Neurogastroenterology & Motility

Neurogastroenterol Motil (2013) **25,** 331–e252 doi: 10.1111/nmo.12078

A randomized, double-blind, controlled study and pooled analysis of two identical trials of fermented milk containing probiotic *Bifidobacterium lactis* CNCM I-2494 in healthy women reporting minor digestive symptoms

P. MARTEAU,* D. GUYONNET,† P. LAFAYE DE MICHEAUX‡ & S. GELU§

*Department of digestive diseases, AP-HP, Lariboisière Hospital, University Denis Diderot, Paris, France
†Digestive Health Department, Danone Research, Palaiseau, France
‡Mathematics and Statistics Department, Montreal University, Montreal, QC, Canada
§RPS France, Caen, France

Abstract

Background *The probiotic fermented milk (PFM) containing Bifidobacterium lactis CNCM I-2494 improved gastrointestinal (GI) well-being and digestive symptoms in a previous trial involving women reporting minor digestive symptoms. Our objective is to confirm these findings in a second study and in a pooled analysis of both studies.* ***Methods*** *In this double-blind, controlled, parallel design study, subjects without diagnosed GI disorders consumed PFM or control dairy product daily for 4 weeks. Endpoints comprised weekly assessment of GI well-being (primary endpoint), rate of responders and digestive symptoms. Data were analyzed on full analysis set population (n = 324) and on the pooled data of randomized subjects of this study with those of the first study (n = 538).* ***Key Results*** *In this second study, no significant difference was observed in the percentage of women reporting an improvement in GI well-being [OR = 1.20 (95% CI 0.87, 1.66)] and rate of responders [OR = 1.38 (95% CI 0.89, 2.14)]. Composite score of digestive symptoms was significantly (P < 0.05) reduced in PFM when compared to the control group [LSmean = −0.42 (95% CI −0.81, −0.03)]. In the pooled analysis, significant differences were observed in favor of PFM group for all endpoints: percentage of women with improved GI well-being [OR = 1.36 (95% CI 1.07, 1.73)], rate of responders [OR = 1.53 (95% CI 1.09, 2.16)] and composite score of digestive symptoms [LSmean = −0.48 (95% CI −0.80, −0.16)].* ***Conclusions & Inferences*** *This second study did not confirm improvement on the primary endpoint. However, a pooled analysis of the two trials showed improvement in GI well-being and digestive symptoms in women reporting minor digestive symptoms.*

Keywords *adult, digestive symptoms, pooled analysis, probiotic, randomized controlled trials.*

Abbreviations: AUC, area under the curve; cfu, colony forming unit; FAS, full analysis set; FBA, food and benefit assessment; GEE, generalized estimation equations; GI, gastrointestinal; IBS, irritable bowel syndrome; IPAQ, international physical activity questionnaire; ITT, intention to treat; NNT, number needed to treat; OR, odd ratio; PFM, probiotic fermented milk.

INTRODUCTION

Recent observations have supported a role for gut microbiota in functional bowel disorders.[1–3] Probiotic foods and supplements have gained high interest during the past years for their use in intestinal diseases.[4] Some can clearly modulate gut functions[5] and seem capable of alleviating gastrointestinal (GI) symptoms in irritable bowel syndrome (IBS).[6,7]

Address for Correspondence
Philippe Marteau, Department of digestive diseases, AP-HP, Lariboisière Hospital, University Denis Diderot, Paris 7, Paris, France.
Tel: +33 1 49 95 25 78; fax: +33 1 49 95 25 77;
e-mail: philippe.marteau@lrb.aphp.fr
Received: 13 April 2012
Accepted for publication: 2 December 2012

However, studies have also shown conflicting results between different probiotic preparations.[8–11] Moreover, some effects are strain-specific, which makes findings for one particular probiotic food/strain not applicable to another, even if it belongs to the same species (e.g. *Lactobacillus acidophilus*).[12,13] These data support the importance of accumulating scientific evidence on specific and well-defined probiotic preparations in high-quality studies to determine its efficacy on gut functions and disorders.

Probiotic foods target large populations. Beyond well-diagnosed IBS subjects or those with other functional bowel disorders, chronic GI symptoms are remarkably common in the general population.[14–16] Gastrointestinal complaints related to gas such as bloating and flatulence are among the more frequent symptoms in this population.[16] A minority of people complaining about such GI symptoms consult medical professionals[17] but these bowel disturbances are well-known to be very bothersome and may substantially impact the daily life on most of those afflicted. The interest of using food to improve this minor to mild spectrum of GI symptoms needs to be assessed as no drug is really addressing this population.

Probiotic fermented milk (PFM) with a specific *Bifidobacterium* strain (*Bifidobacterium lactis* CNCM I-2494) with lactic acid bacteria (*Lactobacillus delbrueckii subsp. bulgaricus* and *Streptococcus thermophilus)* has shown beneficial effects on gut functions in several randomized controlled studies.[18–21] Firstly, the consumption of this probiotic food was associated with shortening of colonic transit time in both healthy people[21] and subjects with IBS-C.[19] Secondly, improvement of abdominal distension and other GI symptoms were observed with this product in people with IBS-C.[19,20] Thirdly, the ability of this product to improve GI symptoms and GI comfort was also demonstrated in non-IBS people reporting minor GI troubles.[18,22] So far, such properties to improve colonic transit time and GI symptoms in IBS-C patients and in the general population have not been demonstrated for any other probiotic preparation.

There are very few examples of simple repetition (duplication) of a clinical study evaluating the effect of administering a clearly defined probiotic preparation (single-organism or specific combination of different bacteria strains) for improving a specific health condition in a well-defined population. However, study repetition is an important criterion for clarifying the beneficial effect of a probiotic product as demonstrated by the conflicting results obtained with the strain *Lactobacillus* GG in the primary prevention of atopic dermatitis.[23,24] In such context, combining the results of the trials in a pooled analysis provides results with higher confidence (higher sample size, smaller confidence interval, more robust product effects estimation) for analyzing the efficacy of the tested product and thus, should establish whether or not there is evidence of an effect and resolve discrepancies between repeated studies.[25] In addition, trials on drugs for IBS usually include large number of subjects to take into consideration the placebo effect and the beta risk to fail to show a significant difference just because of the too small number of subjects.

We present the results of a medium-scale study (n = 324) that is a repetition of the Guyonnet *et al.*[18] study with identical design, testing the effect of 4-week consumption of this probiotic food and of the pooled analysis of this new study together with the first study.[18]

EXPERIMENTAL METHODS

Study population

A total of 388 subjects were recruited in France from one clinical center (RPS clinical centre, Caen). Subjects were identified in the database of healthy subjects of the clinical center. They were recruited through advertisement and none of the subjects was derived from primary care or hospital department. Recruitment period was the same in this study and in the study of Guyonnet *et al.*[18]: first recruited subject in September and last subject out in mid-December. Inclusion criteria were also the same in both studies. Women, 18 to 60 years old, with body mass index between 18 and 30 kg m^{-2}, and having a bowel movement frequency within normal range (3–21 $week^{-1}$). During the entry into the study, a screening questionnaire was used to determine the frequency of four different digestive symptoms (i.e. discomfort or abdominal pain, bloating, flatulence/passage of gas, borborygmi/rumbling stomach) in the past month as already described.[18] Subjects were regular consumers of dairy products.

Subjects were excluded if: (i) they had been diagnosed with IBS or any other functional bowel disorders, (ii) they already consulted gastroenterologist or general practitioner for digestive symptoms of the lower tract (colon and small intestine), (iii) they took or were under prescription of treatment for digestive symptoms (e.g. antispasmodic, laxatives or antidiarrheal drugs), and (iv) they had any significant systemic disease. Antibiotic ingestion within the month prior to the entry in the study was also an exclusion criterion.

Individuals with known lactose intolerance or with 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 (including food supplements) or fermented dairy product other than those provided. They were encouraged to continue with all the other aspects of their dietary and physical exercise habits

Study protocol

The study was single-center, randomized, double-blind, controlled, parallel-group assessing the effect of daily consumption

of a PFM containing *B. lactis* CNCM I-2494 (PFM group) *vs* a non-fermented dairy product (control group).

The study design included a baseline period of 2 weeks followed by a 4-week period of intervention and was a repetition of the design used in the Guyonnet *et al.*[18] study, except the absence of a washout period after the intervention. Briefly, baseline values were obtained for the outcome parameters with a weekly assessment of frequency of digestive symptoms (abdominal pain/discomfort, bloating, flatulence/passage of gas and borborygmi/rumbling stomach), bowel function (bowel movement and stool consistency using the Bristol stool form scale)[26] and health-related quality of life using the Food and Benefit Assessment (FBA) questionnaire.[27] Only subjects having a mean composite score between 2 and 12 [score ranging from 0 (no symptom) to 16 (all symptoms every day)] during this 2-week baseline period and also meeting the other randomization criteria (normal bowel movement frequency, no consumption of antibiotics) were randomized to consume 2 cups day^{-1} (one at breakfast and one at the evening meal) for the 4 weeks of intervention.

The study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee Nord Ouest III (Caen, France). All volunteers gave written informed consent before inclusion in the study. The study is registered at Clinicaltrials.gov: NCT01388010.

Study products

The test product was a fermented milk containing *Bifidobacterium lactis* [strain number I-2494 in French National Collection of Cultures of Microorganisms (CNCM, Paris, France)], referred as DN-173 010 in previous publications such as in the Guyonnet *et al.*[18] paper, together with the two classical yogurt starters, *S. thermophilus* (CNCM strain number I-1630) and *L. bulgaricus* (CNCM strain numbers I-1632 and I-1519), and *Lactococcus lactis* ssp. *lactis* (CNCM strain number I-1631). The test product contains 1.25×10^{10} colony forming unit (cfu) of *Bifidobacterium lactis* CNCM I-2494/DN-173 010 per cup and 1.2×10^{9} cfu cup^{-1} of *S. thermophilus* and *L. bulgaricus*.

The control product was a milk-based non-fermented dairy product without probiotics and with a lactose content of < 4 g cup^{-1} which is similar to the content of lactose in the test product. The control product was acidified using an enzymatic process, which mimics the acidification process that occurs during fermentation of milk by the bacteria strains. Both the test and control products were without flavor and had a similar appearance, texture, and taste. Each cup contained 125 g. Both products were specifically prepared for the study and provided by Danone Research (Palaiseau, France).

Compliance was assessed on the basis of the data reported by subjects on their diaries and of the number of non-used servings returned.

Study endpoints

GI well-being The overall assessment of GI well-being was self-evaluated by subjects weekly from the first week of product consumption (week 1) to the end of the study, as previously described.[18] Subjects indicate if their GI well-being has remained the same, improved, or worsened (3-point Likert scale). The construct validity of this single item 'patient-reported outcome' has been shown in the adult population reporting minor digestive symptoms.[28] It is associated with a broad range of GI symptom changes.

A responder for GI well-being was defined as a subject reporting an improvement in GI well-being for at least 50% of the intervention, i.e. at least 2 weeks over the 4-week product consumption. This definition of responders was prespecified; it is also consistent with the recommendations of drug agencies for IBS trials[29,30] and Rome Foundation for overall assessment of symptom relief in IBS trials.[31]

Digestive symptoms The frequency of individual digestive symptoms (abdominal pain/discomfort, bloating, flatulence/passage of gas and borborygmi/rumbling stomach) was evaluated weekly throughout the study; a composite score was calculated ranging from 0 to 16.[18]

Diet Food consumption and nutrient intakes were measured during two separate time periods in the study: during the second week of the baseline period and during the final week of the intervention period. During each time period, three non-consecutive multiple pass 24-h dietary recalls were undertaken by dieticians over the telephone. Dieticians were trained to standardize procedures and data collection techniques. Data were entered directly into a web-based tool developed specifically for nutritional epidemiological studies by Medical Expert Systems.[32] This tool was linked to a comprehensive food and nutrient composition database containing almost 5000 foods, which was used to calculate the nutrient intake of the subjects.

Food and nutrient intakes (macro and micronutrients) as well as dietary components that may specifically impact on digestive symptoms (e.g. fiber) were analyzed.

Subjects also recorded the consumption of study products, medications during the study and forbidden products (e.g. other fermented dairy products), as well as any adverse event on a daily basis.

Physical activity Physical activity was assessed with the international physical activity questionnaires (IPAQ).[33] This instrument has been developed to obtain comparable estimates of physical activity that can be used internationally. Subjects are classified in three categories of physical activity: high, moderate, and low.

Statistical methods

Statistical analyses were performed using GENMOD, mixed or logistic procedures available in the SAS System package (SAS Institute Inc., Cary, NC, USA) version 9.2.

The sample size calculation of the replication study was based on the data of the primary study endpoint, overall assessment of GI well-being, in the first study of Guyonnet *et al.*[18] In this study, the percentage of women reporting an improvement in their GI well-being was higher in PFM group *vs* control group with an odd ratio (OR) of 1.69 [95% CI (1.17–2.45)]. A significance of 5% and a power level of 80% were used to obtain a sample size of 160 subjects per group (320 subjects in total). Taking into account premature withdrawals observed in the previous study (5%), the number of randomized subjects was 168 per product group (i.e. 336 randomized subjects).

The assignment of subjects to PFM or control groups was carried-out by a well-balanced blocks randomization, performed by the statistician of the CRO in charge of the biometry prior to study onset. This randomization list used for assigning each subject to a product group was prepared and kept confidentially on the sponsor's premises. It was forwarded to the person responsible for the preparation of study products and their labeling. Subjects were included in chronological order as per the randomization list (incrementally by randomization number). The study was conducted using double-blinding methods until data analyses were completed. Study products were delivered to the investigative site

in blank pots with only references to randomization number and legal information for such kind of products.

For this study, all the analyses of efficacy were performed on the full analysis set (FAS) population which corresponds to all the randomized subjects who received the study product and had the primary criteria assessed.[34] Diet analyses were performed only on a subpopulation, (n = 308) which was defined as the subjects of FAS population with valid data, subjects with at least one visit with less than 2 days of diet assessment or with extreme values being excluded.

Measurement on a 3-point scale of the overall assessment of GI well-being, being an ordinal and repeated response, was analyzed with a proportional odds model.[35,36] We used generalized estimation equations (GEE). The model provides an overall fixed effects estimate of product difference. This GEE was performed with product group and group × time interaction as explicative factors, and time as repeated variable. The responder rates for overall assessment of GI well-being were analyzed at week 4 by a logistic regression to compare the products. In both analysis, when the interaction term was not conclusive (significance level at 10%), the model was performed without this interaction.

Odds ratio (OR) was used to report GI well-being results (overall assessment and responders). An OR of 1 indicates that the overall assessment of GI well-being is similar in both groups. An OR greater than 1 indicates that the overall assessment of GI well-being is more likely to occur in PFM group when an OR less than 1 is in favor to control group.

Frequency of digestive symptoms were analyzed using a repeated-measure analysis of variance (on change from baseline) with time, product group, interaction time × product, and baseline score as covariate. In case of non-normality of errors, a mixed linear model on the area under the curve (AUC) was performed on the same fixed effects as in the parametric approach and adjusted on the value of baseline AUC. The number needed to treat (NNT) was calculated from the mean percentage of responders for overall assessment of GI well-being and results are expressed with confidence intervals.[37]

Dietary parameters at baseline were analyzed with a Student's t-test. Raw change of dietary parameters between baseline and end of study were analyzed using an ANCOVA with product and baseline value as fixed effects.

As this study was a duplication of the study of Guyonnet *et al.*[18] the data from these two studies were combined in a pooled analysis. The aim of the pooled analysis was to provide results with higher confidence (higher sample size, smaller confidence interval, more robust product effects estimations) for analyzing the efficacy of the tested product on the different outcomes (GI well-being and digestive symptoms).

This pooled analysis was performed following the strict intention to treat (ITT) principle based on all randomized subjects. This ITT population differs slightly (<4%) from the FAS populations used for the independent analysis of the two studies (ITT n = 538 *vs*. FAS n = 521; this study, ITT n = 336, FAS n = 324; first study,[18] ITT population n = 202, FAS n = 197).

The objective of this pooled analysis was to assess the consistency from the two twin studies as well as consolidate the results from these studies. This pooled analysis was not quoted as meta-analysis, but the methods used were similar and applied with the same rigor as in an individual patient data meta-analysis. Individual data were analyzed together as if they came from one study and the pooled analysis models are an extension of the models used in the individual studies, in the same way as the analysis of a multicenter study.[38] The pooled analysis model provides an overall fixed effects estimate of product difference and is adjusted with the same covariate as the ones used in the new study. The heterogeneity was controlled and tested: the models were fitted on the study parameter and a test of heterogeneity was performed using interaction between product group and study parameter. An absence of significance of the interaction (significance level set at 10%) indicates a low heterogeneity between the studies, making relevant the interpretation of results from the pooled analysis.

Results are graphically displayed with forest plots showing OR or LS means with confidence intervals for each study and for the pooled results. Each individual study is represented by a square with an area proportional to the inverse variance of the estimate, as advised by Whitehead.[38] A different symbol (diamond-shaped) is used for the overall estimates of the pooled analysis. The 95% CI is represented by the length of the line.

RESULTS

New study

Fig. 1 describes the flow of subjects through the protocol. The first subject was included on September 14, 2009 and the last subject was completed on December 18, 2009. From the 388 contacted subjects, 380 were included in this study and 336 were randomized (168 subjects assigned to each group). Two subjects did not complete the study and 10 subjects had missing main outcomes, giving a FAS population of 324. No difference between the PFM product and control groups was observed for baseline data (Table 1). The population for diet analysis was 308.

The compliance during the study was 99.7% and 99.5% for control and PFM group, respectively.

The percentage of women reporting an improvement in their GI well-being (primary endpoint) was not statistically significantly different in PFM group *vs* control group [OR = 1.20; 95% CI (0.87; 1.66)] (Table 2).

The percentage of responders for GI well-being was higher in the test group *vs* the control group without statistically significant difference 54.3% *vs* 46.3%, respectively, (OR = 1.38; 95% CI [0.89; 2.14]) with a NNT (benefit) of 12.5 [NNT (benefit) 5.37 to ∞ to NNT (harmed) 35.3] (Table 2).

Weekly changes during the period of product consumption and the mean change over this 4-week period for the composite score of frequency of digestive symptoms are shown in Table 2. A significantly (P = 0.033) more pronounced decrease in the composite score of frequency of digestive symptoms was observed over the 4-week period in PFM group [LSmean = −0.42; 95% CI (−0.81; −0.03)].

No difference was shown in nutrient intake at baseline between the PFM and control groups (Table 3). The 4-week intervention did not result in significant changes in nutrient intake between the PFM and control groups.

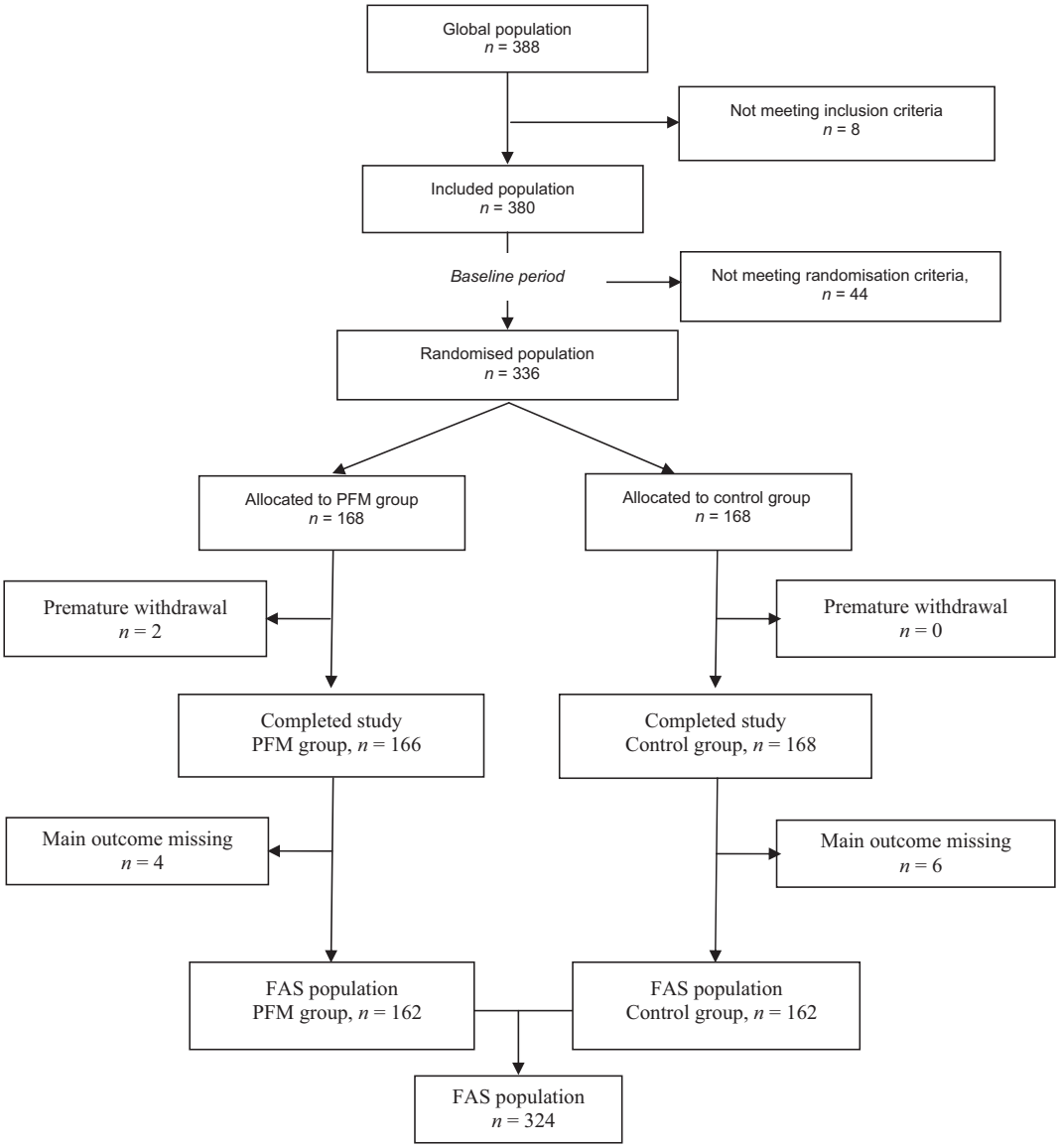

Figure 1 Study flow design. FAS = full analysis set population defined as all randomized subjects with main outcome available.

Pooled analysis

The analyses on the pooled data were conducted in an ITT population of 538 subjects. The percentage of missing data for efficacy parameters (overall GI well-being, GI well-being responders and composite score) was considered as sufficiently low (from 0.6% to 1.7%) to avoid any replacement of missing data. The interaction between study and product was not significant ($P > 0.10$) for the three outcomes and confidence intervals of individual studies results show good overlaps. Results are displayed, for each study and pooled analysis, in Figs 2–4. The percentage of women reporting an improvement in their GI well-being was significantly ($P = 0.014$) higher in PFM group [OR = 1.36; 95% CI (1.07; 1.73)]. The percentage of responders was significantly ($P = 0.015$) higher in PFM group *vs* control group [53.2% *vs* 42.6% for PFM and control group, respectively, OR = 1.53; 95% CI (1.09; 2.16)] with a NNT of 9.5 [95% CI (5.3; 49.1)]. A significant ($P = 0.003$) higher decrease of the composite score over the 4-week was shown in PFM group when comparing with the control group [LSmean = −0.48; 95% CI (−0.80; −0.16)].

Table 1 Baseline characteristics of subjects: comparison between groups

	PFM group (n = 162)	Control group (n = 162)
Age (years)	32.3 ± 10.2	33.0 ± 10.9
Range (years)	18–57	18–59
BMI (kg m^{-2})	22.9 ± 2.5	22.9 ± 2.9
Borborygmi score†	1.75 ± 1.06	1.69 ± 1.01
Bloating score†	1.44 ± 0.84	1.52 ± 0.77
Flatulence score†	2.62 ± 0.97	2.66 ± 1.04
Abdominal pain score†	1.08 ± 0.78	1.07 ± 0.70
Composite score‡	6.90 ± 2.17	6.94 ± 2.10
FBA digestive comfort score*	61.3 ± 13.6	61.3 ± 13.8
Stool frequency (n° $week^{-1}$)	5.77 ± 1.82	5.94 ± 2.71
Physical activity§		
Missing	19 (11.7%)	20 (12.3%)
Low	22 (13.6%)	18 (11.1%)
Moderate	62 (38.3%)	73 (45.1%)
High	59 (36.4%)	51 (31.5%)

PFM, probiotic fermented milk.
All data are expressed as means ± SD except range for years. *FBA: Food and Benefit Assessment questionnaire; digestive comfort score from 0 to 100 (best). †Frequency of individual digestive symptom assessed with a 5-pt Likert scale from 0 (never) to 4 (every day of the week). ‡Composite score of frequency of digestive symptoms ranged from 0 to 16. §Physical activity expressed as percentage and number of subjects by class [n (%)].

DISCUSSION

We performed this study which repeated a first randomized trial.[18] We also performed a pooled analysis of these two studies to provide additional evidence in a large cohort of subjects that the PFM containing *Bifidobacterium lactis* CNCM I-2494 and lactic acid bacteria is able to improve GI well-being and decrease frequency of GI symptoms when consumed by women reporting minor GI symptoms.

The beneficial effect of the PFM on GI well-being was not statistically significant in the new study. This can be explained by a higher response in the control group. Although the percentage of responders in the PFM group is similar in this study *vs* first study (54.3% and 52%, respectively), the control group exhibited a much higher responders rate in this study than in the first trial (46.3% *vs* 36%, respectively). Consequently, an 8% difference in responders' rate was observed in this study compared to 16% in the first study. It has been shown that GI well-being improvement is strongly correlated with decrease in digestive symptoms.[28] However, significant decrease of digestive symptoms was also observed in this study within a similar magnitude of effect than in the first trial.[18] Baseline frequency of digestive symptoms was also comparable in the two studies. It is therefore unlikely that the discrepancies in GI well-being response observed between the two studies may origin from digestive symptoms.

A potential effect of diet or physical activity is unlikely as we have checked that no significant difference in diet occurred between groups at baseline and during the 4-week intervention. A plausible explanation for the findings of this new study, that is nonsignificant trend toward a benefit of the tested PFM, is an underestimation of the magnitude of the placebo response rate and then an underpowered trial.

Trials of large sample size are required to show benefits from interventions (including probiotics) in the GI discomfort area including IBS. Our pooled analysis on 538 subjects represents the largest cohort of subjects for a specific probiotic combination in this area of research. The same probiotic combination in

Table 2 Overall assessment of GI well-being and composite score of digestive symptoms in the FAS population (n = 324)

	Week 1	Week 2	Week 3	Week 4
GI well-being by class†				
PFM group (n = 162)	6.2/60.5/33.3	7.4/50.0/42.6	10.5/44.4/45.1	9.9/45.7/44.4
Control group (n = 162)	16.1/56.8/27.2	9.9/53.1/37.0	8.0/50.6/41.4	5.6/51.2/43.2
Odds ratio‡		1.20 [0.87; 1.66]		
GI well-being – rate of responders§				
PFM group (n = 162)		54.3%		
Control group (n = 162)		46.3%		
Odds ratio‡		1.38 [0.89; 2.14]		
Digestive symptoms¶				
PFM group (n = 162)	−0.76 ± 1. 79	−1.01 ± 2.10	−1.36 ± 2.26	−1.58 ± 2.37
Control group (n = 162)	−0.40 ± 2.13	−0.49 ± 2.30	−1.03 ± 2.40	−1.30 ± 2.57
LS mean		−0.42* [−0.81; −0.03]		

PFM, probiotic fermented milk; GI, gastrointestinal; FAS, full analysis set.
*P < 0.05. †Percentages of subjects by class are expressed at each week as follows: worsened/no change/improved. ‡Odds ratio values are given with 95% CI in brackets. §A responder was defined as a subject having an improvement of their GI well-being at least 2 weeks among the 4 weeks; results are expressed as percentage. ¶Results of composite score of frequency of digestive symptoms are expressed as changes from baselines and were compared between groups over the 4 weeks (LSmean).

Table 3 Mean nutrient intake (unit day^{-1}) at baseline and at the end of the intervention in the FAS population

	PFM group (n = 155)		Control group (n = 153)	
	Baseline	End of study	Baseline	End of study
Energy (kcal)	1710 ± 390	1747 ± 401	1740 ± 443	1767 ± 459
Protein (g)	70 ± 17	74 ± 17	74 ± 18	74 ± 17
Total fat (g)	69 ± 20	74 ± 21	71 ± 22	74 ± 22
Carbohydrate (g)	194 ± 52	191 ± 52	194 ± 57	195 ± 61
Fiber (g)	15 ± 5	15 ± 5	16 ± 5	15 ± 5
Alcohol (g)	4.0 ± 6.6	2.8 ± 4.9	3.6 ± 7.8	3.1 ± 6.3
Water (g)	2153 ± 607	2222 ± 591	2200 ± 550	2253 ± 510
Calcium (mg)	759 ± 257	953 ± 267	794 ± 280	983 ± 265
Iron (mg)	7.9 ± 2.7	7.5 ± 2.4	8.5 ± 2.7	7.8 ± 2.9
Magnesium (mg)	218 ± 70	207 ± 65	228 ± 58	207 ± 61
Potassium (mg)	2114 ± 568	1969 ± 570	2185 ± 622	2007 ± 613
Sodium (mg)	2756 ± 737	2796 ± 736	2695 ± 856	2892 ± 919
Vitamin C (mg)	79 ± 43	77 ± 49	81 ± 45	77 ± 45

PFM, probiotic fermented milk; FAS, full analysis set.
All data are expressed as means ± SD.

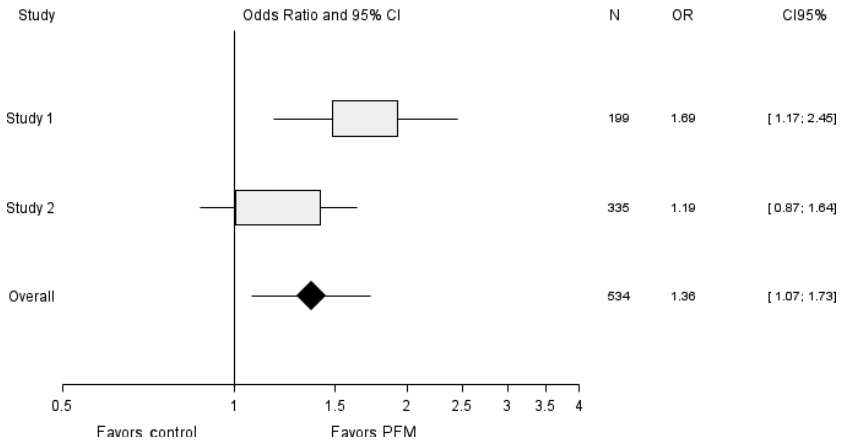

Figure 2 Forest plot of main outcome, weekly overall assessment of gastrointestinal well-being. OR = Odds ratio; CI, confidence interval; test for heterogeneity, $P > 0.10$; test for overall effect, $P = 0.014$.

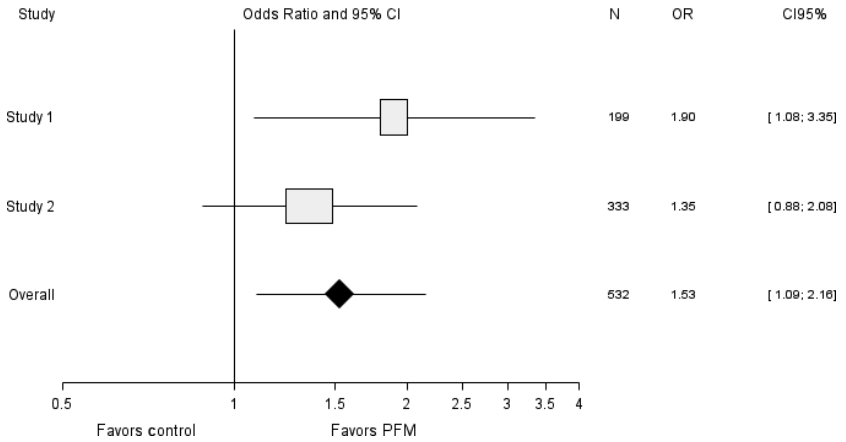

Figure 3 Forest plot of secondary outcomes, rate of responders for gastrointestinal well-being. OR = Odds ratio; CI, confidence interval; test for heterogeneity, $P > 0.10$; test for overall effect, $P = 0.015$.

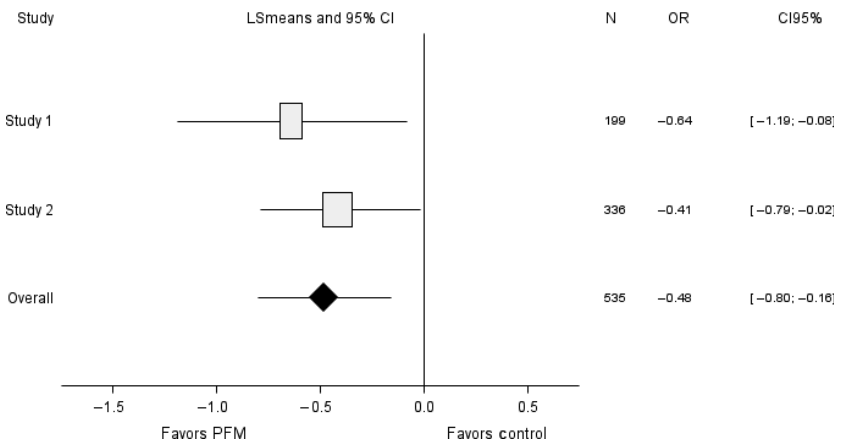

Figure 4 Forest plot of secondary outcomes, composite score of digestive symptoms. CI, confidence interval; test for heterogeneity, $P > 0.10$; test for overall effect, $P = 0.003$.

the same fermented dairy matrix at the same dosage and the same study design were used in both studies justifying pooling of the data. The pooled analysis showed a significant higher percentage of responders in the PFM group with a difference of 10.6% corresponding to a NNT of 9.5. The absence of data on probiotics or other dietary interventions in the general population makes the assessment of the biological relevance of such effect difficult. We acknowledge that the observed benefit is modest, but is within the spectrum (10–15%) of what is considered clinically relevant in IBS.[6,39,40] Although quality of life was not measured in this study, a positive trend in this domain observed in the first study[18] supports further assessment in long-term studies as quality of life is known to change slowly.

The validity of the beneficial effect on global assessment of GI comfort is supported by consistent and significant decreases in frequency of digestive symptoms in this new study, as well as in the first study.[18] Interestingly, the tested probiotic food has been shown to improve symptoms in IBS-C.[19] Taking together, these results suggest that this specific PFM is able to improve digestive symptoms in people with different degrees of digestive symptoms and discomfort, ranging from the general population with minor digestive symptoms to patients fulfilling all IBS criteria. To our best knowledge, these properties have not been shown for another probiotic food or strains.

The effect of this PFM could be mediated by different mechanisms of action. The beneficial effect of probiotics is mediated by a variety of mechanisms involved in the control of GI functions and interactions with gut microbiota.[7] Gut microbiota modulates intestinal motility, barrier function, gas metabolism, and visceral perception.[4,41] This PFM product was shown to accelerate colonic transit time.[19,21] A change in short chain fatty acids may explain the effect on colonic motility.[42] Interestingly, in an unrelated study in an ulcerative colitis mice model, this PFM modified the profile of short chain fatty acids, increased levels of butyric acid, decreased levels of lactic acid, and increased levels of lactic acid-consuming and butyrate-producing bacteria.[43]

Altered visceral sensitivity has been identified as a mechanism involved in GI symptoms.[44] This PFM reduced stress-induced visceral hypersensitivity in an animal model of stress-induced hypersensitivity and restored gut paracellular permeability impairment in this model.[45] These effects located at gut barrier level may explain the decrease in the composite score of GI symptoms by modulating signals arising from the gut.

Gastrointestinal symptoms related to intestinal gas content (bloating, flatulence, and borborygmi) account for more than 80% of the composite score of GI symptoms. Gas metabolism being dependent on the metabolic activities of gut microbiota,[41] the modulation of gut microbiota is a likely candidate to explain the effects of this PFM on GI symptoms. Recent investigations in healthy humans and gnotobiotic mice have shown the ability of this PFM and the consortium of five bacterial strains of this PFM alone to modulate expression of bacterial genes involved in different metabolic pathways including those of carbohydrates.[46] New molecular methods have recently shown significant correlations between the microbiota and common intestinal symptoms such as abdominal pain and bloating in healthy subjects.[47] This opens new avenues to investigate the precise role of the modulation of gut microbiota in the clinical effects of this probiotic food.

In conclusion, the pooled analysis of two medium-sized human studies supports the ability of the tested PFM product to improve GI well-being and reduce digestive symptoms in a population of women reporting minor digestive symptoms. Further research is warranted to know which subjects or patients would most benefit from the consumption of this specific probiotic food. Studies focusing on specific symptoms (e.g. bloating/distension, flatulence) and understanding the mode of action at gut microbiota level and gut-brain axis should help answer this important question.

ACKNOWLEDGMENTS

The authors thank Eline Perrin for providing products, Frédérique Grapin for the study management, Bridget Holmes and Béatrice Trotin for dietary records, Romain Raymond and Jérôme Tanguy for their contribution to the statistical analysis.

FUNDING

This study was funded by Danone Research. D. Guyonnet is employee of Danone. P. Marteau is a consultant of Danone Research. P. Lafaye de Micheaux has been a sporadic consultant of Danone Research.

DISCLOSURE

S. Gelu has no competing interests.

AUTHOR CONTRIBUTIONS

PM and DG were responsible for the study design and for the interpretation of the study results; PLM was the supervisor of the statistical analysis; SG performed the research. All the authors were involved in the writing and validation of the paper. All the authors had complete access to the data.

REFERENCES

1 Salonen A, de Vos WM, Palva A. Gastrointestinal microbiota in irritable bowel syndrome : present state and perspectives. *Microbiology* 2010; **156**: 3205–15.
2 Pimentel M, Lembo A, Chey WD *et al.* TARGET Study Group. Rifaximin therapy for patients with irritable bowel syndrome without constipation. *N Engl J Med* 2011; **364**: 22–32.
3 Quigley EM. Therapies aimed at the gut microbiota and inflammation: antibiotics, prebiotics, probiotics, synbiotics, anti-inflammatory therapies. *Gastroenterol Clin North Am* 2011; **40**: 207–22.
4 Gareau MG, Sherman PM, Walker WA. Probiotics and the gut microbiota in intestinal health and disease. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 503–14.
5 Marteau P. Probiotics in functional intestinal disorders and IBS: proof of action and dissecting the multiple mechanisms. *Gut* 2010; **59**: 285–6.
6 Moayyedi P, Ford AC, Talley NJ *et al.* The efficacy of probiotics in the treatment of irritable bowel syndrome: a systematic review. *Gut* 2010; **59**: 325–32.
7 Clarke G, Cryan JF, Dinan TG, Quigley EM. Review article: probiotics for the treatment of irritable bowel syndrome – focus on lactic acid bacteria. *Aliment Pharmacol Ther* 2012; **35**: 403–13.
8 Ringel-Kulka T, Palsson OS, Maier D *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.
9 Guglielmetti S, Mora D, Gschwender M *et al.* Randomised clinical trial: *Bifidobacterium bifidum* MIMBb75 significantly alleviates irritable bowel syndrome and improves quality of life–a double-blind, placebo-controlled study. *Aliment Pharmacol Ther* 2011; **33**: 1123–32.
10 Simrén M, Öhman L, Olsson J *et al.* Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome – a randomized, double-blind, controlled study. *Aliment Pharmacol Ther* 2010; **31**: 218–27.
11 Søndergaard B, Olsson J, Ohlson K *et al.* Effects of probiotic fermented milk on symptoms and intestinal flora in patients with irritable bowel syndrome: a randomized, placebo-controlled trial. *Scand J Gastroenterol* 2011; **46**: 663–72.
12 Fernandez EM, Valenti V, Rockel C *et al.* Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut* 2011; **60**: 1050–9.
13 Shanahan F. Molecular mechanisms of probiotic action: it's all in the strains! *Gut* 2011; **60**: 1026–7.
14 Frexinos J, Denis P, Allemand H *et al.* Descriptive study of digestive functional symptoms in the French general population. *Gastroenterol Clin Biol* 1998; **22**: 785–91.
15 Janssen HA, Borghouts JA, Muris JW *et al.* Health status and management of chronic non-specific abdominal complaints in general practice. *Br J Gen Pract* 2000; **50**: 375–9.
16 van Kerkhoven LA, Eikendal T, Laheij RJ *et al.* Gastrointestinal symptoms are still common in a general Western population. *Neth J Med* 2008; **66**: 18–22.
17 Talley NJ. Functional gastrointestinal disorders as a public health problem. *Neurogastroenterol Motil* 2008; **20**(Suppl 1): 121–9.
18 Guyonnet D, Schlumberger A, Mhamdi L *et al.* Fermented milk containing *Bifidobacterium lactis* DN-173 010 improves gastrointestinal well-being and digestive symptoms in women reporting minor digestive symptoms. A randomised, double-blind, parallel, controlled study. *Br J Nut* 2009; **102**: 1654–62.
19 Agrawal A, Houghton LA, Morris J *et al.* Clinical trial: the effects of a fermented milk product containing *Bifidobacterium lactis* DN-173-010 on abdominal distension and gastrointestinal transit in irritable bowel syndrome with constipation. *Aliment Pharmacol Ther* 2009; **29**: 104–14.
20 Guyonnet D, Chassany O, Ducrotté P *et al.* 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. *Aliment Pharmacol Ther* 2007; **26**: 475–86.
21 Marteau P, Cuillerier E, Méance S *et al.* *Bifidobacterium animalis* strain DN-173 010 shortens the colonic transit time in healthy women: a double-blind, randomized, controlled study. *Aliment Pharmacol Ther* 2002; **16**: 587–93.
22 Guyonnet D, Woodcock A, Stefani B *et al.* Fermented milk containing *Bifidobacterium animalis* DN-173 010 improved self-reported digestive comfort amongst a general population of adults. A randomized, open-label, controlled, pilot study. *J Dig Dis* 2009; **10**: 61–70.
23 Kalliomäki M, Salminen S, Arvilommi H *et al.* Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial. *Lancet* 2001; **357**: 1076–9.
24 Kopp MV, Hennemuth I, Heinzmann A *et al.* Randomized, double-blind, placebo-controlled trial of probiotics for primary prevention: no clinical effects of *Lactobacillus* GG supplementation. *Pediatrics* 2008; **121**: e850–6.
25 Higgins JPT, Green S (eds) Cochrane Handbook for Systematic Reviews of Interventions, Version 5.0.1 (updated September 2008). The Cochrane Collaboration 2008. Available at: http://www.cochrane-handbook.org
26 Heaton KW, O'Donnell LJ, Braddon FE *et al.* Symptoms of irritable bowel syndrome in a British urban community: consulters and non consulters. *Gastroenterology* 1992; **102**: 1962–7.
27 Guyonnet D, Chassany O, Picard C *et al.* Perceived subject outcomes and impact on health-related quality of life associated with diet using the new Food Benefits Assessment (FBA©) questionnaire: development and psychometric validation. *Public Health Nutr* 2008; **11**: 1163–72.
28 Guyonnet D, Naliboff BD, Rondeau P, Mayer EA, Chassany O. Gastrointestinal well-being in subjects reporting mild gastrointestinal discomfort: characteristics and properties of a global assessment measure. AGA abstracts. *Gastroenterology* 2012; **142**: S817–8.
29 Guidance for Industry Irritable Bowel Syndrome – Clinical Evaluation of Drugs for Treatment. Draft guidance. U.S. Department of Health and Human Services. Food and Drug Administration. Center for Drug Evaluation and Research (CDER). Available at: http://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/default.htm. May 2012.
30 Committee for Proprietary Medicinal Products (CPMP). Points to consider on the evaluation of medicinal products for the treatment of irritable bowel syndrome. CPMP/EWP/785/97. London, final document, March 19 2003.
31 Irvine EJ, Whitehead WE, Chey WD *et al.* Design of treatment trials for functional gastrointestinal disorders. *Gastroenterology* 2006; **130**: 1538–51.
32 Biró G, Hulshof KF, Ovesen L *et al.* Selection of methodology to assess food intake. *Eur J Clin Nutr* 2002; **56**(Suppl 2): S25–32.
33 Craig CL, Marshall AL, Sjöström M *et al.* International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* 2003; **35**: 1381–95.
34 Committee for Proprietary Medicinal Products (CPMP). Statistical Principles for Clinical Trials (ICH Topic E 9). CPMP/ICH/363/96. European Medicines Agency, London, 1998
35 McCullagh P. Regression models for ordinal data. *J R Statist Soc B* 1980; **42**: 109–42.
36 Agresti A. *Categorical Data Analysis*. 2nd edn. Hoboken, New Jersey: John Wiley & Sons, Inc., 2002.
37 Altman DG. Confidence intervals for the number needed to treat. *BMJ* 1998; **317**: 1309–12.
38 Whitehead A. Meta-analysis using individual patient data. In: *Meta-Analysis of Controlled Clinical Trials*. (*Statistics in Practice*). Chichester; New York: John Wiley & Sons, 2002.
39 Corazziari E, Bytzer P, Delvaux M *et al.* Clinical trial guidelines for pharmacological treatment of irritable bowel syndrome. *Aliment Pharmacol Ther* 2003; **18**: 569–80.
40 Hoveyda N, Heneghan C, Mahatani KR *et al.* A systematic review and meta-analysis: probiotics in the treatment of irritable bowel syndrome. *BMC Gastroenterol* 2009; **9**: 15.
41 Nakamura N, Lin HC, McSweeney CS *et al.* Mechanisms of microbial hydrogen disposal in the human colon and implications for health and disease. *Annu Rev Food Sci Technol* 2010; **1**: 363–95.
42 Cherbut C. Motor effects of short-chain fatty acids and lactate in the gastrointestinal tract. *Proc Nut Soc* 2003; **62**: 95–9.
43 Veiga P, Gallini CA, Beal C *et al.* Bifidobacterium animalis subsp. lactis fermented milk product reduces inflammation by altering a niche for colitogenic microbes. *Proc Natl Acad Sci USA* 2010; **107**: 18132–7.
44 Kellow JE, Azpiroz F, Delvaux M *et al.* Applied principles of neurogastroenterology: physiology/motility sensation. *Gastroenterology* 2006; **130**: 1412–20.
45 Agostini S, Goubern M, Tondereau V *et al.* A marketed fermented dairy product containing *Bifidobacterium lactis* CNCM I-2494 suppresses gut hypersensitivity and colonic barrier disruption induced by acute stress in rats. *Neurogastroenterol Motil* 2012; **24**: 376–e172. (doi: 10.1111/j.1365-2982.2011.01865.x).
46 McNulty NP, Yatsunenko T, Hsiao A *et al.* The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* 2011; **3**: 106ra106.
47 Jalanka-Tuovinen J, Salonen A, Nikkilä J *et al.* Intestinal microbiota in healthy adults: temporal analysis reveals individual and common core and relation to intestinal symptoms. *PLoS ONE* 2011; **6**: e23035.