

Randomized Control Trials

Effect of synbiotic in constipated adult women – A randomized, double-blind, placebo-controlled study of clinical response[☆]

Dan L. Waitzberg^{a,b}, Luciana C. Logullo^b, Amanda F. Bittencourt^b, Raquel S. Torrinhas^{a,*},
 Glauca M. Shiroma^b, Natalia P. Paulino^b, Maria L. Teixeira-da-Silva^b

^a University of São Paulo Scholl of Medicine, Department of Gastroenterology, Surgical Gastroenterology Discipline (LIM 35), Sao Paulo, Brazil

^b GANEP - Nutrição Humana, Sao Paulo, Brazil

ARTICLE INFO

Article history:

Received 15 August 2012

Accepted 15 August 2012

Keywords:

Synbiotic
 Fructooligosaccharides
 Lactobacillus
 Bifidobacteria
 Constipation
 Probiotic

SUMMARY

Background & aims: Synbiotic intake may selectively change microbiota composition, restore microbial balance in the gut and improve gastrointestinal functions. We have assessed the clinical response of chronically constipated women to a commercially available synbiotic, combining fructooligosaccharides with *Lactobacillus* and *Bifidobacterium* strains (LACTOFOS[®]).

Methods: Following 1 week of non-interventional clinical observation, 100 constipated adult women, diagnosed by ROME III criteria, were randomized to receive two daily doses (6 g) of synbiotic or maltodextrin (placebo group), for 30 days. Treatment response was evaluated by patient's daily record of evacuation (stool frequency, consistency and shape, according to Bristol scale), abdominal symptoms (abdominal pain, bloating and flatulence) and constipation intensity (Constipation Scoring System AGACHAN).

Results: Patients treated with synbiotic had increased frequency of evacuation, as well as stool consistency and shape nearer normal parameters than the placebo group, with significant benefits starting during the second and third weeks, respectively (interaction group/time, $P < 0.0001$). There were no significant differences in abdominal symptoms, but AGACHAN score was better in the synbiotic than in the placebo group.

Conclusions: Dietary supplementation with a synbiotic composed of fructooligosaccharides with *Lactobacillus* and *Bifidobacterium* improved evacuation parameters and constipation intensity of chronically constipated women, without influencing abdominal symptoms. NCT01286376

© 2012 Elsevier Ltd and European Society for Clinical Nutrition and Metabolism. All rights reserved.

1. Introduction

Constipation is a chronic disease estimated to affect about 16% of the worldwide general population and to be 2–3 times more prevalent and symptomatic in women than men.¹ Constipation frequency appears to augment with increasing age, particularly after 65 years old, and large amounts of healthcare resources are expended on its diagnosis and treatment.^{1,2} In addition, available therapies are unsatisfactory in one-third of patients.²

Although constipation disorder is usually defined as ≤ 3 bowel movements per week, patients often equate constipation with abnormal stool consistency, feelings of incomplete emptying, straining, and urge to defecate. Therefore, the ROME III criteria, developed in 2006, include these symptoms in diagnosing constipation.³ In addition, for Rome III criteria patients should rarely pass loose stools without laxatives and have symptoms distinct from those of irritable bowel syndrome (IBS).³

Constipation can be a consequence of intestinal dysbiosis, with an increase of potentially pathogenic microorganisms and a decrease of potentially beneficial microorganisms.⁴ These alterations may affect large bowel motility and secretory functions by changing the metabolic environment of the colon and the amount of available physiologically active substances.⁴

The ingestion of soluble fibers with prebiotic effects, such as inulin and fructooligosaccharides (FOS), stimulates the growth of beneficial bifidobacteria and lactobacilli in the colon.⁵ Oral intake, at adequate concentrations, of specific strains of lactobacilli,

[☆] The results of this study were presented at Clinical Nutrition Week 2012, the annual meeting of The American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.), in Orlando, FL.

* Corresponding author. Laboratório de Nutrição e Cirurgia Metabólica do Aparelho Digestivo – LIM 35 – Faculdade de Medicina da Universidade de São Paulo (FMUSP), Av. Dr. Arnaldo, 455 2º andar, sala 2108 – Cerqueira César, CEP 01246-903, São Paulo – SP, Brazil. Tel.: +55 11 3061 7459; fax: +55 11 3061 7459.

E-mail addresses: torrinhas@uol.com.br, metanutri@usp.br (R.S. Torrinhas).

bifidobacteria and other living commensal microorganisms, generically defined as probiotic, may be associated with health benefits, including improvements in bowel movements, permeability and microbial profile, improved function of intestinal immune barrier and prevention of colon cancer.⁶

The combination of probiotic strains and prebiotic fibers may provide synergistic effects, after which they are named: synbiotics.⁷ The interaction between probiotics and prebiotics *in vivo* may improve the survival of probiotics.⁷ Synbiotic intake has been shown to modify microbiota composition and restore intestinal microbial balance, which may have positive effects on gastrointestinal functions.⁸

This prospective, randomized, double-blind, parallel study was designed to evaluate the effects of a synbiotic, consisting of FOS, *Bifidobacterium lactis* and three different strains of *Lactobacillus*, on chronic constipation in women. Although the individual benefits of FOS and probiotic strains in the treatment of chronic constipation have been reported, the effects of the specific synbiotic associations we utilized have not yet been assessed.

Our aim was to evaluate the clinical response to synbiotic in chronically constipated women, through comparisons of changes in: (1) Frequency of bowel movements and stool consistency and shape; (2) Abdominal symptoms; and (3) Constipation states according to a standard constipation scoring system.

2. Methods

2.1. Ethical issues

The current study was registered in the Clinical Trials Database (ID: NCT01286376) and performed according to the ethical recommendations of the Declaration of Helsinki and the Ethical Committee of the Real e Benemérita Associação Portuguesa de Beneficência do Hospital São Joaquim, which approved the study protocol. All enrolled patients provided written informed consent.

2.2. Patients

Adult (aged 18–75 years) literate female patients from GANEP – Human Nutrition of the Real e Benemérita Associação Portuguesa de Beneficência do Hospital São Joaquim with bowel constipation, as diagnosed by ROME III criteria,³ were screened between May 2010 and February 2011 for eligibility to participate in our study. Exclusion criteria were bowel constipation due to pharmacologic interventions; diagnosis of gastrointestinal diseases (e.g., cancer, previous abdominal surgery, inflammatory bowel diseases) by endoscopic or radiologic evaluation 5 years before or after the symptoms started; disorders of respiratory, cardiologic, renal, hepatic, gastroenterological, hematologic, neurologic or psychiatric functions; other diseases that, in the opinion of the investigator, could significantly affect intestinal transit; lactose intolerance or allergy to any ingredient of fiber supplements; dependence on laxatives; alcohol or drugs use; regular use of antidepressants, opioid narcotic analgesics, anticholinergic or anti-spasmodic agents; use of investigative drugs one month before or during the study; regular use of medications affecting intestinal motility; regular intake (≥ 3 times/week) of products containing pre or probiotics (e.g. yoghurts, dairy drinks, supplements); and antibiotic ingestion in the last 3 months. A computer-generated (GraphPad statistical software) sequence with a block size of 10 patients (1:1 allocation) was employed by an independent investigator to assign the participants to either of the groups (synbiotic and placebo). The patients were enrolled and assigned by one exclusive investigator (L.C.L.) and randomization sequence was concealed until the end of statistical analysis.

2.3. Synbiotic treatment

After a week of non-interventional clinical observation, all included patients were randomized to receive two daily doses of 6 g LACTOFOS[®] (synbiotic group) or maltodextrin (control group), each diluted in 100 ml of water with a minimum of 4 h between doses, for 30 days. Each LACTOFOS[®] sachet contained 6 g of fructooligosaccharide (FOS) and 10^8 – 10^9 bacteria of the strains *Lactobacillus paracasei* (Lpc-37), *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (NCFM) and *Bifidobacterium lactis* (HN019). The control and experimental sachets were prepared by the manufacturer of LACTOFOS[®] and were identical in appearance, taste and smell.

2.4. Baseline demographic and clinical data

One week before treatment, each patient was interviewed by a trained dietitian. Data regarding body weight and height, waist circumference (WC), body mass index (BMI), percentage of fat, water, lean mass and bone were collected using the MEA Slim[®] – 02.510 balance (Plenna). Regular physical activity was also recorded.

2.5. Clinical response evaluation

Clinical response to treatment was evaluated throughout the study period. Each patient kept a self-report daily record of evacuation data, abdominal symptoms and constipation intensity, after being instructed by one exclusive trained dietitian (L.C.L.) during a first consultation, immediately before treatment. Patients were monitored weekly by phone calls, to verify synbiotic/placebo consumption, any issues regarding recording of data, and to assess adverse events. After treatment, patients attended a final consultation with the same dietitian to verify the reported data and patient impressions regarding treatment.

2.6. Evacuation categorization

Analysis of evacuation included determination of stool frequency, consistency and shape. Stool consistency and shape were classified by the patient using the scale of Bristol,⁹ which classifies stool form into seven categories: 1, nut-like; 2, lumpy sausage; 3, sausage with cracks; 4, smooth snake; 5, soft blobs; 6, fluffy pieces; and 7 watery. Stool consistency and shape were assessed by determining the average difference from category 4 (smooth snake), which was regarded as ideal stool form and consistency.

2.7. Abdominal symptoms categorization

Patients recorded their perception of abdominal pain, bloating and flatulence according to four classifications of symptoms (0, no symptoms; 1, tolerable symptoms; 2 bothersome symptoms; 3, symptoms impairing daily activities). For each patient, intestinal symptoms were recorded as the highest score per week.

2.8. Grading of constipation intensity

Constipation intensity was determined using the AGACHAN Constipation Scoring System,¹⁰ which considers at the same time the following set of symptoms: frequency of bowel movements, difficulty/straining to evacuate, pain on evacuation, sensation of incomplete evacuation, abdominal pain, time taken to start the evacuation, type of assistance (digital assistance or enema) for evacuation, attempts per day and duration of constipation. The

lower is the AGACHAN score; the lower is considered the constipation intensity. The intensity of each symptom was scored from zero to four, except for "type of assistance for evacuation", which ranged from zero (without assistance) or two (with digital assistance or enema). The AGACHAN score (sum of all scores obtained from each symptom) was assessed before (at the first consultation) and at the end (at the last consultation) of treatment for each patient. AGACHAN scores of 0–10, 11–20, and 21–30 were classified as mild, moderate, and severe, respectively.

2.9. Sample size and statistical analysis

Sample size (minimum, 84 patients; 42 per group) was calculated by using the frequency of evacuation as the main variable and considering the standard deviation of the difference between the periods assessed in each treatment to be one evacuation per week (mean) and the mean difference between treatments of 0.8 evacuation per week. For all statistical analysis, the significance level for all tests was 5%, using two-tailed alternative hypotheses.

Data distributions were evaluated using the Kolmogorov–Smirnov test for normality. Differences between groups at baseline were evaluated by Student's *t*-tests, Wilcoxon signed rank tests or Fisher's exact tests, as appropriate. Clinical data were analyzed by analysis of variance (ANOVA) for repeated measures or nonparametric analysis of ordinal data with repeated measures.¹¹ Multiple comparisons were used to check for differences between groups during each week. Changes in patients' AGACHAN Score graduation were evaluated by McNemar's test. All statistical analyses were performed using IBM SPSS 18.0 for Windows (SPSS, Chicago, IL, USA) or a specific EXCEL function, available at <http://www.ime.usp.br/%7Ejmsinger/Medidas%20repetidas%20NP.zip>.

3. Results

3.1. Patients

We randomized a total of 100 patients, 50 per group, but one patient in the synbiotic group voluntarily withdraw during the first week of the study. Of the 99 enrolled patients, 49 patients (25 in the synbiotic and 24 in the control group) did not provide AGACHAN scores at the end of treatment. The CONSORT diagram shows the flow of the participants (Fig. 1).

The study groups were well matched (Table 1) and it was not observed any demographic and clinical characteristics differences, even between the 50 patients that provided AGACHAN scores at the beginning and at the end of treatment (Table 2). The two groups did also have similar frequency of physical activity (24% of patients for synbiotic and 23% of patients for placebo, $P > 0.999$).

3.2. Clinical response

Patients did not report feeling any relevant additional discomfort during the ingestion of either synbiotic or placebo.

3.2.1. Evacuation

Patients from placebo and synbiotic group had similar basal values for stool frequency (0.39 and 0.34 times, respectively, $P = 0.4315$) but not for stool consistency and shape, which was nearer to normal parameters (Bristol scale = 4) in placebo group (average distance: 1.32 vs 1.63 points, $P = 0.0056$). Even though patients from synbiotic group started at a point more departed from normal consistency and shape, after treatment they had increased frequency of evacuation and stool consistency and shape nearer normal parameters than patients in the placebo group (Figs. 2 And 3, respectively). These benefits became significant during the second and third weeks of treatment, respectively (interaction group/time, $P < 0.0001$).

3.2.2. Abdominal symptoms

There were no significant differences between groups in the frequency of abdominal symptoms neither before nor after treatment (Table 3).

3.2.3. Constipation intensity

Patients from synbiotic and placebo groups had similar basal values of AGACHAN score (15.29 vs 15.88 points, respectively – $P = 0.501$). A significant number of patients changed their AGACHAN Score from moderate to mild after synbiotic treatment (McNemar $P = 0.003$, Table 4), while no changes were observed in placebo-treated patients ($P = 0.39$, Table 4). Even though only 50 patients (50.5%) provided their AGACHAN scores at the end of the study, we observed a low chance of type II error ($\beta = 0.0007$, when evaluated at a 5% significance level) and thus a high power for the test (99.92%). The chance of error in concluding that there is a difference between groups with such data seems very low (low P values, low β). In addition, patients in the synbiotic group had a better (lower) final AGACHAN score than those in the placebo group (Fig. 4).

4. Discussion

We found those 30 days of supplementation with a commercially available synbiotic FOS, plus the prebiotic strains *Lactobacillus paracasei* (Lpc-37), *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (NCFM) and *Bifidobacterium lactis* (HN019), improved clinical parameters relative to placebo in constipated women who met the ROME III criteria. Maltodextrin was chosen as the placebo control because it is an easily absorbed and digested carbohydrate

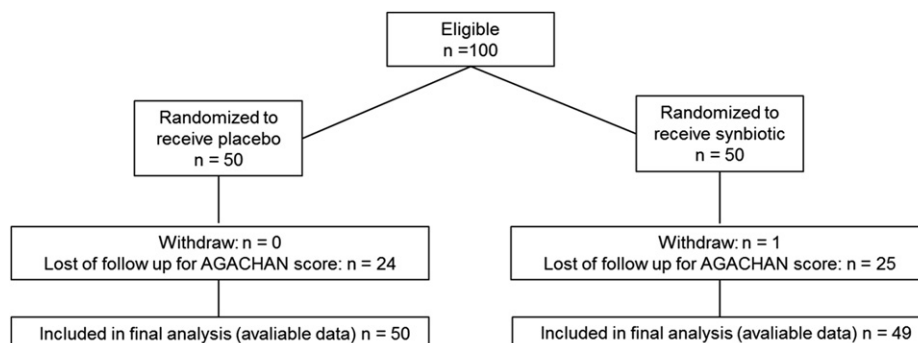


Fig. 1. CONSORT diagram of patient selection, treatment and analysis.

Table 1

Demographic and clinical characteristics of 99 constipated women treated for 4 weeks with synbiotic ($n = 49$) or placebo ($n = 50$).

Variable	Synbiotic ($n = 49$)	Placebo ($n = 50$)	<i>P</i> value
Age (≥ 65 years)	0 (0.0%)	3 (6.0%)	0.24 ^a
Waist circumference (cm)	86.0 \pm 11.5	85.8 \pm 10.1	0.97 ^b
Body weight (kg)	69.7 \pm 14.9	67.5 \pm 13.0	0.39 ^b
Height (m)	1.64 \pm 0.07	1.63 \pm 0.06	0.32 ^c
BMI	25.9 \pm 5.8	25.3 \pm 4.4	0.98 ^b
Body fat mass (%)	31.8 \pm 7.6	32.9 \pm 5.4	0.68 ^b
Body water mass (%)	48.3 \pm 8.2	48.7 \pm 3.9	0.62 ^b
Body lean mass (%)	31.7 \pm 8.0	32.7 \pm 2.2	0.40 ^b
Body bone mass (%)	7.1 \pm 1.6	7.0 \pm 1.2	0.29 ^b

Data are expressed as mean \pm standard deviation for continuous variables, and categorical variables as absolute frequency and percent (for group).

^a Fisher's test exact.

^b Wilcoxon test (or Mann–Whitney test).

^c Student's *t*-test.

not fermented by colonic bacteria and does not interfere with the microbial ecology of the gastrointestinal tract, or with gut metabolism and function.

The prebiotic strains and probiotic FOS contained in our synbiotic mixture have been associated with intestinal health. *L. rhamnosus* HN001 and *L. acidophilus* NCFM positively changed the intestinal microbiota of elderly volunteers, mainly affecting specific subpopulations of intestinal lactobacilli and *Clostridium difficile*¹²; whereas *L. paracasei* Lpc-37 and *B. lactis* were able to transiently colonize the intestines of healthy subjects and of patients with atopic dermatitis.¹³ *L. acidophilus* NCFM also improved symptoms of bloating in patients with functional bowel diseases¹⁴; and *B. lactis* HN019 shortened whole gut transit time in a dose-dependent manner and reduced the frequency of functional gastrointestinal symptoms in adults.¹⁵ FOS is associated with relief from constipation, formation of preferable intestinal microbiota and intestinal immunomodulation.¹⁶

The ideal concentration of probiotic microorganisms providing beneficial effects in humans has not been determined. Shah¹⁷ and Kailasapathy¹⁸ have suggested a minimum concentration of 10^6 colony-forming units (CFU)/ml or per gram of viable probiotics, but recommend the oral ingestion of 10^8 – 10^9 CFU/g to compensate for the reduction in number resulting from passage through the GI tract into the gut. Our protocol, consisting of synbiotic supplementation for 30 days, was designed to achieve adequate probiotic concentrations (ranging from 10^8 to 10^9 CFU) and prebiotic amount (6 g twice) to improve intestinal responses. These amounts and the period of treatment are in agreement with the results of previous

Table 2

Demographic and clinical characteristics of 50 constipated women who provided AGACHAN score in the beginning and at the end of the 4-week treatment with synbiotic ($n = 24$) or placebo ($n = 26$)

Variable	Synbiotic ($n = 24$)	Placebo ($n = 26$)	<i>P</i> value
Age (≥ 65 years)	0 (0.0%)	2 (7.7%)	0.49 ^a
Waist circumference (cm)	86.4 \pm 12.8	86.7 \pm 11.3	0.94 ^c
Body weight (kg)	69.3 \pm 16.9	67.4 \pm 12.6	0.84 ^b
Height (m)	1.63 \pm 0.07	1.62 \pm 0.05	0.57 ^b
BMI	26.0 \pm 6.7	25.5 \pm 4.0	0.83 ^b
Body fat (%)	30.6 \pm 9.4	33.4 \pm 4.9	0.34 ^b
Body water (%)	47.5 \pm 11.2	48.3 \pm 3.5	0.62 ^b
Body lean (%)	32.6 \pm 7.4	32.5 \pm 2.3	0.17 ^b
Body bone (%)	6.9 \pm 1.9	7.0 \pm 1.2	0.86 ^b

Data are expressed as mean \pm standard deviation for continuous variables, and categorical variables as absolute frequency and percent (for group).

^a Fisher's test exact.

^b Wilcoxon test (or Mann–Whitney test).

^c Student's *t*-test.

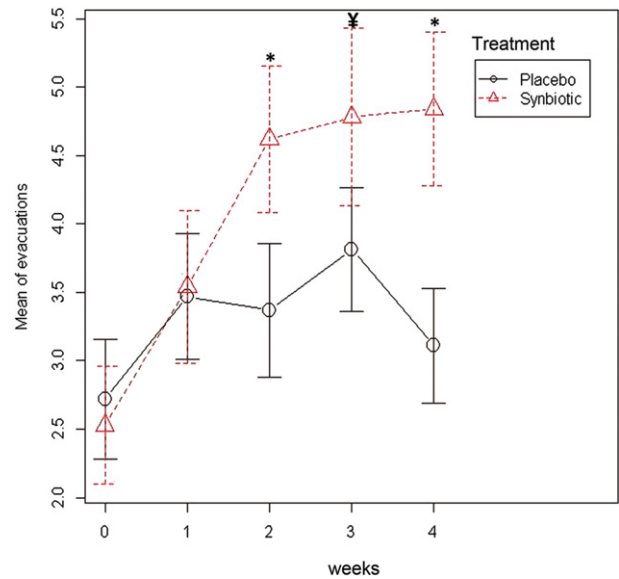


Fig. 2. Mean and confidence interval, in absolute numbers, of self-reported evacuations, in each week of the 4-week treatment of constipated women with synbiotic ($n = 49$) or placebo ($n = 50$). Nonparametric ANOVA for repeated measures, * $P < 0.0001$ and † $P = 0.016$ vs placebo.

clinical trials, which showed similar clinical benefits in constipated subjects treated with other synbiotic mixtures.^{19,20}

We found that the use of synbiotic was associated with an increased number of bowel movements and better stool consistency and shape, without influencing abdominal symptoms. Similar results have been reported with other probiotics. For example, treatment of patients with chronic constipation for 4 weeks with a probiotic beverage containing *L. casei* Shirota resulted in significant reductions in the occurrence of moderate and severe constipation, degree of constipation, and occurrence of hard stools, and increased defecation frequency, compared with placebo-treated

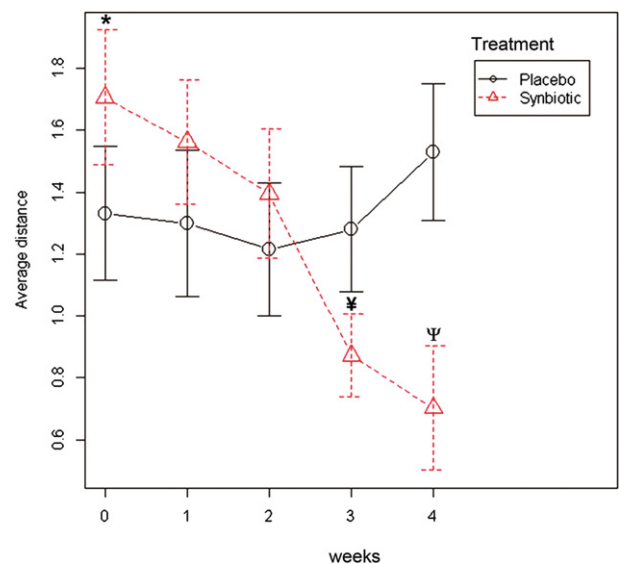


Fig. 3. Average and confidence interval, in absolute numbers, of the difference between the ideal form of stool consistency and shape (Bristol scale = 4) and those self-reported in each week of the 4-week treatment of constipated women with synbiotic ($n = 49$) or placebo ($n = 50$). Nonparametric ANOVA for repeated measures, * $P = 0.0056$, † $P = 0.0001$ and ‡ $P < 0.0001$ vs placebo.

Table 3

Abdominal symptoms of 99 constipated women treated with synbiotic ($n = 49$) or placebo ($n = 50$), assessed considering the highest self-reported score per week.

Week	Group	Abdominal symptoms		
		Pain	Bloating	Flatulence
Basal	Synbiotic	1.30 ± 0.86	1.30 ± 0.81	1.38 ± 0.81
	Placebo	1.26 ± 0.78	1.20 ± 0.86	1.26 ± 0.90
One	Synbiotic	1.04 ± 0.88	0.94 ± 0.87	1.16 ± 0.96
	Placebo	1.30 ± 0.74	1.20 ± 0.73	1.32 ± 0.74
Two	Synbiotic	0.84 ± 0.82	1.02 ± 1.00	1.08 ± 0.94
	Placebo	0.96 ± 0.78	1.06 ± 0.89	1.34 ± 0.77
Three	Synbiotic	0.92 ± 0.90	1.14 ± 1.03	1.24 ± 1.04
	Placebo	1.22 ± 0.86	1.24 ± 0.94	1.38 ± 0.81
Four	Synbiotic	0.98 ± 0.96	1.04 ± 0.90	1.14 ± 1.01
	Placebo	1.20 ± 0.88	1.14 ± 0.83	1.36 ± 0.83

Data are expressed as mean ± standard deviation. Nonparametric ANOVA for repeated measures, $P > 0.05$ for all.

patients.²¹ Moreover, in agreement with our findings, these benefits were not accompanied by changes in the occurrence and degree of flatulence and bloating sensation.²¹

Two additional randomized, placebo-controlled clinical trials also showed that supplementation with probiotics increased defecation frequency and improved stool consistency in adults. In one of these trials, constipated adults treated for 4 weeks with *Escherichia coli* Nissle 1917 showed an increase in the average number of stools per week and a lower incidence of hard stools, compared with subjects who received placebo.²² Moreover, effectiveness and tolerance were significantly greater in the probiotic group.²² In the other study, adult women treated for 2 weeks with a fermented milk product containing *B. lactis* DN-173 010 and yoghurt strains (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) experienced increased stool frequency and improvements in defecation condition and stool consistency when compared with subjects who received acidified milk containing non-living bacteria, not including *B. lactis* DN-173 010 or yoghurt strains.²³ Since abdominal symptoms were not assessed in either study, direct comparison with our findings was not possible.

Clinical benefits have also been observed in constipated subjects treated with prebiotic. Supplementation with FOS of constipated elderly nursing-home residents was found to result in increased daily output of *Bifidobacterium*.²⁴ In addition, students with deregulated gastrointestinal and immune function induced by acute psychological stress during academic exams showed improvements in constipation and abdominal pain after galactooligosaccharide supplementation.²⁵ We previously showed

Table 4

Cross table of the number and percentage of 50 constipated women distributed in the different AGACHAN score categories (mild, moderate and severe) as self-reported before and after 4-week treatment with synbiotic ($n = 24$) or placebo ($n = 26$).

AGACHAN before treatment	AGACHAN after treatment						Total	
	Mild		Moderate		Severe		n	%
	n	%	n	%	n	%		
Placebo group								
Mild	1	100	0	0	0	0	1	100
Moderate	1	4.5	19	86.4	2	9.1	22	100
Severe	0	0	0	0	3	100	3	100
Total	2	7.7	19	73.1	5	19.2	26	100
Synbiotic group								
Mild	3	100	0	0	0	0	3	100
Moderate	12	63.2	7	36.8	0	0	19	100
Severe	0	0	2	100	0	0	2	100
Total	15	62.5	9	37.5	0	0	24	100

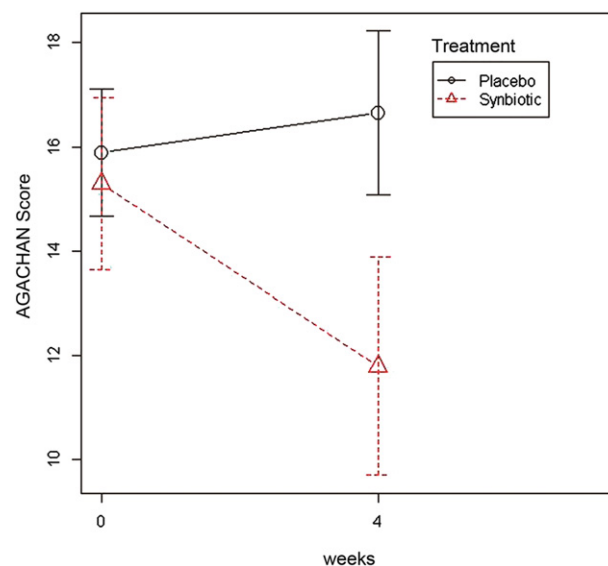


Fig. 4. Average and confidence interval of the AGACHAN score of constipated women self-reported at the basal week and after 4-weeks treatment with synbiotic ($n = 24$) or placebo ($n = 26$). Nonparametric ANOVA for repeated measures and Wilcoxon, $P < 0.0001$ synbiotic vs placebo.

that 3 weeks of treatment with a fiber mixture of inulin and agar gum reduced the fecal pathogenic bacteria *Clostridium* sp. in constipated women relative to placebo, with no changes in fecal short-chain fatty acids and bowel movements.²⁶

It is difficult to compare our present findings with those of earlier studies, because we evaluated the effects of a synbiotic, not its individual components. Synbiotic therapy may have a synergistic effect in the treatment of constipation and was shown to be more effective than either a prebiotic or a probiotic alone in modulating gut microbiota in anaerobic fecal batch cultures.²⁷ Moreover, patients with ulcerative colitis on synbiotic therapy experienced greater quality-of-life changes than patients who received probiotic or prebiotic treatment alone.²⁸

To our knowledge, only two previous randomized clinical trials have evaluated the effect of a synbiotic supplement in constipated individuals.^{19,20} These studies evaluated the effects of a commercial synbiotic mixture, composed of a combination of FOS and *L. casei*, *L. rhamnosus*, *S. thermophilus*, *Bifidobacterium breve*, *L. acidophilus*, and *Bifidobacterium infantis* strains in children¹⁹ and adult men²⁰ satisfying the Rome III criteria for constipation. Both studies had the same intervention protocol, consisting of 4 weeks of treatment with synbiotic plus placebo, synbiotic plus liquid paraffin or liquid paraffin plus placebo.^{19,20} In agreement with our findings, synbiotic (plus placebo) treatment increased the frequency of bowel movements and stool consistency, but differed from our findings, in that these benefits in children were accompanied by reduced abdominal pain and painful defecation.¹⁹ Individuals who received liquid paraffin had the same effects as those who received synbiotic plus placebo, except that treatment with liquid paraffin led to oil seepage, absent from individuals who received synbiotic plus placebo.^{19,20}

A large multicenter open-label trial in Italy of patients with the constipation variant of IBS found that a synbiotic preparation consisting of the probiotic *Bifidobacterium longum* (W11) and the short-chain oligosaccharide prebiotic Fos Actilight, increased stool frequency, as well as reducing abdominal pain and bloating in patients with moderate-severe symptoms.²⁹ In addition, other open-label, uncontrolled, multicenter trials of patients meeting Rome II criteria for constipation predominant IBS found that

treatment with a synbiotic preparation increased stool frequency and reduced abdominal pain and bloating in patients with moderate to severe symptoms.³⁰ Although these studies assessed the effects of synbiotic preparations, they were performed in patients with IBS, preventing comparisons with our findings.

Taken together, our data are in agreement with studies showing that treatment with probiotics and synbiotics benefits patients with constipation, increasing stool frequency and consistency.^{19–23} Our findings also showed an absence of additional benefits on abdominal symptoms observed in part of these previous clinical trials.^{20,21}

Intensity of abdominal symptoms may involve the subjective perception of an unpleasant experience, which can be influenced by physiological, sensory, affective, cognitive, behavioral and socio-cultural factors.³¹ Therefore, differences in improvements in abdominal symptoms observed in clinical trials of pre- and synbiotics in treating constipation may be influenced by uncontrolled personal differences in how participants rate their symptoms, mainly because of the complexity of these multidimensional factors.

Although we did not observe a direct improvement in abdominal symptoms, we found that AGACHAN scores were better in patients who received synbiotic than placebo. The AGACHAN score, a standard scoring system specifically designed to evaluate constipated patients, measures in conjunct abdominal pain and other clinical symptoms, such as painful evacuation effort and feeling of incomplete evacuation, as well as stool frequency and consistency.¹⁰

All patients were required to provide their initial AGACHAN score for inclusion in this study, but unfortunately many refused to provide it after treatment. This may have been due to the inclusion in AGACHAN scores of questions embarrassing for Brazilians (e.g. Did you need digital help to evacuate?). Nevertheless, no demographic or clinical differences were found between the remaining patients and the statistics presented a low *P* value and a high power.

Increased stool frequency is a main goal of treatment for constipated patients. Interestingly, we found that, during the first week of treatment, all patients experienced an increase in stool frequency, regardless of whether they received placebo or synbiotic. This finding indicates a possible placebo effect, which has been widely documented in randomized, placebo-controlled drug trials. Brain imaging has demonstrated that placebo can mimic the effect of active drugs by activating the same brain areas. This effect has been observed with a dopamine placebo in patients with Parkinson's disease and with analgesic placebos.³² These findings suggest that, to observe the actual effects of synbiotic treatment in patients with constipation, would require treatment for more than 2 weeks.

This study had several limitations. It included only young adult females and involved only one specific commercial synbiotic mixture. Similar results may therefore not be achieved in other age groups or in men or when using other synbiotic mixtures containing different individual amounts of pre- and probiotics. Treatment compliance was assessed by phone calls and also by looking at each participant's diary, without performing an intention to treat analysis. Although constipation is often related to diet, our patients' regular diet and fluid consumption were not analyzed by specific questions. The main contribution of our study was to verify whether, regardless of patient eating habits, these patients could benefit from supplementation with synbiotic. Physical activity was assessed as an external habit (different from the natural diet and fluid ingestion) that could interfere in our results. In addition, we did not assess changes in fecal microbiota and short fatty acids resulting from synbiotic treatment, changes that could strengthen our associative observation.

In conclusion, we found that 3 weeks of treatment with a synbiotic containing FOS as a prebiotic and *L. paracasei* (Lpc-37), *L. rhamnosus* (HN001), *L. acidophilus* (NCFM) and *B. lactis* (HN019) as probiotics facilitated evacuation in constipated women by increasing stool frequency, improving stool consistency and shape, and decreasing constipation intensity. Further studies, in particular large, randomized clinical trials, are needed to confirm these results and to define the clinical role of synbiotic administration in constipated patients.

Conflict of interest

The authors do not have any additional conflict of interest to declare.

Acknowledgment

The authors thank the statisticians Prof. Júlio Cesar Rodrigues Pereira and João Italo Dias França for the statistical discussion. *Statement of Authorship:* DLW conceived the study, and participated in its design and coordination and helped to interpret the data and to draft the manuscript; LCL performed the enrollment, assignment and clinical evaluation of patients and participated in data analysis; AFB performed the database relating to abdominal symptoms and constipation intensity and participated in the analysis and interpretation of these data; RST participated in data analysis and interpretation and helped to write the manuscript; GMS performed the database relating to stool frequency, consistency and shape and participated in the analysis and interpretation of these data; NPP monitored the patients weekly, by phone calls, and participated in overall organization of data; MLT participated in the study design and conception, oriented the data collection and revised the manuscript critically. All authors read and approved the final manuscript. *Funding sources:* This study was supported by SKL Functional Nutrition that kindly provided the synbiotic LACTOFOS®.

References

- Mugie SM, Benninga MA, Di Lorenzo C. Epidemiology of constipation in children and adults: a systematic review. *Best Pract Res Clin Gastroenterol* 2011;**25**: 3–18.
- Rantis Jr PC, Vernava 3rd AM, Daniel GL, Longo WE. Chronic constipation—is the work-up worth the cost? *Dis Colon Rectum* 1997;**40**:280–6.
- Drossman DA. Rome III: the new criteria. *Chin J Dig Dis* 2006;**7**:181–5.
- Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. *Genes Nutr* 2011;**6**:209–40.
- Macfarlane S, Macfarlane GT, Cummings JH. Review article: probiotics in the gastrointestinal tract. *Aliment Pharmacol Ther* 2006;**24**:701–14.
- Sanders ME. Probiotics: considerations for human health. *Nutr Rev* 2003;**61**: 91–9.
- MacFarlane GT, Cummings JH. Probiotics and prebiotics: can regulating the activities of intestinal bacteria benefit health? *Br Med J* 1999;**318**:999–1003.
- Morelli L, Zonenschain D, Callegari ML, Grossi E, Maisano F, Fusillo M. Assessment of a new synbiotic preparation in healthy volunteers: survival, persistence of probiotic strains and its effect on the indigenous flora. *Nutr J* 2003;**2**:11.
- Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 1997;**32**:920–4.
- Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD. A constipation scoring system to simplify evaluation and management of constipated patients. *Dis Colon Rectum* 1996;**39**:681–5.
- Brunner E, Langer F. Nonparametric analysis of ordered categorical data in designs with longitudinal observations and small sample sizes. *Biometr J* 2000;**42**:663–75.
- Lahtinen SJ, Forssten S, Aakko J, Granlund L, Rautonen N, Salminen S, et al. Probiotic cheese containing *Lactobacillus rhamnosus* HN001 and *Lactobacillus acidophilus* NCFM(®) modifies subpopulations of fecal lactobacilli and *Clostridium difficile* in the elderly. *Age (Dordr)* 2012;**34**:133–43.
- Roessler A, Forssten SD, Gleis M, Ouweland AC, Jahreis G. The effect of probiotics on faecal microbiota and genotoxic activity of faecal water in patients with atopic dermatitis: a randomized, placebo-controlled study. *Clin Nutr* 2012;**31**:22–9.

14. Ringel-Kulka T, Palsson OS, Maier D, Carroll I, Galanko JA, Leyer G, et al. Probiotic bacteria *Lactobacillus acidophilus* NCFM and *Bifidobacterium lactis* Bi-07 versus placebo for the symptoms of bloating in patients with functional bowel disorders: a double-blind study. *J Clin Gastroenterol* 2011;**45**:518–25.
15. Waller PA, Gopal PK, Leyer GJ, Ouweland AC, Reifer C, Stewart ME, et al. Dose-response effect of *Bifidobacterium lactis* HN019 on whole gut transit time and functional gastrointestinal symptoms in adults. *Scand J Gastroenterol* 2011;**46**:1057–64.
16. Tokunaga T. Novel physiological function of fructooligosaccharides. *Biofactors* 2004;**21**:89–94.
17. Shah NP. Probiotic bacteria: selective enumeration and survival in dairy foods. *J Dairy Sci.* 2000;**83**:894–907.
18. Kailasapathy K, Chin J. Survival and therapeutic potential of probiotic organisms with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. *Immunol Cell Biol.* 2000;**78**:80–8.
19. Khodadad A, Sabbaghian M. Role of synbiotics in the treatment of childhood constipation: a double-blind randomized placebo controlled trial. *Iran J Pediatr* 2010;**20**:387–92.
20. Fateh R, Iravani S, Frootan M, Rasouli MR, Saadat S. Synbiotic preparation in men suffering from functional constipation: a randomised controlled trial. *Swiss Med Wkly* 2011;**141**:w13239.
21. Koebnick C, Wagner I, Leitzmann P, Stern U, Zunft HJ. Probiotic beverage containing *Lactobacillus casei* Shirota improves gastrointestinal symptoms in patients with chronic constipation. *Can J Gastroenterol.* 2003;**17**:655–9.
22. Möllenbrink M, Bruckschen E. Treatment of chronic constipation with physiologic *Escherichia coli* bacteria. Results of a clinical study of the effectiveness and tolerance of microbiological therapy with the *E. coli* Nissle 1917 strain (Mutaflor). *Med Klin (Munich)* 1994;**89**:587–93.
23. Yang YX, He M, Hu G, Wei J, Pages P, Yang XH, et al. Effect of a fermented milk containing *Bifidobacterium lactis* DN-173010 on Chinese constipated women. *World J Gastroenterol* 2008;**14**:6237–43.
24. Yen CH, Kuo YW, Tseng YH, Lee MC, Chen HL. Beneficial effects of fructooligosaccharides supplementation on fecal bifidobacteria and index of peroxidation status in constipated nursing-home residents—a placebo-controlled, diet-controlled trial. *Nutrition* 2011;**27**:323–8.
25. Hughes C, Davoodi-Semiromi Y, Colee JC, Culpepper T, Dahl WJ, Mai V, et al. Galactooligosaccharide supplementation reduces stress-induced gastrointestinal dysfunction and days of cold or flu: a randomized, double-blind, controlled trial in healthy university students. *Am J Clin Nutr* 2011;**93**:1305–11.
26. Waitzberg DL, Pereira CCA, Logullo L, Jacintho TM, Almeida D, Teixeira Da Silva ML, et al. Microbiota benefits after inulin and partially hydrolyzed guar gum supplementation – a randomized clinical trial in constipated women. *Nutr Hop* 2012;**27**:123–9.
27. Saulnier DM, Gibson GR, Kolida S. *In vitro* effects of selected synbiotics on the human faecal microbiota composition. *FEMS Microbiol Ecol* 2008;**66**:516–27.
28. Fujimori S, Gudis K, Mitsui K, Seo T, Yonezawa M, Tanaka S, et al. A randomized controlled trial on the efficacy of synbiotic versus probiotic or prebiotic treatment to improve the quality of life in patients with ulcerative colitis. *Nutrition* 2009;**25**:520–5.
29. Colecchia A, Vestito A, La Rocca A, Pasqui F, Nikiforaki A, Festi D, Symbiotic Study Group. Effect of a symbiotic preparation on the clinical manifestations of irritable bowel syndrome, constipation-variant. Results of an open, uncontrolled multicenter study. *Minerva Gastroenterol Dietol* 2006;**52**:349–58.
30. Dughera L, Elia C, Navino M, Cisarò F, ARMONIA Study Group. Effects of symbiotic preparations on constipated irritable bowel syndrome symptoms. *Acta Biomed* 2007;**78**:111–6.
31. Sousa FF, Silva JA. A métrica da dor (dormetria): problemas teóricos e metodológicos. *Revista DOR* 2005;**6**:469–513.
32. Miwa H. Placebo effect in Parkinson's disease. *Brain Nerve* 2007;**59**:139–46.