

# Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study

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## SUMMARY

### Background

The efficacy of probiotics in alleviating the symptoms of irritable bowel syndrome (IBS) appears to be both strain- and dose-related.

### Aim

To investigate the effect of LAB4, a multistrain probiotic preparation on symptoms of IBS. This probiotic preparation has not previously been assessed in IBS.

### Methods

Fifty-two participants with IBS, as defined by the Rome II criteria, participated in this double blind, randomized, placebo-controlled study. Participants were randomized to receive either a probiotic preparation comprising two strains of *Lactobacillus acidophilus* CUL60 (NCIMB 30157) and CUL21 (NCIMB 30156), *Bifidobacterium lactis* CUL34 (NCIMB 30172) and *Bifidobacterium bifidum* CUL20 (NCIMB 30153) at a total of  $2.5 \times 10^{10}$  cfu/capsule or a placebo for 8 weeks. Participants reported their IBS symptoms using a questionnaire fortnightly during the intervention and at 2 weeks post-intervention.

### Results

A significantly greater improvement in the Symptom Severity Score of IBS and in scores for quality of life, days with pain and satisfaction with bowel habit was observed over the 8-week intervention period in the volunteers receiving the probiotic preparation than in the placebo group.

### Conclusion

LAB4 multistrain probiotic supplement may benefit subjects with IBS.

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## INTRODUCTION

Irritable bowel syndrome (IBS) is a chronic relapsing gastrointestinal condition characterized by abdominal discomfort, bloating and changes in bowel habit. It has a significant negative impact on quality of life and social functioning, but does not lead to the development of serious disease and associated mortality. Nevertheless, IBS does generate significant direct and indirect healthcare costs.<sup>1</sup> IBS symptom pathogenesis is far from clearly defined and most hypotheses focus on one or more of the following: altered intraluminal milieu, immune activation, enteric neuromuscular dysfunction and/or brain-gut axis dysregulation. It has been proposed that IBS may result from a dysfunctional interaction between the indigenous flora and the intestinal mucosa leading to immune activation in the colonic mucosa.<sup>2</sup> Changes in the colonic microbiota could result in the proliferation of gas-producing organisms or in organisms that facilitate deconjugation of bile acids thereby impacting upon water and electrolyte transport within the colon. Alleviation of the symptoms of the bacterial overgrowth (small intestinal bacterial overgrowth) observed in some IBS sufferers by the use of antibiotics also provides evidence for the contribution of microbial abnormalities to IBS symptoms.<sup>3</sup>

Dysregulation of the microbiota is also linked to the growing evidence for the onset of IBS following an attack of acute gastroenteritis, which is associated with on-going inflammation induced by the infecting organisms.<sup>4</sup>

Pharmacological therapy for IBS has primarily targeted individual symptoms by means of antidiarrhoeals, laxatives and antispasmodics with some successes using antidepressants and serotonergic agents, but the latter are associated with some safety issues.<sup>5, 6</sup> Success with these drugs has been limited and although potential therapeutic targets have been identified, new drugs are not available, which has led many IBS sufferers to seek alternative remedies.

Data are accumulating to suggest that the use of probiotic-based products may be beneficial for the control of IBS symptoms. Probiotics are live microorganisms which, when administered in adequate amounts, confer health benefits on the host.<sup>6</sup>

Quigley and Flourie<sup>7</sup> reviewed the use and efficacy of probiotics in IBS and suggested a clear rationale for probiotic usage in response to a dysfunctional relationship between the indigenous microbiota and the host. The authors further suggested the feasibility of

probiotics for bacterial displacement and alteration of luminal content. However, clarification is required regarding the need for clear definition of strains, dosage and viability of the probiotic organisms in use.

The probiotic product used in this study comprises a consortium of lactobacillus and bifidobacterial organisms. Kassinen *et al.*<sup>8</sup> have shown that both the lactobacillus and the bifidobacterial components of the microbiota of IBS sufferers were present in lower numbers than in the controls suggesting a value for intervention strategies comprising both organisms. The consortium and dose used in this study had previously proved effective in both prevention of *Clostridium difficile in vivo*<sup>8, 9</sup> and the modulation of the composition of the intestinal re-growth population following antibiotic therapy.<sup>10, 11</sup>

The aim of this randomized, double-blind placebo-controlled trial was to assess the potential of the LAB4 multistrain probiotic (comprising two strains of *Lactobacillus acidophilus* CUL60 and CUL21 together with *Bifidobacterium lactis* CUL34 and *Bifidobacterium bifidum* CUL20) to attenuate the symptoms of IBS.

## MATERIALS AND METHODS

### Participants

Ethical approval for this study was granted by The University of Sheffield Research Ethics Committee (Ref.: SMBRER 13). Volunteers reporting active IBS symptoms were recruited to the study through advertisements in a local newspaper and posters placed around The University of Sheffield. Volunteers were informed at recruitment that the study was to investigate the effect of a probiotic on the symptoms of IBS in accordance with ethical requirements. Subjects were excluded if they had a history of abdominal surgery, were pregnant or lactating, had other gastrointestinal disorders, were already taking prebiotic or probiotic products or were receiving medication for symptoms of IBS. All volunteers reported a previous diagnosis of IBS by their general practitioner (GP), but GP records were not checked. Self-reported symptoms of IBS were used to confirm the presence of IBS according to the Rome II criteria.<sup>12</sup> Volunteers provided written, informed consent.

### Study design

This was a double-blind placebo-controlled study to evaluate the efficacy of a multistrain probiotic

preparation in the treatment of IBS. The study was conducted over a 10-week period. Subjects were asked to complete a validated questionnaire to assess IBS symptoms<sup>13</sup> at baseline (0) and fortnightly throughout an 8-week intervention (2, 4, 6 and 8). IBS symptoms were again assessed at week 10 to investigate if there was an effect beyond the period of supplementation. The questionnaire assesses severity and duration of abdominal pain (abdominal pain, days with pain), abdominal distension (bloating), satisfaction with bowel habits (bowel habit) and quality of life. Volunteers were asked to record the number of days they had experienced abdominal pain over the previous 2 weeks and then this was calculated as a percentage. All other components were assessed using a visual analogue scale and scored out of 100. Individual scores were combined to give the total Symptom Severity Score with a maximum score of 500. This score classifies subjects as having no symptoms (<75), mild (75–175), moderate (175–300) or severe IBS (>300). The questionnaire has been shown to be reproducible, sensitive to change and is easy to complete.<sup>13</sup> The primary endpoint was the IBS Symptom Severity Score during the intervention and follow up and its components were the secondary endpoints.

### Sample size and randomization

Fifty-six subjects were recruited to the study and randomized (stratified by age and gender) to receive probiotic treatment ( $n = 28$ ) and placebo treatment ( $n = 28$ ). The sample size was calculated based on a 15% reduction in severity of symptoms. It was calculated that 50 subjects (25 in each group of the study) were needed to detect a difference between the two groups with a power of 80% at the 5% level of statistical significance. The sample size was increased to 56 subjects to allow for just over 10% drop out rate.

### Probiotic intervention

The probiotic and the placebo preparations were prepared as identically packaged, cellulose capsules by Cultech Ltd, Port Talbot, UK. The probiotic preparation contained two strains of *L. acidophilus*, CUL-60 (NCIMB 30157), CUL-21 (NCIMB 30156), *B. bifidum* CUL-20 (NCIMB 30153) and *B. lactis* CUL-34 (NCIMB 30172) at a total of  $2.5 \times 10^{10}$  colony forming units (cfu) per capsule. The placebo contained 300 mg maltodextrin. Volunteers were instructed to ingest one

capsule per day with water for 8 weeks. Compliance was assessed by counting the number of capsules remaining at the end of the intervention and checked against self-reported capsule diaries.

### Compliance

Of the fifty-six volunteers recruited, four subjects in the placebo arm withdrew from the study (one due to ill health, two for deviation from protocol and one for unknown reasons) (Figure 1). Fifty-two subjects participated in the intervention comprising 28 subjects in the treatment group and 24 subjects in the control group (Table 1). Four subjects (two in each treatment arm) failed to return all the questionnaires.

### Side effects

One subject in the treatment group reported an increase in flatulence throughout the duration of the study. No other side-effects were reported.

### Statistical analysis

The primary and secondary endpoints were analysed by an ANOVA model with repeated measurements. In this model, the baseline measurement of an endpoint, treatment, time and interaction between time and treatment were treated as fixed effects whereas the subject was treated as a random effect. During the trial, four subjects failed to complete all questionnaires, resulting in some incomplete observations. These incomplete

Table 1. Baseline characteristics of the study population.

	Probiotic	Placebo
No. of subjects	28	24
Female % ( $n$ )	89 (25)	83 (20)
Mean age in years $\pm$ s.d.	40 (12)	38 (11)
Predominant bowel habit % ( $n$ )		
Alternating	61 (17)	62.5 (15)
Constipation	29 (8)	25 (6)
Diarrhoea	11 (3)	12.5 (3)
Mean IBS severity score $\pm$ s.d.	283 $\pm$ 61	252 $\pm$ 60
IBS classification % ( $n$ )		
Mild	7 (2)	12.5 (3)
Moderate	57 (16)	75 (18)
Severe	36 (10)	12.5 (3)

IBS, irritable bowel syndrome.

observations are not imputed but are assumed to be missing at random in the ANOVA model analysis. The estimated treatment differences from the ANOVA model are therefore reported together with their 95% confidence intervals. Reported *P*-values are two-sided and a *P*-value of <0.05 was considered statistically significant and all statistical analyses were carried out by using the Statistical Analysis System (SAS) version 9.1 (SAS Institute, Inc., Cary, NC, USA).

## RESULTS

The demographic and baseline characteristics of the subjects are shown in Table 1. The two groups of subjects were similar in terms of age, gender, type of bowel habit and symptoms. Subjects receiving the probiotic preparation had a higher IBS severity score at baseline than the subjects receiving placebo (mean  $\pm$  s.d.  $283 \pm 61$  vs.  $252 \pm 60$  respectively).

Significant improvements from baseline for Symptom Severity Score were seen throughout the intervention period in both active and placebo groups from weeks 2 to 10 (Table 2). The overall *P*-value for the ANOVA analysis evaluating all time points was 0.0008. More detailed analysis of these symptoms showed that significant improvements from the baseline were reported in quality of life and satisfaction with bowel habit in both groups throughout the study. Abdominal pain/bloating symptoms did not show significant improvements from the baseline in the probiotic group until week 4 (*P* = 0.0002; LS mean  $-16.10$ ; 95% CI:

$-24.64$  to  $-7.56$ ) of intervention and that in the placebo group, significant improvements in these symptoms were only recorded at week 6 (*P* = 0.0218; LS mean  $-11.04$ ; 95% CI:  $-20.46$  to  $-1.62$ ) and week 8 (*P* = 0.0028; LS mean  $-14.74$ ; 95% CI:  $-24.38$  to  $-5.11$ ). The number of days with pain improved significantly by week 2 in the probiotic group (*P* = 0.0176; LS mean  $-8.46$ ; 95% CI:  $-15.44$  to  $-1.49$ ), but did not reduce significantly for the placebo group until 4 weeks into the study (*P* = 0.0058; LS mean  $-10.64$ ; 95% CI:  $-18.17$  to  $-3.11$ ). The severity of abdominal pain reduced significantly in both groups from week 4 onwards (probiotic: *P* < 0.0001, LS mean  $-19.05$ , 95% CI:  $-27.41$  to  $-10.70$ ; placebo: *P* = 0.0185, LS mean  $-10.88$ , 95% CI:  $-19.93$  to  $-1.84$ ). The overall placebo effect for the Symptom Severity Score in this study was 33% ranging from 23% to 45% for the individual symptoms.

Comparison of the effectiveness of the probiotic in the presence of significant placebo effect in this study showed a significant difference in the Symptom Severity Score in favour of the probiotic at 6 weeks (*P* = 0.0347; difference between groups:  $-47.82$ ; 95% CI:  $-92.18$  to  $-3.4$ ) and 8 weeks (*P* = 0.0217; difference between groups:  $-52.73$ ; 95% CI:  $-97.67$  to  $-7.78$ ) but by 2 weeks postintervention, no significant differences could be detected between the probiotic and placebo groups (Figure 2).

Figure 3 shows that greater improvements were recorded for all symptoms for the probiotic group than for the placebo group throughout the study.

**Table 2.** Symptom severity at baseline and change in symptom severity in treatment and placebo group at weeks 8 and 10 (2 weeks postintervention)

	Baseline (mean $\pm$ s.d.)	Week 8 (mean $\pm$ s.d.)	Change at week 8	<i>P</i> -value	Week 10 (mean $\pm$ s.d.)	Change at week 10	<i>P</i> -value
<b>Placebo group</b>							
Symptom Severity Score	252.08 $\pm$ 59.92	172.00 $\pm$ 99.51	-80.66	<0.0001	193.41 $\pm$ 75.49	-59.25	0.0005
Abdominal distension/bloating	46.71 $\pm$ 21.83	32.05 $\pm$ 29.64	-14.74	0.0028	39.27 $\pm$ 25.00	-7.52	0.1259
Satisfaction with bowel habit	68.04 $\pm$ 20.08	44.36 $\pm$ 21.60	-24.41	<0.0001	48.68 $\pm$ 17.15	-20.09	<0.0001
Number of days with pain	42.67 $\pm$ 23.74	27.68 $\pm$ 23.31	-14.27	0.0004	32.14 $\pm$ 22.52	-9.82	0.0148
Quality of life	61.88 $\pm$ 11.64	47.41 $\pm$ 17.58	-16.07	<0.0001	48.59 $\pm$ 14.40	-14.89	0.0001
Abdominal pain	32.79 $\pm$ 15.04	20.50 $\pm$ 26.05	-16.16	0.0009	24.73 $\pm$ 23.59	-11.94	0.0134
<b>Active group</b>							
Symptom Severity Score	282.68 $\pm$ 60.59	150.23 $\pm$ 101.96	-133.39	<0.0001	189.19 $\pm$ 84.28	-94.43	<0.0001
Abdominal distension/bloating	48.54 $\pm$ 25.77	25.88 $\pm$ 25.05	-22.80	<0.0001	36.65 $\pm$ 23.51	-12.04	0.0080
Satisfaction with bowel habit	73.39 $\pm$ 17.73	39.65 $\pm$ 23.83	-32.34	<0.0001	48.38 $\pm$ 20.01	-23.61	<0.0001
Number of days with pain	48.64 $\pm$ 21.81	26.12 $\pm$ 24.29	-22.94	<0.0001	28.27 $\pm$ 21.09	-20.79	<0.0001
Quality of life	67.61 $\pm$ 15.70	37.50 $\pm$ 22.40	-29.65	<0.0001	48.58 $\pm$ 19.81	-18.57	<0.0001
Abdominal pain	44.50 $\pm$ 18.03	21.08 $\pm$ 24.06	-21.20	<0.0001	27.31 $\pm$ 21.09	-14.97	0.0008

Significant improvements in quality of life (Figure 3a) were recorded for those receiving the probiotic at the end of the intervention period ( $P = 0.0068$ ; difference between groups:  $-13.58$ ; 95% CI:  $-23.38$  to  $-3.78$  at week 8) and this was associated with significantly improved satisfaction with bowel habit (Figure 3c) for the probiotic subjects over the placebo group at 6 weeks ( $P = 0.0422$ ; difference between groups:  $-11.05$ ; 95% CI:  $-21.70$  to  $-0.39$ ).

The number of days with pain (Figure 3d) recorded was significantly lower in the probiotic group at week 10 than in the placebo group ( $P = 0.0448$ ; difference between groups:  $-10.97$ ; 95% CI:  $-21.69$  to  $-0.26$ ).

## DISCUSSION

Significant differences in the Symptom Severity Score were recorded between the probiotic and placebo groups correlating with improved quality of life and bowel habit together with fewer numbers of days with pain for the probiotic group. No differences in abdominal pain or bloating were discernible between the two groups. The use of the probiotic was well tolerated and free from significant adverse effects. The effect of the probiotic on the different groups of bowel habit could not be ascertained in this study because of lack of numbers in each group.

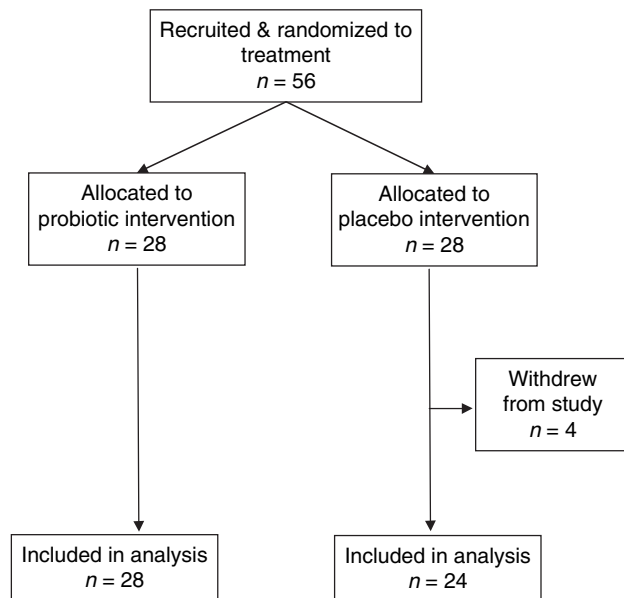


Figure 1. Flow chart of subject progression through the study.

The overall placebo response rate observed in this study (33%) is comparable with that seen in many other IBS studies. Patel *et al.*<sup>14</sup> concluded that placebo effects in IBS clinical trials measuring global outcome were highly variable ranging from 16 to 71%, whereas Dorn *et al.*<sup>15</sup> found a placebo response rate of 42.6% in complementary and alternative medicine IBS trials. Several factors are thought to contribute towards the placebo effect including Pavlovian conditioning and the expectation of a positive outcome.<sup>16</sup> In this trial all, volunteers had been informed that the purpose of the study was to investigate the possible benefits of a probiotic preparation, although they knew that they may be receiving a placebo. Owing to the nature of the intervention the volunteers may have been anticipating an improvement in their IBS symptoms which is likely to have contributed towards the placebo effect.

Several randomized controlled trials (RCTs) have been set up with IBS sufferers to assess the efficacy of multistrain probiotic preparations containing a variety of organisms at different doses and for different study periods and the responses have been variable. Most of the products provided daily doses in the range of  $5\text{--}9 \times 10^9$  cfu and, in most cases, reductions in symptom severity score were observed<sup>17–21</sup> and some, but not all, of the products significantly reduced abdominal pain symptoms. Guyonnet *et al.*<sup>22</sup> demonstrated improvements in symptoms among a constipation-predominant group of IBS sufferers receiving a daily dose of  $2.5 \times 10^{10}$  cfu of *Bifidobacterium animalis*

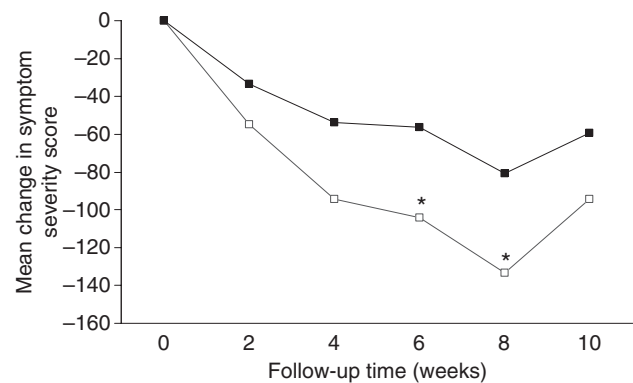


Figure 2. Effects of LAB4 multistrain probiotic on Symptom Severity Score in subjects with irritable bowel syndrome. There was a reduction in total symptom severity (mean) after the probiotic intervention (□) and in control (■) groups from baseline. Repeat measures analysis showed that there was a significant difference between the treatment groups ( $*P < 0.05$ ).



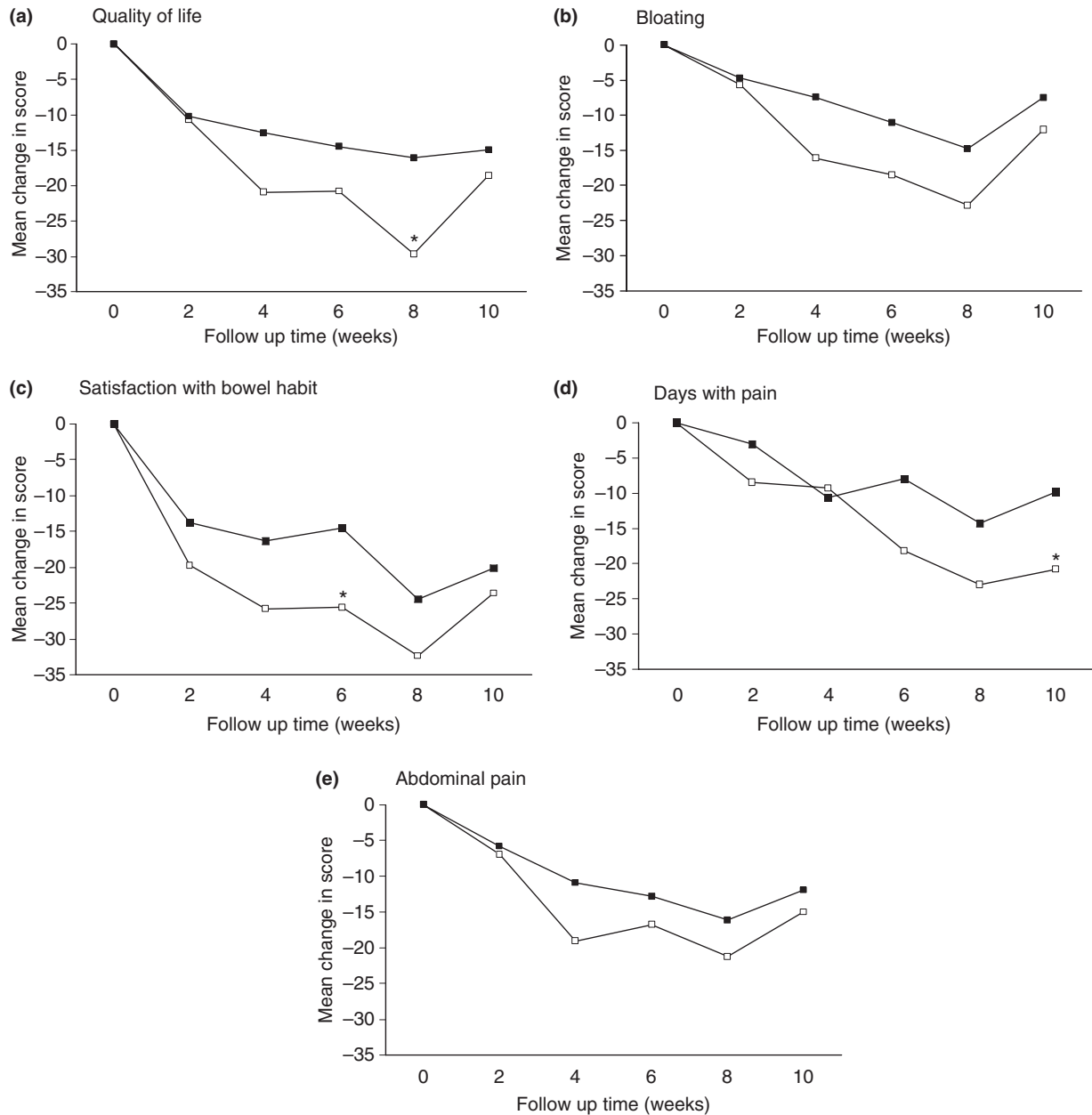


Figure 3. Change (mean) in scores for (a) quality of life, (b) bloating, (c) satisfaction with bowel habit, (d) days with pain and (e) abdominal pain during the 10-week study ( $*P < 0.05$ ).

DN-173010, *Streptococcus thermophilus* and *Lactobacillus bulgaricus* for a period of 6 weeks.

O'Mahoney *et al.*<sup>23</sup> demonstrated greater IBS symptom relief with the administration of *Bifidobacterium infantis* rather than *Lactobacillus salivarius* as single strain products and the results of Whorwell *et al.*<sup>24</sup> indicated that there may be a dose responsiveness to the administration of *B. infantis* (but formulation issues with the higher dosage in this study necessitate

further clarification). Reduction in abdominal pain was demonstrated during an RCT with *L. acidophilus*-SDC 2012, 2013 at a daily dosage of  $2 \times 10^9$  cfu by Sinn *et al.*<sup>25</sup>, whereas in the current study with the LAB4 consortium, there was a significant reduction observed in the number of days with pain for the probiotic group. Rousseaux *et al.*<sup>26</sup> demonstrated that *L. acidophilus* NCFM induced MOR1 and CB2 expression through the NF- $\kappa$ B pathway when in contact with epithelial cells,

which contributes to the modulation of visceral pain. This potential of *L. acidophilus* to alleviate pain supports the reduction in days of pain observed for the IBS sufferers receiving the LAB4 product.

In conclusion, this study shows the potential benefit of the LAB4 multistrain probiotic supplement at a daily dosage of  $2.5 \times 10^{10}$  cfu in the management of IBS. Future studies will aim to identify the mechanism of the probiotics' potential beneficial effect.

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