of short chain fatty acids (SCFA), and in the long-term this stimulation is rather oriented toward butyrate production.^{43,44} Experimental studies showed that rectal instillation of butyrate increased colonic sensitivity in rats.⁴⁵ However, in healthy subjects colonic administration of butyrate, at physiologically relevant concentrations, dose-dependently decreased visceral sensitivity.³⁴ Contrary with our hypothesis, the effect of scFOS on rectal sensitivity in the group of IBS patients as a whole was similar to that of placebo. Our study included only patients with proven rectal hypersensitivity at prescreening and applied state-of-the-art methodology. Indeed, the tensostat has been shown to be a reliable tool for the evaluation of visceral sensation (detection of hypersensitivity with 95% sensitivity and 72% specificity).⁴⁶ The tensostat applies fixed tension levels to the gut wall; since gut perception relays activation of tension receptors, the tensostat standardizes the stimulus regardless of the size or contractile activity of the organ.^{15,16}

The 5 g dose was shown to be effective in healthy populations with minor digestive disorders, but may be too low for patients fulfilling IBS criteria. Indeed, scFOS stimulates the growth of Bifidobacteria in a dose-dependent manner from 2.5 to 10 g d^{-1} in humans.⁷ Therefore, a dose of between 5 and 10 g d^{-1} should be considered for future studies with IBS patients.

However, it is interesting to note that the effects of scFOS on digestive symptoms in our study and elsewhere⁴⁷ were more prominent in constipated subjects. It was not evaluated in our study but already reported elsewhere that constipated subjects usually present a lower proportion of *Roseburia-E rectale* in their fecal microbiota in comparison to healthy subjects.^{21,30} It has also been shown by an in vitro fermentation study and in gnotobiotic mice with human fecal slurry, that scFOS stimulate this group of bacteria.^{23,42} However in our study *Roseburia-E rectale* was not modified by scFOS and, again, it might be related to the low dose tested and could explain the lack of effect on rectal sensitivity.

Our study failed to prove the primary outcome, but still provides useful data suggesting potential beneficial effects of scFOS in IBS, and provides hints for the proper design of studies in terms of sensitive target population (constipation) and effective dose (>5 g d^{-1}).

ACKNOWLEDGMENTS

Delphine Roux for her technical assistance.

FUNDING

The study was financially supported by Beghin-Meiji Company and by the Spanish Ministry of Economy and Competitiveness (Direccion General de Investigacion Cientifica y Tecnica, SAF 2013-43677-R; Ciberherd is funded by the Instituto de Salud Carlos III).

CONFLICTS OF INTEREST

FA, CD, ABD, JMC, AA, JS, and MD declare no conflicts of interest. AW and FR are employed by Tereos.

AUTHOR CONTRIBUTION

FA, CD, ABD, JMC, AW, FR, and MD were responsible for the study design; FA, CD, ABD, AA, JS, and MD carried out the study; FA, ABD, FR wrote the manuscript; CD, JMC carried out the statistical analysis; FA, CD, ABD, JMC, AA, JS, AW, FR, and MD reviewed and approved the final manuscript.

REFERENCES

- Fukudo S, Kaneko H, Akiho H, et al. Evidence-based clinical practice guidelines for irritable bowel syndrome. *J Gastroenterol*. 2015;50:11–30.
- Ohman L, Tornblom H, Simren M. Crosstalk at the mucosal border: importance of the gut microenvironment in IBS. *Nat Rev Gastroenterol Hepatol*. 2015;12:36–49.
- Kerckhoffs APM, Samsom M, van der Rest ME, et al. Lower Bifidobacteria counts in both duodenal mucose-associated and faecal microbiota in irritable bowel syndrome patients. *World J Gastroenterol*. 2009;15:2887–2892.
- Ponnusamy K, Choi JN, Lee SY, Lee CH. Microbial community and metabolomic comparison of irritable bowel syndrome faeces. *J Med Microbiol*. 2011;60:817–827.
- Moloney RD, Johnson AC, O'Mahony SM, Dinan TG, Greenwood-Van Meerveld B, Cryan JF. Stress and the microbiota-gut-brain axis in visceral pain: relevance to irritable bowel syndrome. *CNS Neurosci Ther*. 2016;22:102–117.
- Bouhnik Y, Flourié B, Riottot M, et al. Effects of fructo-oligosaccharides ingestion on fecal Bifidobacteria and selected metabolic indexes of colon carcinogenesis in healthy humans. *Nutr Cancer*. 1996;26:21–29.
- Bouhnik Y, Raskine L, Simoneau G, Paineau D, Bornet F. The capacity of short-chain fructo-oligosaccharides to stimulate fecal Bifidobacteria: a dose-response relationship study in healthy humans. *Nutr J*. 2006;5:8.
- Hidaka H, Eida T, Takizawa T, Tokunaga T, Tashiro Y. Effects of fructo-oligosaccharides on intestinal flora and human health. *Bifidobacteria Microflora*. 1986;5:37–50.
- Tokunaga T, Nakada Y, Tashiro Y, Hirayama M, Hidaka H. Effects of fructooligosaccharides (FOS) intake on the intestinal microflora and defecation in healthy volunteers. *Bifidus*. 1993;6:143–150.
- Rycroft CE, Jones MR, Gibson GR, Rastall RA. A comparative in vitro evaluation of the fermentation properties of prebiotic oligosaccharides. *J Appl Microbiol*. 2001;91:878–887.
- Boutron-Ruault MC, Marteau P, Lavergne-Slove A, et al. Effects of a 3-mo consumption of short-chain fructo-oligosaccharides on parameters of colorectal carcinogenesis in patients with or without small large colorectal adenomas. *Nutr Canc*. 2005;53:160–168.
- Paineau D, Payen F, Panserieu S, et al. The effects of regular consumption of short-chain fructo-oligosaccharides on digestive comfort of subjects with minor functional bowel disorders. *Br J Nutr*. 2008;99:311–318.
- Drossman DA. The functional gastrointestinal disorders and the Rome III process. *Gastroenterology*. 2006;130:1377–1390.
- Distrutti E, Azpiroz F, Soldevilla A, Malagelada J-R. Gastric wall tension determines perception of gastric distension. *Gastroenterology*. 1999;116:1035–1042.
- Distrutti E, Salvio B, Azpiroz F, Malagelada JR. Rectal function and bowel habit in irritable bowel syndrome. *Am J Gastroenterol*. 2004;99:131–137.
- Caldarella MP, Azpiroz F, Malagelada J-R. Selective effects of nutrients on gut sensitivity and reflexes. *Gut*. 2007;56:37–42.
- Aspiroz F. Intestinal perception: mechanisms and assessment. *Brit J Nutr*. 2005;93:S7–S12.
- Lewis S, Heaton K. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol*. 1997;32:920–924.
- Zigmond AS, Snaith RP. The Hospital Anxiety Depression scale. *Acta Psychiatr Scand*. 1983;67:361–370.
- Francis CY, Morris J, Whorwell PJ. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. *Aliment Pharmacol Ther*. 1997;11:395–402.
- Chassard D, Dapoigny M, Scott KP, et al. Functional dysbiosis within the gut microbiota of patients with constipated-irritable bowel syndrome. *Aliment Pharmacol Ther*. 2012;35:828–838.
- Crouzet L, Gaultier E, Del'Homme C, et al. The hypersensitivity to colonic distension of IBS patients can be transferred to rats through their fecal microbiota. *Neurogastroenterol Motil* 2013; 25: 272–282.
- Saulnier DMA, Gibson GR, Kolida S. In vitro effects of selected probiotics on the human faecal microbiota composition. *FEMS Microbiol Ecol*. 2008;66:516–527.
- Edwards JE, Huws SA, Kim EJ, Kingston-Smith AH. Characterization of the dynamics of initial bacterial colonization of nonconserved forage in the bovine rumen. *FEMS Microbiological Ecology*. 2007;62:323–335.
- Matsuki T, Watanabe K, Fujimoto J, et al. Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal Bifidobacteria. *Appl Environ Microbiol*. 2004;70:167–173.
- Furet JP, Firmesse O, Gourmelon M, et al. Comparative assessment of human and farm animal faecal microbiota using real-time quantitative PCR. *FEMS Microbiol Ecol*. 2009;68:351–362.
- Bartsch S, Fite A, Macfarlane GT, McMurdo MET. Characterization of bacterial communities in feces from healthy elderly volunteers and hospitalized elderly patients by using real-time PCR and effects of antibiotic treatment on the fecal microbiota. *Appl Environ Microbiol*. 2004;70:3575–3581.
- Ramirez-Farias C, Slezak K, Fuller Z, Duncan A, Holtrop G, Louis P. Effect of inulin on the human gut microbiota: stimulation of *Bifidobacterium adolescentis* and *Faecalibacterium prausnitzii*. *Br J Nutr*. 2009;101:541–550.
- Rajilic-Stojanovic M, Biagi E, Heilig HG, et al. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. *Gastroenterology*. 2011;141:1792–1801.
- Maukonen J, Satokari R, Mattö J, Söderlund H, Mattila-Sandholm T, Saarela M. Prevalence and temporal stability of selected clostridial groups in irritable bowel syndrome in relation to predominant faecal bacteria. *J Med Microbiol*. 2006;55:625–633.
- Mattö J, Maunuksela L, Kajander K, et al. Composition and temporal stability of gastrointestinal microbiota in irritable bowel syndrome – a longitudinal study in IBS and control subjects. *FEMS Immunol Med Microbiol*. 2005;43:213–222.
- Roberfroid MB, Gibson GR, Hoyles L, et al. Prebiotic effects: metabolic and health benefits. *Br J Nutr*. 2010;104: 111–111.
- Tana C, Umesaki Y, Imaoka A, Handa T, Kanazawa M, Fukudo S. Altered profiles of intestinal microbiota and organic acids may be the origin of symptoms in irritable bowel syndrome. *Neurogastroenterol Motil*. 2010;22:512–519.
- Vanhoutvin SALW, Troost FJ, Kilkens TOC, et al. The effects of butyrate enemas on visceral perception in healthy volunteers. *Neurogastroenterol Motil*. 2009;21:e952–e976.
- Messaoudi M, Lalonde R, Vioille N, et al. Assessment of psychotropic-like properties of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in rats and human subjects. *Br J Nutr*. 2011;105:755–764.
- Silk DBA, Davis A, Vulevic J, Tzortzis G, Gibson GR. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol Ther*. 2009;29:508–518.

37. Jalanka-Tuovinen J, Salonen A, Nikkila J, et al. Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS ONE*. 2011;6:e23035.
38. Mertz H, Naliboff B, Munakata J, Niazi N, Mayer E. Altered rectal sensitivity is a biological marker of patients with irritable bowel syndrome. *Gastroenterology*. 1995;109:40–52.
39. Delvaux M. Do we need to perform rectal distension tests to diagnose IBS in clinical practice? *Gastroenterology*. 2002;122:2075–2078.
40. Posserud I, Syrous A, Lindström L, Tack J, Abrahamsson H, Simren M. Altered rectal perception in irritable bowel syndrome is associated with symptom severity. *Gastroenterology*. 2007;133:1113–1123.
41. Van der Veen PP, Van Rood YR, Masclee AA. Symptom severity but no psychopathology predicts visceral hypersensitivity in irritable bowel syndrome. *ClinGastroenterol Hepatol*. 2008;6:321–328.
42. Respondex F, Gerard P, Bossis M, et al. Short-chain fructooligosaccharides modulate intestinal microbiota and metabolic parameters of humainized gnotobiotic diet induced obesity mice. *PLoS ONE*. 2013;8:e71026.
43. Le Blay G, Michel C, Blottière HM, Cherbut C. Prolonged intake of fructo-oligosaccharides induces a short-term elevation of lactic acid-producing bacteria and a persistent increase in caecal butyrate in rats. *J Nutr*. 1999;129:2231–2235.
44. Tsukahara T, Iwasaki Y, Nakayama K, Ushida K. Stimulation of butyrate production in the large intestine of weaning piglets by dietary fructooligosaccharides and its influence on the histological variables of the large intestinal mucosa. *J Nutr Sci Vitaminol*. 2003;49:414–421.
45. Bourdu S, Dapoigny M, Chapuy E, et al. Rectal instillation of butyrate provides a novel clinically relevant model of non-inflammatory hypersensitivity in rats. *Gastroenterology*. 2005;128:1996–2008.
46. Bouin M, Plourde V, Boivin M, et al. Rectal distension testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology*. 2002;122:1771–1777.
47. Tominaga S, Hirayama M, Adachi T, Tokunaga T, Iino H. Effects of ingested fructooligosaccharides on stool frequency in healthy female volunteers: a placebo-controlled study. *Bifidobacteria Microflora*. 1999;18:49–53.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.