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Effect of a fermented milk containing *Bifidobacterium animalis* DN-173 010 on the health-related quality of life and symptoms in irritable bowel syndrome in adults in primary care: a multicentre, randomized, double-blind, controlled trial

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SUMMARY

Background
Health-related quality of life (HRQoL) has been rarely evaluated as a primary endpoint in the assessment of the effect of probiotics on the irritable bowel syndrome (IBS).

Aim
To study the effects of fermented milk containing *Bifidobacterium animalis* DN-173 010 and yoghurt strains on the IBS in a multicentre, double-blind, controlled trial.

Methods
A total of 274 primary care adults with constipation-predominant IBS (Rome II) were randomized to consume for 6 weeks either the test fermented milk or a heat-treated yoghurt (control). HRQoL and digestive symptoms were assessed after 3 and 6 weeks on an intention-to-treat population of 267 subjects.

Results
The HRQoL discomfort score, the primary endpoint, improved ($P < 0.001$) in both groups at weeks 3 and 6. The responder rate for the HRQoL discomfort score was higher (65.2 vs. 47.7%, $P < 0.005$), as was the decrease in bloating score [$0.56 \pm$ (s.d.)1.01 vs. 0.31 ± 0.87, $P = 0.03$], at week 3 in the test vs. the control group. In those subjects with <3 stools/week, stool frequency increased ($P < 0.001$) over 6 weeks in the test vs. control group.

Conclusions
This study suggests a beneficial effect of a probiotic food on discomfort HRQoL score and bloating in constipation-predominant IBS, and on stool frequency in subjects with <3 stools/week.

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INTRODUCTION

Irritable bowel syndrome (IBS), a part of the larger group of functional gastrointestinal (GI) diseases,[1] stays among the most common diseases known. Its prevalence in the general adult population from Western countries ranges from <5% up to 20% depending on the criteria used (from Manning to Rome III)[2–5] and seems to be as high in Asia, Indian subcontinent and South America as in Western countries.[1, 4] IBS is defined by abdominal pain or discomfort and an alteration in bowel habit.[6] Bloating, a supportive symptom of IBS diagnosis and a functional bowel disorder *per se* in the Rome II[7] and the very recent Rome III classifications,[8] is the most common abdominal complaint in IBS subjects, being reported and considered as the most bothersome symptom by 96% and 60% of them respectively.[9] Although IBS is not a life-threatening disease, health-related quality of life (HRQoL) is impaired.[10–13]

Some probiotic strains, either single (*Bifidobacterium Infantis* 36524, *Lactobacillus Plantarum 299v*) or combined (VSL#3), have been associated with a significant alleviation of IBS symptoms,[14–17] while others proved ineffective.[18–20] The rationale for the use of probiotics in IBS may clearly be the correction, first, of a dysfunctional shift in the host-gut indigenous flora relationship with its subsequent effect on motility, sensitivity and/or gas production by the intestine[6] and, second, of a low-grade inflammation or immune activation, which leads to enteric nerve or muscle dysfunction – with normalization of a Th1-like proinflammatory cytokine profile.[16] Unfortunately, even if the use of probiotics seems to be very promising, the very great majority of clinical trials on probiotics in IBS over the past years had methodological weaknesses, particularly due to the small number of study subjects, *i.e.* most often <35 per group.[20, 21] The most significant studies have been conducted on *Bifidobacterium infantis* 35624.[16, 17] After a pilot study in a total of 80 male/female IBS subjects,[16] *B. infantis* 36524 proved capable in a large-scale trial on 362 (90 per group) primary care female subjects[17] to alleviate major symptoms of IBS, a clinical effect associated with an anti-inflammatory one as suggested by the correction of an abnormal interleukin (IL) 10/IL12 blood ratio.[16]

In healthy adults as well as elderly subjects, a fermented milk containing *Bifidobacterium animalis* DN-173 010 alone or in association with the yoghurt starters *Lactobacillus bulgaricus* and *Streptococcus thermophilus* has been shown to decrease orofecal and/or colonic, especially sigmoid, transit time;[22–25] *B. animalis* DN-173 010 survives complete transit through the digestive tract and is recovered live in stools in large quantities relative to the quantity initially ingested.[26–28]

The present large-scale, multicentre, randomized, controlled clinical trial aimed to assess, in primary care IBS adult subjects with predominant constipation, the effects of a fermented milk combining *B. animalis* DN-173 010 and yoghurt strains on digestive symptoms and HRQoL as evaluated by an IBS-specific questionnaire.

SUBJECTS AND METHODS

Study population

A total of 276 subjects were recruited from 18 February 2005 to 18 May 2005 from 35 primary care centres (general practitioners) across France. They were male and female subjects aged 18–65 years with a diagnosis of IBS according to the Rome II criteria,[7] of the constipation-predominant type as defined by <3 bowel movements (BM) per week and/or hard stools and/or straining.[7] A subgroup of subjects was defined on the single criterion of <3 bowel movements per week. In addition to fulfilling the Rome II criteria in the preceding 12 months, subjects were to have presented symptoms (abdominal pain, bloating, or global digestive discomfort) at least once per week during the last month. Organic GI diseases, including inflammatory bowel disease and GI involvement of systemic diseases, were excluded. To be considered for inclusion, all subjects had to have undergone within the last 5 years a colonoscopy, which had proved normal. Subjects were usual consumers of dairy products.

Subjects were excluded from the study if they had a diarrhoea-predominant (more than 3 stools/day and/or loose or watery stools, and/or urgency) IBS type, clinical signs of alarm (rectorragy, fever, recent weight loss), or current severe abdominal pain as defined by the need of analgesics or antispasmodics for 5 days or more. Subjects were not eligible if they had started psychotropic drugs or drugs for IBS-symptoms since <1 month.

Pregnant or breast-feeding women, women planning to become pregnant during the study, individuals with known lactose intolerance, dietary habits which might interfere with the assessment of the study product (e.g.

slimming or vegetarian diets) or known allergy to the study product components were also excluded.

Throughout the study, the subjects were not allowed to consume any probiotic or fermented dairy product other than those provided, fibres or complete cereal products, or prebiotics. They were encouraged to continue with all the other aspects of their dietary and physical exercise habits.

Study protocol

During a 1- to 3-week baseline run-in period, symptoms, stool frequency and consistency/form were recorded, and subjects answered a specific HRQoL questionnaire (the Functional Digestive Disorders Quality of Life (FDDQL) questionnaire).[29] Thereafter, eligible IBS subjects were randomized to consume, daily for 6 weeks, 2 pots (one at breakfast, one at dinner) of either the study fermented milk (test product) or the control, heat-treated product. The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the ''Pays de la Loire n °2 Region'' of Nantes, France. All subjects provided written informed consent before inclusion in the study.

Study products

The test product was a fermented milk (Activia, Danone), containing *Bifidobacterium animalis* DN-173 010 (1.25×10^{10} colony forming unit (cfu) per pot) together with the two classical yoghurt starters, *S. thermophilus* and *L. bulgaricus* (1.2×10^{9} cfu/pot). The control product was heat-treated yoghurt containing non-living bacteria ($<10^{4}$ cfu/pot). Both the test and control products were without flavour, and had a similar appearance, colour, texture and taste. Each serving, corresponding to one pot, contained 125 g. Both products were specifically prepared for the study and provided by Danone Research (Palaiseau, France).

Assessments and study endpoints

Health-related quality of life

This was assessed by the self-administration of the FDDQL questionnaire as developed and validated by Chassany *et al.*,[29] at baseline (i.e. the day before starting the consumption of the test or control product) and after 3 and 6 weeks of product consumption. The primary endpoint as prespecified in the protocol was the discomfort HRQoL dimension that measures the problem impact of the whole IBS symptoms on HRQoL at week 3. This *a priori* specification of a dimension of interest among a multidimensional questionnaire has been done in accordance with the recent guidance documents released by the Food and Drug Administration[30] and the European Agency for the Evaluation of Medicinal Products.[31]

The secondary endpoints of HRQoL were the seven other dimensions (daily activities, anxiety, diet, sleep, health perceptions, coping with disease and stress impact) investigated by the FDDQL, and the global HRQoL score. Dimension scores and global score ranged from 0 (worse HRQoL) to 100 (best HRQoL). Subjects reporting an improvement at least 10% vs. baseline of their discomfort dimension score were defined as responders. In the absence of consensus, the threshold has been set accordingly with the definitions of responders reported in the literature for HRQoL questionnaires.[32, 33] The rate of responders was calculated at the end of weeks 3 and 6 of the study period.

IBS symptoms, stool characteristics

These parameters were defined as secondary endpoints. Subjects completed a diary to evaluate their symptoms (bloating, abdominal pain, and evolution of global digestive symptoms) and stool characteristics (stool frequency and consistency/form). Bloating and abdominal pain were evaluated, at baseline and at the end of weeks 3 and 6 of the study period, using a 6-point Likert scale. Bowel movements were reported daily, and their number expressed per week. The evolution of global digestive symptoms was evaluated using a 7-point Likert scale: results were regrouped into three categories, i.e. worsened, no change and improved. Stool consistency form was determined using the Bristol stool scale.[34]

Subjects recorded daily in their diary about the consumption of study products, medications started during the study, and forbidden products (fibres, other fermented dairy products), as well as any adverse events.

Statistical methods

The trial sample size, required to give a power of 85% for detecting a 'between group' effect size of 0.4 for the primary endpoint, was calculated to be at least

114 subjects per treatment group. This effect size figure was chosen to allow the detection of a moderate effect, seeing that sizes of 0.2 and 0.8 are classically associated with a weak and important effect, respectively.[35] To achieve a number of 114 subjects per group, 138 subjects per group were finally recruited to take into account premature withdrawals and missing values for main criteria.

All analyses were done on the intention-to-treat (ITT) population corresponding to subjects having consumed at least one pot of product. In addition, analyses of HRQoL, IBS symptoms and stool characteristics data were performed in the subgroup of those definitely constipated subjects ($n = 19$), as defined on the only basis of <3 stools per week; a lower significance level ($P < 0.001$) was applied for judging results in this subgroup, as recommended.[36]

Baseline demographic data were compared between groups using Student's *t*-test, χ^2-test or Fisher's exact test, when appropriate.

For all the dimensions of the FDDQL questionnaire, the last observation carried forward method was used to replace missing data at 6 weeks. The analysis of the score differences, at weeks 3 and 6 vs. baseline, between test and control groups was done using a non-parametric covariance analysis (treatment group and baseline score as fixed factors and primary care centre as random factor). The responder rates for the discomfort HRQoL dimension were analysed at weeks 3 and 6 by χ^2-test.

For IBS-symptoms, differences between groups were analysed using a non-parametric analysis of variance at weeks 3 and 6 (for bloating and abdominal pain) with treatment group and baseline score as fixed factors. Stool frequency and consistency were analysed using a repeated-measure analysis of variance with treatment group and baseline score as fixed factors. The global digestive symptoms assessment at weeks 3 and 6 was analysed by the Mann–Whitney–Wilcoxon test. All analyses of the evolution of scores of dimensions of FDDQL questionnaire and IBS-symptoms (3- or 6-week values vs. baseline) within each group were done using the Mann–Whitney–Wilcoxon test.

RESULTS

Subjects

Figure 1 describes the flow of subjects through the protocol. From the 276 enrolled subjects, 274 were randomized. Seven subjects were subsequently excluded (premature withdrawal) leaving 135 subjects assigned to the test product group and 132 to the control group giving an ITT population of 267.

Baseline characteristics of subjects

They showed no significant differences between the test product and control groups (Table 1).

Compliance to product consumption

The self-reported levels of product consumption were 96.3 ± 9.3% and 96.7 ± 6.1% in the test product and control groups, respectively.

Health-related quality of life

Results (within and between groups, and rates of responders) of the HRQoL discomfort dimension are shown in Table 2. Results (within and between groups) of the seven other HRQoL dimension scores and the global HRQoL score are shown in Table 3.

Discomfort dimension score, the primary HRQoL endpoint, improved ($P < 0.001$) at weeks 3 and 6, as compared to baseline, in both groups but the improvement did not significantly differ between groups (Table 2). The effect size between groups at week 3 is 0.22 in favour of the test group. The percentage of responders for the discomfort dimension was significantly higher (65.2 vs. 47.7%, $P = 0.003$) at week 3, in the test product as compared to the control group.

The scores of daily activities and anxiety improved ($P < 0.001$) at weeks 3 and 6, as compared to baseline, in both groups; the improvement was significantly higher in the control vs. the test product group (Table 3). The stress impact dimension was more ($P < 0.05$) improved, at week 6, in the control as compared to the test product group. Score changes, at weeks 3 and 6 vs. baseline, of diet, sleep, health perception and coping with disease dimensions, and the global score did not differ significantly between groups (Table 3).

IBS symptoms and stool characteristics

Bloating, abdominal pain and global digestive symptom assessment

Score values within the test product and control groups at baseline and weeks 3 and 6, as well as

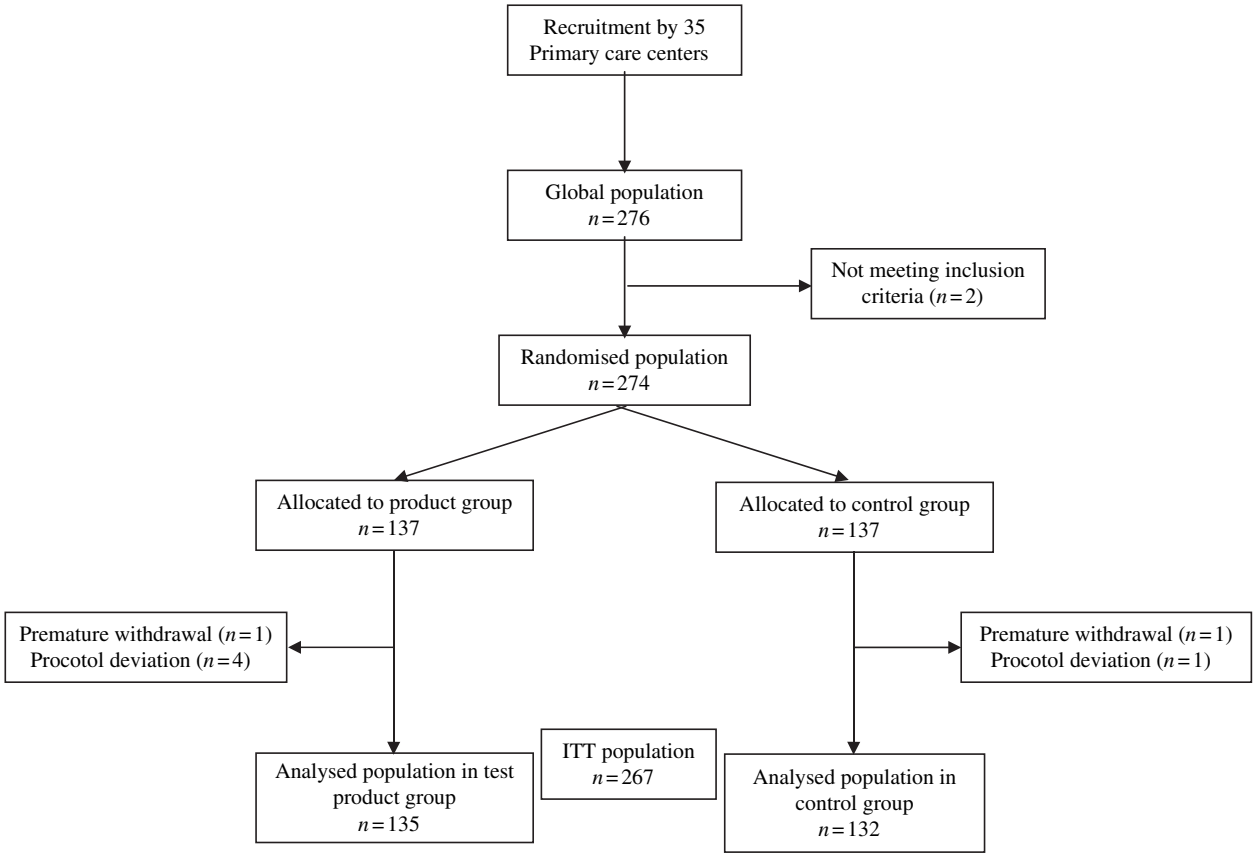

Figure 1. Study flow design.

Table 1. Baseline characteristics of subjects: comparisons between groups

	Test product group (n = 135)	Control group (n = 132)	P value
Age (years)	49.4 ± 11.4	49.2 ± 11.4	NS
Range (years)	23–65	20–65	
Sex ratio (female/male)	106/29	93/39	NS
HRQoL discomfort score*	49.7 ± 18.8	52.5 ± 18.2	NS
Bloating score†	3.79 ± 1.09	3.73 ± 1.04	NS
Abdominal pain score†	3.47 ± 1.14	3.24 ± 1.09	NS
Stool frequency	7.07 ± 5.13	6.79 ± 3.92	NS
Stool consistency‡	3.23 ± 1.59	3.38 ± 1.74	NS

All data are expressed as means ± s.d. No significant ($P > 0.05$) differences were found between groups in all the variables tested.
HRQoL : health-related quality of life.
*According to Chassany *et al.*[29]; †Assessed with a 6-pt Likert scale; ‡According to Lewis and Heaton.[34]

differences between groups at weeks 3 and 6 vs. baseline are shown in Table 4. Bloating and abdominal pain scores at weeks 3 and 6 significantly ($P < 0.001$) improved as compared to baseline within both groups. The improvement in bloating score was significantly ($P = 0.03$) higher at week 3 in the test

Table 2. Functional digestive disorders quality of life questionnaire*: discomfort dimension in the intention-to-treat population (n = 267) at baseline and weeks 3 and 6 of the study period

	Test product group (n = 135)			Control group (n = 132)		
	Baseline	Week 3	Week 6	Baseline	Week 3	Week 6
Score (0-100)	49.7 ± 18.8	60.8 ± 18.1†	62.0 ± 18.4†	52.5 ± 18.2	59.9 ± 17.9†	65.8 ± 18.9†
Change from baseline		10.7 ± 14.5	12.2 ± 16.2		7.5 ± 14.7	13.5 ± 19.3
Responder rate (%)		65.2% ‡ (88/135)	63.0% (85/135)		47.7% (63/132)	56.8% (75/132)

Scores (0: worse; 100: best) and changes from baseline are expressed as means ± s.d. Rates of responders are expressed as percentages and as the ratio (in brackets) of the number of responders to the number of all the corresponding group subjects. Analysis of the evolution of scores (3- and 6-week vs. baseline values) in each group was done using the Mann–Whitney–Wilcoxon test († $P < 0.001$). Changes from baseline at weeks 3 and 6 were compared between groups with a non-parametric covariance analysis adjusted on the baseline discomfort score. Rates of responders were compared by χ^2-test (‡ $P < 0.005$).
*According to Chassany *et al.*[29]

Table 3. Functional digestive disorders quality of life questionnaire*: 7 (other than discomfort, shown in Table 2) quality-of-life dimensions and global score in the intention-to-treat population (n = 267) at baseline and weeks 3 and 6 of the study period

	Test product group (n = 135)			Control group (n = 132)		
	Baseline	Week 3	Week 6	Baseline	Week 3	Week 6
Daily activities	79.0 ± 18.2	83.6 ± 17.0†	86.0 ± 15.3†	81.7 ± 17.3	87.7 ± 14.5†	89.6 ± 13.1†
		(4.5 ± 13.8)	*(7.0 ± 16.5)*		*(5.8 ± 14.3‡)*	*(7.8 ± 16.4)*
Anxiety	63.3 ± 22.9	67.2 ± 21.9†	69.3 ± 20.1†	63.9 ± 21.0	69.7 ± 20.3†	73.8 ± 19.8†
		(3.9 ± 15.6)	*(6.0 ± 16.3)*		*(5.3 ± 14.1‡)*	*(9.6 ± 17.0§)*
Diet	60.4 ± 24.3	62.5 ± 23.7	65.4 ± 22.4†	63.2 ± 22.8	69.0 ± 22.1†	72.3 ± 20.1†
		(2.6 ± 16.3)	*(5.5 ± 15.2)*		*(5.7 ± 14.8)*	*(9.0 ± 18.6)*
Sleep	68.9 ± 19.4	77.5 ± 20.4†	78.2 ± 20.5†	71.4 ± 17.7	77.5 ± 18.5†	79.7 ± 18.6†
		(9.1 ± 16.8)	*(9.6 ± 19.1)*		*(5.6 ± 15.6)*	*(8.1 ± 20.0)*
Health perception	62.1 ± 19.4	63.4 ± 18.8	63.7 ± 18.5	61.8 ± 21.3	64.1 ± 20.2	66.0 ± 19.6¶
		(1.2 ± 13.1)	*(1.4 ± 16.3)*		*(1.8 ± 14.7)*	*(3.7 ± 16.8)*
Coping with disease	52.6 ± 23.5	57.2 ± 23.6	57.0 ± 23.5¶	49.1 ± 24.4	51.9 ± 23.9¶	56.4 ± 24.4¶
		(4.5 ± 24.2)	*(4.7 ± 24.0)*		*(3.1 ± 22.5)*	*(7.4 ± 25.6)*
Impact of stress	27.5 ± 24.6	27.4 ± 23.3	28.2 ± 23.3	30.3 ± 25.7	34.1 ± 26.8**	35.3 ± 27.1†
		(−0.6 ± 20.7)	*(0.5 ± 23.5)*		*(3.7 ± 23.5)*	*(4.5 ± 23.1§)*
Global score	62.3 ± 15.8	67.7 ± 14.0†	68.8 ± 14.7†	63.0 ± 14.2	68.7 ± 13.2†	72.1 ± 12.4†
		(5.2 ± 9.8)	*(6.5 ± 11.2)*		*(4.9 ± 9.0)*	*(8.7 ± 12.1)*

Scores (0: worse; 100: best) and changes from baseline (in brackets and italics) are expressed as means ± s.d. Analysis of the evolution of score (3 and 6 weeks value vs. baseline) in each group was done using the Mann–Whitney–Wilcoxon test († $P < 0.001$; ¶ $P < 0.01$; ** $P < 0.05$). Changes from baseline at weeks 3 and 6 were compared between groups with a non-parametric covariance analysis adjusted on the baseline discomfort score (‡ $P < 0.005$; § $P < 0.05$).
*According to Chassany *et al.*[29]

product as compared to the control group. The evolution of global digestive symptoms scores (Table 4) as well as the percentage of subjects with global digestive symptom score improvement (data not shown) did not differ between groups at weeks 3 and 6.

Table 4. Irritable bowel syndrome symptoms: bloating*, abdominal pain* and global digestive symptoms† in the intention-to-treat population (n = 267) at baseline and weeks 3 and 6 of the study period

	Test product group (n = 135)			Control group (n = 132)		
	Baseline	Week 3	Week 6	Baseline	Week 3	Week 6
Bloating	3.79 ± 1.09	3.27 ± 1.03‡ *(−0.56 ± 1.01)§*	3.13 ± 1.12‡ *(−0.69 ± 1.24)*	3.73 ± 1.04	3.44 ± 0.95‡ *(−0.31 ± 0.87)*	3.06 ± 1.17‡ *(−0.67 ± 1.25)*
Abdominal pain	3.47 ± 1.14	2.94 ± 1.10‡ *(−0.55 ± 1.03)*	2.80 ± 1.12‡ *(−0.68 ± 1.20)*	3.24 ± 1.09	2.85 ± 1.14‡ *(−0.39 ± 0.95)*	2.73 ± 1.19‡ *(−0.52 ± 1.27)*
Evolution of global digestive symptoms		4.88 ± 1.04	5.07 ± 1.14		4.75 ± 0.99	5.22 ± 1.26

Scores and change from baseline (in brackets and italics) are expressed as mean ± s.d. Analysis of the evolution of bloating and abdominal pain score (3- and 6-week vs. baseline values) in each group was done using the Mann–Whitney–Wilcoxon test (‡ $P < 0.001$). Changes from baseline at weeks 3 and 6 were compared between groups with a non-parametric covariance analysis adjusted on the baseline score (§ $P < 0.05$). Global digestive symptoms were compared by the Mann–Whitney–Wilcoxon test.
* Assessed with a 6-pt likert scale from; † Assessed with a 7-pt likert scale.

Stool characteristics

Data are shown in Figure 2. In the ITT population (n = 267), stool frequency (Figure 2a) and consistency (data not shown) did not differ between the test product and control groups. In the subgroup (n = 19) of constipated IBS subjects, defined on the basis of <3 bowel movements per week, stool frequency increased ($P < 0.001$) over the 6-week period of product consumption, showing effect as soon as week 1, in the test product subgroup as compared to the control subgroup (Figure 2b). Results of the analysis of HRQoL discomfort dimension and IBS symptom scores in this subject subgroup are given in Table 5. HRQoL discomfort score and responder rate, bloating, abdominal pain and global digestive symptom scores improved ($P < 0.05$) at week 6 in the test product but not in the control subgroup.

Adverse events

Ten subjects from the control group and 13 from the test product group reported minor adverse events throughout the study. Four subjects in the control group and three in the test group stopped the consumption of the product after an adverse event. Two subjects reported serious adverse events in the control group.

DISCUSSION

In this study involving a large cohort of adults with IBS of the constipation-predominant type according to Rome II criteria,[7] HRQoL was chosen as a primary endpoint in order that results should be as close as possible to the subjects' own perception of their symptoms and to the impact of the latter on their daily life. The discomfort dimension of FDDQL, a validated questionnaire,[29] was able to capture the improvement in multiple IBS symptoms, which has been one of the guidelines for selecting the primary endpoint in the design of treatment trials for functional gastrointestinal disorders in Rome II[37] as well as in the recent Rome III[38] criteria. HRQoL remains as an important criterion since IBS significantly impacts it.[10–13] HRQoL goes beyond symptoms and functional status and thus measures broader concepts; therefore it is quite logical that the expected improvement over time and the expected differences between groups of the HRQoL endpoint are smaller than the ones expected for symptoms.[39] Based on this consideration, the definition of responder in terms of HRQoL is also different than that for symptoms, i.e. the threshold of improvement of HRQoL score is lower than that of the improvement of symptoms, such as abdominal pain.[7] This has been observed across many disease areas.[40–42] Finally, while using HRQoL as a primary endpoint in a randomized clinical trial may appear as a challenge, it is however, considered by regulatory authorities as an important endpoint in the evaluation of therapeutics.[30, 31] Indeed, one study with alosetron has reported a significant improvement of HRQoL, clearly showing the sensitivity of HRQoL instruments to detect positive effects in trials.[43]

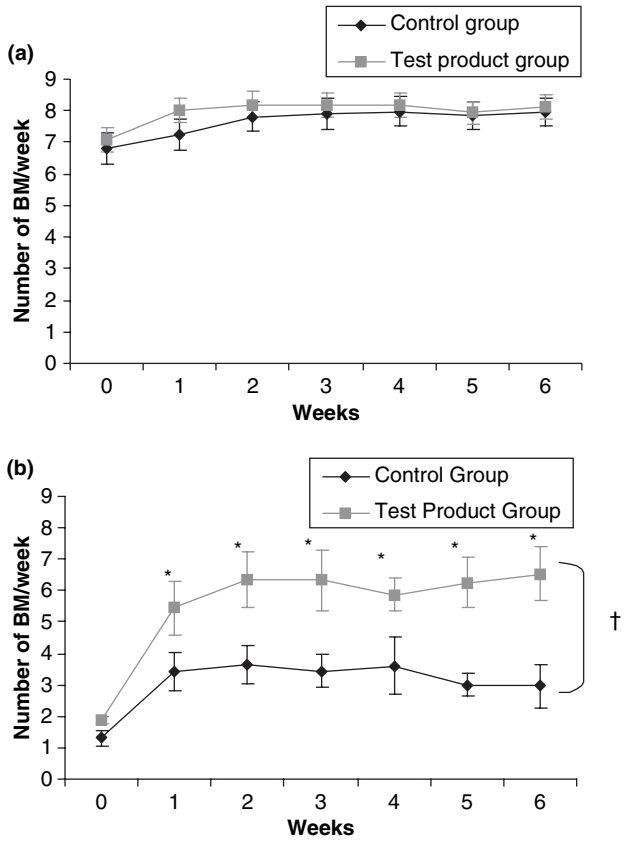

Figure 2. Stool frequency: (a) intention-to-treat population (n = 267); (b) subgroup of subjects (n = 19) with <3 bowel movements (BM) per week. Values are expressed as means ± s.d. Analysis of the evolution of the stool frequency was done using a repeated-measure analysis of variance, either comparing each time-points (*$P < 0.05$) or global evolution throughout the 6-week period of consumption (†$P < 0.001$).

To the best of our knowledge, only two studies[16, 17] reporting the effects of probiotics in IBS have used a specific IBS HRQoL questionnaire: this is in line with the limited number of subjects in most of the previously published trials on the probiotics-IBS topic. In the present study, the product efficacy was also assessed, as recommended,[38] by using a responder definition based on the answer to the main criterion.

Our recruitment strategy was defined to select adults with mild-to-moderate forms of the constipation-predominant spectrum of IBS, in order to study subjects close to those seen in the community rather than at referral centres.[8] In clinical trials on IBS, the initial degree of symptom severity is a key point, since it may influence the improvement of these symptoms and HRQoL;[44] although subjects with mild symptoms are the most likely to report satisfactory relief, they showed, in a recent study, no average decrease in symptom severity or improvement in IBS-QoL, while, conversely, subjects with severe-symptoms had the largest reductions in IBS symptom severity and the largest improvements in IBS-QoL but were the least likely to report satisfactory relief.[44]

A high "placebo" effect was observed in the control group in the present work, which confirms well-known data reported in IBS placebo-controlled trials showing an average placebo response rate of 40–45%.[45, 46] For some dimensions of the HRQoL questionnaire (daily activities, anxiety and impact of stress), a significantly more pronounced increase was observed in the control group. Moreover, we observed an increasing placebo effect in the control group from week 3 to week 6, which may account for the non-significant effect of test product on bloating and responder rate for discomfort dimension of FDDQL at week 6. Similar findings have been reported in the evolutionary profile of the placebo effect in functional dyspepsia and other functional GI disorders,[47] and in Crohn's disease.[48] The reason for the increase in placebo response with time, as well as for the high placebo response at week 6 (around 60%), is uncertain but might be due to the natural variation of the disease, regression to the mean, and subject's expectations.[45, 49] Understanding the placebo response in IBS trials represents an important challenge that is currently the object of specific research.[49] In addition, in the present study, the given control product (heat-treated yoghurt) may not have been a true placebo and may have influenced primary and secondary outcomes due to its own properties: one work has suggested that non-living probiotic strains may have an effect in IBS[50] and another recent laboratory study in a post-parasite infection mouse model, has shown that the spent culture medium for *Lactobacillus paracasei* could normalize inflammatory changes as well as improve smooth muscle hypercontractility to levels similar to those treated with the live bacteria.[51] Moreover, the high placebo effect could be partly due to the advertising (TV, magazines, posters…) done on the health benefit of fermented dairy products, such as the one tested in the present study. Consequently, this may have led to an underestimate of the observed effect of the test product. However, in spite of this, the test product exhibited a difference over the control group of 17.5% in the rate of responders for HRQoL discomfort score, which in turn results in a number needed to treat of

Table 5. Health-related quality-of-life (HRQoL) discomfort dimension and symptoms of the irritable bowel syndrome in the subgroup of subjects (n = 19) with <3 bowel movements/week

	Test product group (n = 9)			Control group (n = 10)		
	Baseline	Week 3	Week 6	Baseline	Week 3	Week 6
HRQoL discomfort*						
Score	44.8 ± 15.7	64.0 ± 17.5	63.0 ± 21.0§	53.6 ± 12.5	55.8 ± 14.0	56.9 ± 19.6
Changes from baseline		*(16.7 ± 16.7)*	*(18.2 ± 13.5¶)*		*(3.7 ± 10.0)*	*(4.8 ± 18.2)*
Responder rate (%)		67% (6/9)	78%¶ (7/9)		40% (4/10)	30% (3/10)
Bloating†						
Scores	4.0 ± 0.76	3.00 ± 1.0§	2.75 ± 1.28§	4.40 ± 0.84	4.10 ± 0.57	3.56 ± 1.13
Change from baseline		*(−1.0 ± 0.93¶)*	*(−1.25 ± 1.04)*		*(−0.30 ± 0.67)*	*(−0.78 ± 1.30)*
Abdominal pain†						
Scores	4.00 ± 0.76	3.33 ± 1.0	2.50 ± 1.31§	3.10 ± 1.37	2.90 ± 1.10	2.33 ± 1.32
Change from baseline		*(−0.75 ± 0.71)*	*(−1.50 ± 1.20)*		*(−0.20 ± 0.63)*	*(−0.56 ± 1.33)*
Global digestive symptoms‡		5.56 ± 0.53¶	5.88 ± 0.64¶		4.60 ± 1.07	4.67 ± 1.66

Scores (0: worse; 100: best) and changes from baseline (in brackets and italics) are expressed as means ± s.d. Rates of responders are expressed as percentages and ratios (in brackets) of the number of responders to the number of subjects of the whole corresponding group. Analysis of the evolution of score (3- and 6- week vs. baseline values) in each group was done using the Mann–Whitney–Wilcoxon test (§ $P < 0.05$). Change from baseline at weeks 3 and 6 were compared between groups with a non-parametric covariance analysis adjusted on the baseline score († $P < 0.05$). Rates of responders were compared by χ^2-test (¶ $P < 0.05$). Global digestive symptoms were compared by Mann–Whitney–Wilcoxon test (¶ $P < 0.05$).
*According to Chassany *et al.*[29]; †Assessed with a 6-pt Likert scale ; ‡Assessed with a 7-pt likert scale.

5.7. A Cochrane systematic review of antispasmodic agents in IBS yielded a number needed to treat of 6.[52]

Bloating-type symptoms are the most common and bothersome abdominal complaint in subjects with IBS and are reported by up to 96% of them.[53] Bloating, a notoriously difficult symptom to treat,[54] is also one of the most important supportive signs of IBS diagnosis[7] and *per se* a frequent GI functional disorder, i.e. ''functional bloating''.[8] In the present study, we observed a significant relief in abdominal bloating, for the whole probiotic subject group and for the subgroup characterized by a stool frequency of <3 stools/week. The significant reduction of bloating score we observed appears to be of special interest, as the magnitude of symptom reduction by standard medical care has been shown to be significantly greater in subjects with severe IBS as compared to subjects with moderate or mild IBS.[44] A similar alleviating effect on bloating of VSL#3,[55] a mixture of 8 probiotics, and of *B. infantis* 35624[16] has been reported in a study population of IBS subjects (35 and 77 subjects, respectively). The alleviating effect of the latter probiotic has been very recently confirmed and extended, in a large randomized controlled trial.[17]

The mechanisms of the probiotic effects on IBS symptoms, including bloating,[9] are not precisely known, although a shift in pro-inflammatory cytokine profiles is strongly suggested.[16] Effects on bloating might be particularly related to a reduced synthesis of gaseous products of fermentation,[56–58] due to an alteration in the resident colonic microbiota,[57] motility, gas distribution (small intestinal vs. colonic) and/or propulsion along the bowel.[59–61] The positive effects on bloating also reported with non-absorbable antibiotics[62] further supports the evolving role of the enteric microbiota in IBS. But, probiotics, especially probiotic food, such as fermented milk, may ultimately prove much more acceptable and cost-effective than antibiotics for the long-term treatment of a chronic disease such as IBS.[63]

In this study, a striking and significant effect (up to a mean of 6 stools per week) of the test probiotic product on stool frequency was observed in that subgroup of subjects with the quantitatively most severe (<3 stools per week) degree of constipation: even though the sample size was not calculated for the multiple comparisons done, the high level of significance found was consistent with the statistical

criteria required by the number of subgroup analyses performed.[36] The increase in stool frequency, unaccompanied by a significant change in stool consistency scores, showed a rapid onset, i.e. after 1 week of product consumption, and was sustained throughout the 6-week study. Consistent with our observation is the previous finding that the probiotics tested in the present study may accelerate the intestinal, especially colonic and sigmoid, speed of transit.[22–25] Most of the present primary care IBS subjects had mild-to-moderate constipation (which was defined, according to Rome II criteria,[7] more often by hard/lumpy stools and/or straining than solely by <3 bowel motions per week) that probably accounts for the relatively small number of subjects in the above-mentioned subgroup. It is noteworthy that the favourable probiotic effects in this subgroup also included a significant alleviation of bloating and global digestive symptom score. Investigation of a larger IBS population with <3 bowel movements a week, as that seen in referral centres warrants further consideration.

A clear-cut effect of probiotics on constipation *per se* has been quite rarely shown in IBS subjects, except in two studies.[17, 64] The most important one[17] concerned 362 primary care subjects with any bowel habit subtype having received for 4 weeks *B. infantis* 35624, a probiotic which previously showed no effect on stool frequency in a more limited number of subjects.[16]

In conclusion, the present large-scale study strongly suggests a beneficial effect of a probiotic food containing *B. animalis* DN-173010 on HRQoL discomfort score and bloating, and also on stool frequency in those subjects with <3 stools per week. Further studies aiming to confirm the results obtained and to elucidate mechanisms of such effects should be of special interest for providing additional scientific evidence to support the use of such probiotic food to alleviate IBS symptoms and improve HRQoL discomfort.

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