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

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## 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  $\leq 44$  g) a parallel, placebo-controlled, randomized, and double-blind study was performed to assess the effects of dietary supplementation (5 g d<sup>-1</sup>) 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 & Interferences:** 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.

## KEYWORDS

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.<sup>1,2</sup> 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<sup>3,4</sup> and differences in microbiota may be related to their sensory dysfunction.<sup>5</sup>

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

and activity of the intestinal microbiota.<sup>6–8</sup> In humans, increasing doses of scFOS supplementation of between 2.5 and 10 g d<sup>-1</sup> is associated with an increase in fecal bifidobacteria<sup>7</sup> which is generally greater when the initial concentration of bifidobacteria is relatively low.<sup>9,10</sup>

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

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 criteria<sup>13</sup> 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.
- 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<sup>-1</sup>) 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.<sup>14–16</sup> 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.<sup>17</sup> 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.<sup>18</sup> Patients were instructed to fill in at home, validated versions in French and Spanish of the Hospital Anxiety Depression (HAD) scale<sup>19</sup> and Severity Scoring System of IBS.<sup>20</sup>

### 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<sup>®</sup> sachet (Merck, Darmstadt, Germany) to maintain anaerobic conditions, and to store the box below +15°C until the visit. After opening the Anaerocult A<sup>®</sup> sachet, chemical reactions release CO<sub>2</sub> and absorb O<sub>2</sub> 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<sup>21,22</sup> or to be modified by FOS administration,<sup>23</sup> 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; Retsch 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, Mannheim, Germany) with specific primers and under the conditions previously described for Eubacteria,<sup>24</sup> Bifidobacteria,<sup>25</sup> Lactobacilli,<sup>26</sup> Enterobacteriaceae,<sup>27</sup> and Roseburia - *E. rectale* and *Faecalibacterium prausnitzii*<sup>28</sup> (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.

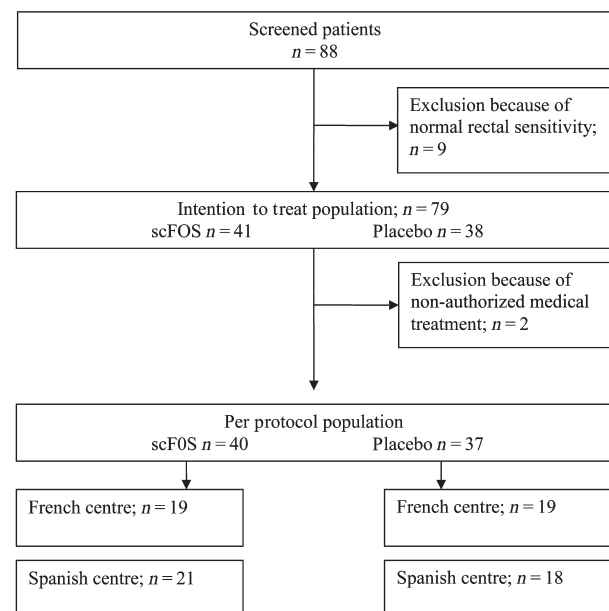
## 3 | RESULTS

### 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 <sup>a</sup> |
| 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 <sup>a</sup> 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              |

<sup>a</sup>An 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

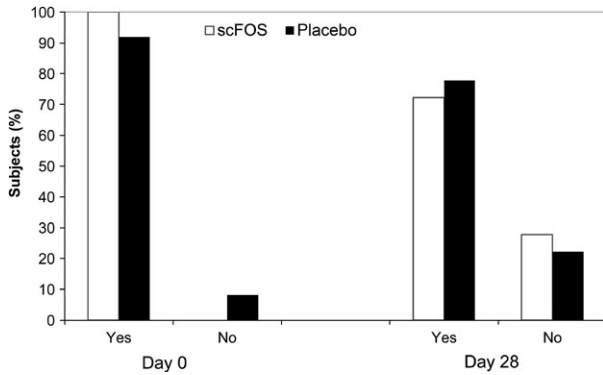
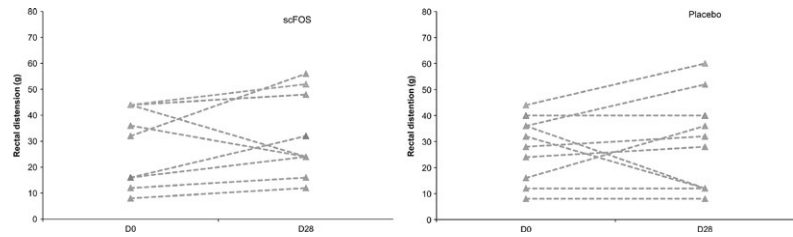
The IBS severity composite score was identical in the two groups prior to treatment (Table 1). This score significantly improved after 28 days ( $P=.001$ ) but with no difference between the scFOS and placebo groups ( $-68.7 \pm 124.4$  and  $-76.2 \pm 123.5$ , respectively;  $P=.721$ ). In the sub-group 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 ( $-122.3 \pm 157.2$  and  $-38.1 \pm 120.7$ , 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).

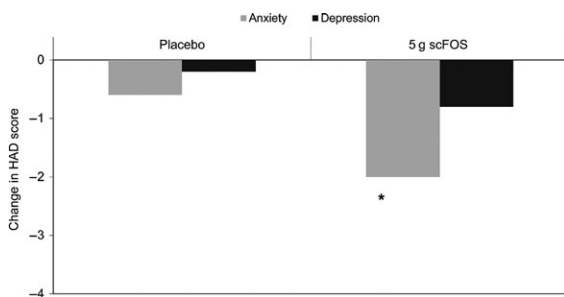
### 3.5 | Fecal microbiota

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 statistical 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<sup>21,29</sup> and appears to be less stable over time.<sup>30,31</sup> 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.<sup>21,32</sup> 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,<sup>33</sup> and this may be pathophysiologically important, because butyrate may be able to lower visceral perception.<sup>34</sup> A functional dysbiosis in IBS-C gut microbiota was recently reported,<sup>21</sup> 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.<sup>22</sup> 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,<sup>7</sup> 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 low<sup>10</sup> 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.<sup>20</sup> 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).<sup>12</sup>

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).<sup>19</sup> 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) |                          | P values<br>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 <sup>a</sup> | 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/Eubacterium rectale group | 9.08 ± 0.91        | 8.84 ± 1.06              | 8.92 ± 0.77          | 9.22 ± 0.93 <sup>a</sup> | .032                         |
| Faecalibacterium prausnitzii            | 9.57 ± 0.92        | 9.34 ± 0.80              | 9.85 ± 0.72          | 9.50 ± 0.90              | .723                         |

<sup>a</sup>Significant 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.<sup>35,36</sup> 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.<sup>5</sup> 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.<sup>37</sup> 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.<sup>5</sup>

Rectal hypersensitivity has been proposed as an IBS marker.<sup>38,39</sup> Abdominal pain could be related to hypersensitivity, and indeed, some studies indicate that hypersensitivity to rectal distension in IBS is predicted by symptom severity.<sup>40,41</sup> 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 coccooides* group,<sup>23,42</sup> 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.<sup>43,44</sup> Experimental studies showed that rectal instillation of butyrate increased colonic sensitivity in rats.<sup>45</sup> However, in healthy subjects colonic administration of butyrate, at physiologically relevant concentrations, dose-dependently decreased visceral sensitivity.<sup>34</sup> 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).<sup>46</sup> 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.<sup>15,16</sup>

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.<sup>7</sup> 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 elsewhere<sup>47</sup> 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.<sup>21,30</sup> 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.<sup>23,42</sup> 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  $g d^{-1}$ ).

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## 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.

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## SUPPORTING INFORMATION

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