# Efficacy and safety of spore-forming probiotics in the treatment of functional dyspepsia: a pilot randomised, double-blind, placebo-controlled trial

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*Lancet Gastroenterol Hepatol* 2021; 6: 784–92

Published Online August 3, 2021 https://doi.org/10.1016/S2468-1253(21)00226-0

This online publication has been corrected. The corrected version first appeared at thelancet.com/gastrohep on August 27, 2021

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## Summary

**Background** Current treatments for functional dyspepsia have limited efficacy or present safety issues. We aimed to assess spore-forming probiotics in functional dyspepsia as monotherapy or add-on therapy to long-term treatment with proton-pump inhibitors.

**Methods** In this single-centre, randomised, double-blind, placebo-controlled pilot trial that took place at University Hospitals Leuven (Leuven, Belgium), adult patients (≥18 years) with functional dyspepsia (as defined by Rome IV criteria, on proton-pump inhibitors or off proton-pump inhibitors) were randomly assigned (1:1) via computer-generated blocked lists, stratified by proton-pump inhibitor status, to receive 8 weeks of treatment with probiotics (*Bacillus coagulans* MY01 and *Bacillus subtilis* MY02, $2·5×10^9$ colony-forming units per capsule) or placebo consumed twice per day, followed by an open-label extension phase of 8 weeks. Individuals with a history of abdominal surgery, diabetes, coeliac or inflammatory bowel disease, active psychiatric conditions, and use of immunosuppressant drugs, antibiotics, or probiotics in the past 3 months were excluded. All patients and on-site study personnel were masked to treatment allocation in the first 8 weeks. Symptoms, immune activation, and faecal microbiota were assessed and recorded. The primary endpoint was a decrease of at least 0·7 in the postprandial distress syndrome (PDS) score of the Leuven Postprandial Distress Scale in patients with a baseline PDS score of 1 or greater (at least mild symptoms), assessed in the intention-to-treat population. This study is registered with ClinicalTrials.gov, NCT04030780.

**Findings** Between June 3, 2019, and March 11, 2020, of 93 individuals assessed for eligibility, we included 68 patients with functional dyspepsia (51 [75%] women, mean age 40·1 years [SD 14·4], 34 [50%] on proton-pump inhibitors). We randomly assigned 32 participants to probiotics and 36 to placebo. The proportion of clinical responders was higher with probiotics (12 [48%] of 25) than placebo (six [20%] of 30; relative risk 1·95 [95% CI 1·07–4·11]; p=0·028). The number of patients with adverse events was similar with probiotics (five [16%] of 32) and placebo (12 [33%] of 36). Two serious adverse events occurring during the open-label phase (appendicitis and syncope in two separate patients) were assessed as unlikely to be related to the study product.

**Interpretation** In this exploratory study, *B coagulans* MY01 and *B subtilis* MY02 were efficacious and safe in the treatment of functional dyspepsia. Participants had potentially beneficial immune and microbial changes, which could provide insights into possible underlying mechanisms as future predictors or treatment targets.

**Funding** MY HEALTH.



## Introduction

Functional dyspepsia is a common chronic gastrointestinal disorder defined by upper abdominal symptoms originating from the gastroduodenal region with no structural disease on routine investigations.[1] However, the presence of subtle pathology is not excluded by the Rome IV criteria and increasing evidence points to local duodenal and systemic changes in functional dyspepsia.[2,3] Impaired duodenal mucosal integrity and low-grade inflammation have been reported in patients with functional dyspepsia, correlating with gastric emptying and postprandial symptoms.[4,5] Moreover, systemic immune activation and increased small-bowel-homing T cells ($CD4^+α4β7^+CCR9^+$) and the correlation with gastric emptying rate and symptom severity were reported.[6] Different underlying mechanisms have been studied, including gastric dysfunction, hypersensitivity to duodenal luminal content, and central factors such as gut–brain signalling.[2,3] Despite the socioeconomic impact to the health service and patient and decreased quality of life, the pathophysiology of functional dyspepsia is incompletely understood and treatment options are limited in efficacy and number.[3,7]

First-line therapy for functional dyspepsia is acid suppression with proton-pump inhibitors and although guidelines advise against dose escalation, inappropriate use of proton-pump inhibitors, even in the absence of clinical benefit, is frequently reported.[2] Long-term intake of proton-pump inhibitors can increase the risk of enteric

## Research in context

### Evidence before this study

Functional dyspepsia is a common and costly gastrointestinal disorder and current treatments have limited efficacy or safety issues. Long-term treatment with proton-pump inhibitors can cause intestinal dysbiosis, and potential beneficial effects of probiotics have been suggested. We searched PubMed for articles published from database inception until June 1, 2021, using the search terms "functional dyspepsia", and "probiotic". Studies in animals and children were excluded. Of the five studies in adult patients identified, only one study included a placebo yoghurt as control for probiotic efficacy in uninvestigated dyspepsia. Besides the absence of rigorous and placebo-controlled probiotic trials, the efficacy and safety of spore-forming probiotics or gastric-acid-resistant endospores have not been assessed in patients with functional dyspepsia.

### Added value of this study

The combination of *Bacillus coagulans* MY01 and *Bacillus subtilis* MY02 strains was effective and safe in patients with functional dyspepsia compared with placebo. Decreased Th17 signalling in blood and increased *Faecalibacterium* in stools were associated with clinical efficacy of probiotics. Beneficial probiotic effects in patients with functional dyspepsia on proton-pump inhibitors included a reduction in small intestinal bacterial overgrowth.

### Implications of all the available evidence

Treatment with spore-forming probiotics can be considered as monotherapy or as add-on to proton-pump inhibitors in patients with functional dyspepsia with refractory symptoms. Changes in immune activation and intestinal microbiota are potential underlying mechanisms of spore-forming probiotics. This study underscores the potential role of microbiota in functional dyspepsia and provides effect sizes to design future trials. Further investigation is needed.

infections (including *Clostridioides difficile*),[8] and changes in faecal microbiota or dysbiosis have been reported.[9] Probiotics are live microorganisms that exert a health benefit on the host.[10] Previous studies suggested efficacy of probiotics for proton-pump inhibitor-related side-effects and uninvestigated dyspeptic symptoms, which could be caused by an altered small intestinal microbiome.[11–13] An intestinal-like bacterial profile in the gastric fluid suggested the presence of small intestinal bacterial overgrowth in at least a subset of patients with functional dyspepsia.[12] Nevertheless, placebo-controlled studies investigating probiotics in functional dyspepsia are scarce.[14] Gram-positive and spore-forming probiotic strains could be more efficacious than traditional probiotic supplements because of gastric-acid resistant endospores with improved storage conditions and survival in the intestine.[15,16] Despite beneficial effects of *Bacillus coagulans* and *Bacillus subtilis* strains on gut permeability and inflammation in in-vitro models,[17] clinical trials on the effect of spore-forming probiotics are absent in human disorders with similar alterations, including functional dyspepsia.

To bridge this gap, we aimed to assess the efficacy and safety of the combination of *B coagulans* MY01 and *B subtilis* MY02 strains in patients with functional dyspepsia. We hypothesised that functional dyspepsia symptoms, measured with a validated daily diary, would be improved by these spore-forming probiotics compared with placebo in patients with functional dyspepsia as add-on to proton-pump inhibitors or as monotherapy. In addition to a comprehensive clinical and safety evaluation, biological markers of immune activation and both relative and quantitative microbiota composition were studied to assess potential underlying mechanisms.

## Methods

### Study design and participants

We did a single-centre study with a randomised, double-blind, placebo-controlled, and parallel-group design with open-label extension (appendix p 5). Patients were recruited from the outpatient department of University Hospitals Leuven (Leuven, Belgium), to which they were referred. Adult patients (≥18 years) with functional dyspepsia, diagnosed according to Rome IV criteria with normal endoscopy including *Helicobacter pylori* testing,[1] were included and divided into two predefined cohorts based on current proton-pump inhibitor status: on proton-pump inhibitors (daily proton-pump inhibitor therapy of any type and dose during the past 4 weeks with insufficient efficacy) or off proton-pump inhibitors (no proton-pump inhibitors during the past 8 weeks or longer). Eligible patients had no history of abdominal surgery, diabetes, coeliac or inflammatory bowel disease, or active psychiatric conditions (stable dose of a single neuromodulator was allowed). Use of immunosuppressant drugs, antibiotics, or probiotics in the past 3 months and alcohol use of more than ten units per week were exclusionary.

See **Online** for appendix

The trial was done in accordance with the Declaration of Helsinki and good clinical practice regulations after approval by the ethics committee of University Hospitals Leuven (number S62043). Written informed consent was obtained from each participant before inclusion. All data were collected at KU Leuven (Leuven, Belgium) and University Hospitals Leuven. The protocol is accessible online.

For **protocol** see http://targid.eu

### Randomisation and masking

Participants were randomly assigned using an online randomisation tool by staff not otherwise involved in the

For the **randomisation tool** see http://www.randomization.com/

study. Patients were randomly assigned (1:1) to probiotics or placebo, stratified by proton-pump inhibitor-status. The list was generated with a block size of five. Double blinding was achieved by packaging probiotics and placebo in the same sealed and consecutively numbered bottles with capsules similar in packaging, smell, and taste. All study participants and on-site study personnel remained masked for the treatment allocation (randomised controlled trial phase) until database lock and signature of the statistical analysis plan.

### Procedures

The probiotic treatment consisted of a 1:1 combination of spray-dried *B coagulans* MY01 and *B subtilis* MY02 endospores (total of $2 \cdot 5 \times 10^9$ colony-forming units per capsule) in a mixture of 50 mg with 300 mg maltodextrin per capsule, taken twice per day with meals. The placebo contained 350 mg maltodextrin per capsule, also taken twice per day. Both products were manufactured by MY RESEARCH (Diepenbeek, Belgium). Patients with functional dyspepsia on proton-pump inhibitors were given placebo or probiotics in combination with their daily proton-pump inhibitor therapy (no change in dose or type) for the entire study period. Treatment compliance was established by counting capsules and defined as good if 80% or more were used after each treatment phase.

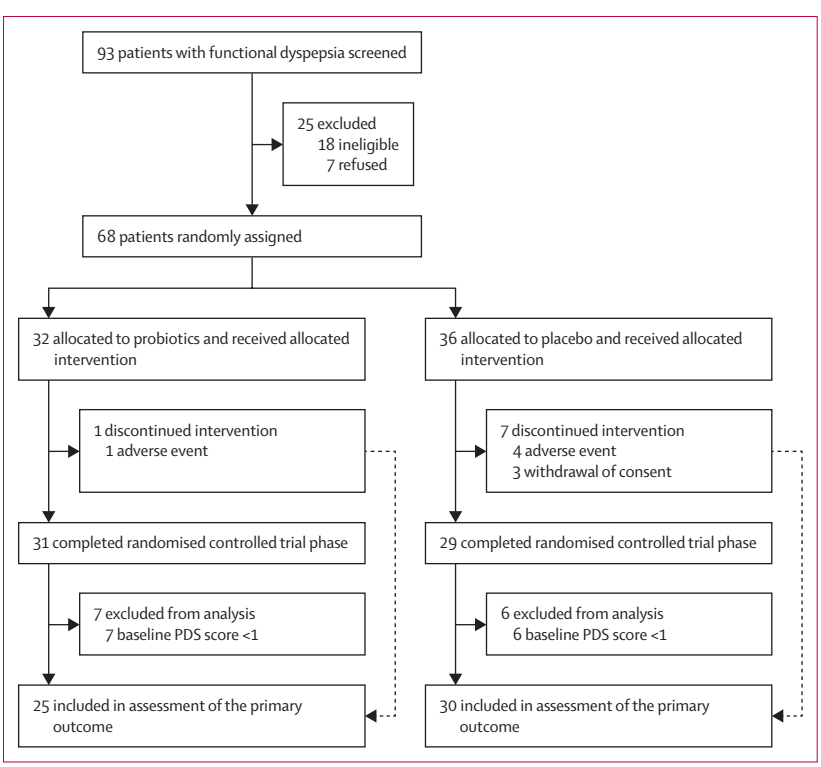


***Figure 1:*** **Trial profile**
Patients with functional dyspepsia discontinuing the intervention or who did not adhere to study guidelines were regarded as non-responders for the primary endpoint (intention-to-treat analysis). LPDS=Leuven Postprandial Distress Scale. PDS=postprandial distress syndrome.

After screening and assessment by a single physician (LW), a run-in period of 1 week took place with completion of a daily diary during run-in and from 1 week onwards. Study procedures were done at baseline (visit 1), after 8 weeks of treatment with probiotics or placebo (visit 2), and after 8 additional weeks of open-label extension treatment with probiotics (visit 3; appendix p 5). The Leuven Postprandial Distress Scale (LPDS) was used as a validated daily diary, including eight items (cardinal or core postprandial distress syndrome [PDS] symptoms and EPS symptoms, nausea, belching, and heartburn).[18] Monthly questionnaires included patient assessment of upper gastrointestinal disorders symptom severity index (PAGI-SYM) and quality of life (PAGI–QOL).[18]

Fasting plasma samples were collected for determination of high-sensitivity C-reactive protein (baseline, week 8) and lipopolysaccharide-binding protein (baseline, week 8, and week 16). Also, systemic cytokines and peripheral blood mononuclear cells (PBMCs) were analysed at each study visit, with subtyping of $CD4^+$ and gut homing ($CD4^+\alpha4\beta7^+CCR9^+$) T-cell subsets after ex-vivo stimulation.

Stool samples were collected and transported within 24 h of each visit under cooled (4–8°C) and anaerobic conditions (AnaeroGen, Thermo Fisher Scientific, Basingstoke, UK) for 16S ribosomal RNA gene amplicon sequencing and flow-cytometry-based quantification of faecal microbiota. In patients with functional dyspepsia on proton-pump inhibitors, glycocholic acid breath tests were done at baseline and week 8 to detect small intestinal bacterial overgrowth, as proton-pump inhibitors have been shown to affect the gut microbiome.[9] No breath tests were done in patients with functional dyspepsia off proton-pump inhibitors.

### Outcomes

The primary endpoint was the proportion of clinical responders, defined as a decrease of at least 0·7 in PDS score at week 8 in patients with functional dyspepsia with baseline PDS scores of 1 or more (at least mild symptom scores) on the LPDS diary in the entire cohort (on and off proton-pump inhibitors). This diary was chosen due to the recall period of 24 h, with good reliability, validity, and responsiveness for PDS symptoms.[18] The responder definition was higher than the reported minimum clinically important difference of 0·5 and calculated as the weekly mean of the cardinal PDS scores or first three questions (early satiation, postprandial fullness, upper abdominal bloating) of the LPDS, which are scored from 0 (none) to 4 (very severe).[18]

Secondary endpoints were the proportion of minimal clinical responders in the first 8 weeks for the entire cohort or a decrease of at least 0·5 for cardinal PDS scores, the proportions of minimal responders (PDS) in three or more weeks of the past 4 weeks (randomised controlled trial phase), and the evolution of weekly minimal responder rates (PDS) or symptom scores (PDS, epigastric pain syndrome [EPS], and individual questions; randomised

controlled trial phase). Cardinal EPS symptoms were defined as the weekly mean of the scores for epigastric pain and burning. Changes in PAGI-SYM and PAGI-QOL were also assessed and secondary biological endpoints comprised changes in plasma high-sensitivity C-reactive protein, lipopolysaccharide-binding protein, cytokines, PBMCs, and faecal microbiota.

Safety was assessed by grading adverse events at every study visit or in case of premature termination using the Common Terminology Criteria for Adverse Events version 4.0, with the relationship for all participants randomly assigned and exposed to the study products (full analysis set).

### Statistical analysis

As there is no previous study investigating the effect of spore-forming probiotics in functional dyspepsia, no reasonable power analysis was possible. Based on an assumed response rate of 50% with probiotics and 20% with placebo using the higher cutoff of the primary endpoint (decrease in PDS score ≥0·7), a sample size of 36 would be required per group (power of 80% and $\alpha$=0·05). Based on feasibility, we aimed to include 30 patients completing the randomised controlled trial phase per group in this pilot study.

Data from the full analysis set were analysed according to the intention-to-treat principle. Responder analyses were done following an extreme case approach (participants with missing data were considered non-responders) in participants with at least mild baseline PDS scores (≥1) as predefined in the statistical analysis plan, which was finalised and signed before unmasking. Proportions were compared with $\chi^2$ or Fisher exact tests and ratios or relative risks (RRs) were calculated and presented with 95% CIs. Mean changes from baseline in continuous clinical and biological endpoints were analysed using linear mixed models with group (probiotic, placebo) as between-participant and visit or week (LPDS) as within-participant factors of interest with their interaction. The interaction effect or between-group difference in changes from baseline (randomised controlled trial assessed at week 8) was the main effect of interest and within-group changes from baseline were also assessed for both groups at week 16 (open-label extension phase). Finally, associations were studied between changes in clinical and biological endpoints. No imputation was done for missing data. Significance tests were based on a two-sided $\alpha$ of 0·05 for the primary outcome in this exploratory study. Results from spore-forming probiotics (eg, changes in metabolic or stool parameters, among others) during the open-label extension phase and the glycocholic acid breath tests (on proton-pump inhibitors) were the prespecified exploratory endpoints. Analyses were implemented using SAS, version 9.4, and least squares means estimates ($\beta$) are given with 95% CIs. Graphs were created with GraphPad, version 8.0. Results are reported in accordance with 2010 CONSORT guidelines, and additional details can be found in the appendix (pp 1–2). No data or safety monitoring committee was used as probiotics are registered as food supplements rather than medicinal products. This study was registered with ClinicalTrials.gov, NCT04030780.

### Role of the funding source

The funder of the study provided feedback on the protocol, which was drafted by LW and TV. The funder provided the spore-forming probiotics and placebo control products as well as information that was given to the participants about the probiotics. The funder had no role in data collection, data analysis, or data interpretation

| | Probiotic group (n=32) | Placebo group (n=36) |
|---|---|---|
| Age, years | 39·63 (15·15) | 40·51 (13·85) |
| Sex | | |
| Female | 24 (75%) | 27 (75%) |
| Male | 8 (25%) | 9 (25%) |
| Body-mass index, kg/m² | 23·26 (3·51) | 22·58 (3·45) |
| Race | | |
| White | 31 (97%) | 32 (89%) |
| African | 0 | 2 (6%) |
| Arabic | 1 (3%) | 0 |
| Asian | 0 | 2 (6%) |
| On proton-pump inhibitors at baseline | | |
| Yes | 17 (53%) | 17 (47%) |
| No | 15 (47%) | 19 (53%) |
| Functional dyspepsia subtypes or irritable bowel syndrome | | |
| PDS | 20 (63%) | 22 (61%) |
| Overlap | 6 (19%) | 8 (22%) |
| EPS | 6 (19%) | 6 (17%) |
| Irritable bowel syndrome | 14 (44%) | 20 (56%) |
| Clinical scores* | | |
| Cardinal PDS | 1·53 (1·00) | 1·65 (0·83) |
| Cardinal EPS | 0·95 (0·82) | 0·91 (0·79) |
| PAGI-SYM | 2·08 (0·83) | 2·15 (0·81) |
| PAGI-QOL | 3·24 (0·96) | 3·39 (0·97) |
| Biological outcomes | | |
| High sensitivity C-reactive protein, mg/L | 2·46 (5·22) | 2·75 (5·77) |
| Lipopolysaccharide binding protein, pg/mL | 14·10 (6·21) | 12·27 (5·53) |
| Richness | 142·6 (49·37) | 163·45 (79·77) |
| Shannon | 37·37 (15·46) | 39·02 (17·75) |
| Inverse Simpson | 17·56 (8·90) | 17·20 (8·78) |

Data are mean (SD) or n (%) for the full analysis set. PDS=postprandial distress syndrome. EPS=epigastric pain syndrome. PAGI-SYM=patient assessment of upper gastrointestinal disorders symptom severity index. PAGI-QOL=patient assessment of upper gastrointestinal disorders quality of life. *Clinical scores could range from 0–4 for PDS and EPS and 0–5 for PAGI.

***Table 1:*** **Baseline characteristics**

| | Probiotic group (n=32) | Placebo group (n=36) |
|---|---|---|
| **Clinical scores*** | | |
| Clinical response† | 48% (30 to 67) | 20% (10 to 37) |
| Minimal clinical response‡ | 56% (37 to 73) | 27% (14 to 44) |
| Cardinal PDS | −0·53 (−0·74 to −0·32) | −0·23 (−0·44 to −0·02) |
| Cardinal EPS | −0·39 (−0·58 to −0·19) | −0·11 (−0·31 to 0·08) |
| PAGI-SYM | −0·42 (−0·66 to −0·17) | −0·45 (−0·69 to −0·21) |
| PAGI-QOL | 1·16 (0·35 to 1·96) | 1·46 (0·67 to 2·24) |
| **Biological outcomes** | | |
| High sensitivity C-reactive protein, mg/L | 0·32 (−1·46 to 2·09) | −0·64 (−2·37 to 1·1) |
| Lipopolysaccharide binding protein, pg/mL | 0·01 (−0·12 to 0·14) | 0·07 (−0·07 to 0·20) |
| Richness | −0·01 (−0·06 to 0·05) | 0·05 (−0·01 to 0·10) |
| Shannon | 0·19 (−0·68 to 1·05) | 0·39 (−0·50 to 1·29) |
| Inverse Simpson | −0·05 (−0·78 to 0·68) | 0·10 (−0·66 to 0·85) |

Data are estimate (95% CI) in the full analysis set. PDS=postprandial distress syndrome. EPS=epigastric pain syndrome. PAGI-SYM=patient assessment of upper gastrointestinal disorders symptom severity index. PAGI-QOL=patient assessment of upper gastrointestinal disorders quality of life. *Clinical scores could range from 0–4 for PDS and EPS and 0–5 for PAGI. †Decrease in PDS score of 0·7 or greater at week 8 in patients with functional dyspepsia with baseline scores of 1 or greater (n=25 probiotic group, n=30 placebo group). ‡Decrease in PDS score of 0·5 or greater at week 8 in patients with functional dyspepsia with baseline scores of 1 or greater (n=25 probiotic group, n=30 placebo group).

***Table 2:*** **Within-group changes in clinical and biological endpoints from baseline after 8 weeks**

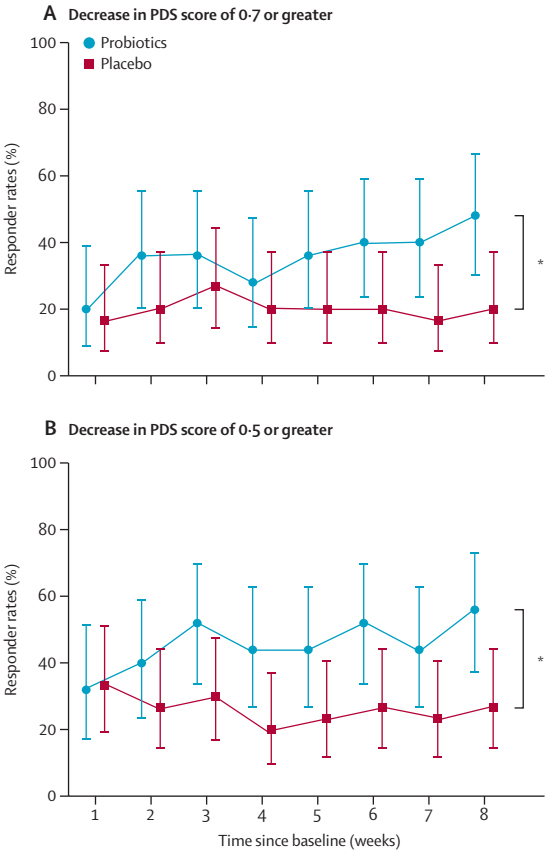


***Figure 2:*** **Weekly evolution of clinical (A) and minimal clinical responders (B) in patients with functional dyspepsia**
Proportions with 95% CI for patients with functional dyspepsia with baseline PDS scores of 1 or more and a decrease (PDS) of 0·7 or more (clinical response) or 0·5 or more (minimal clinical response) at each week and per group (n=25 probiotics, n=30 placebo; intention-to-treat analysis). Significance is given for the difference at week 8 (primary and secondary endpoint; *p<0·05). PDS=postprandial distress syndrome.

and had no access to the individual participant data or samples in agreement with the university policy for investigator-initiated studies.

## Results

Between June 3, 2019, and March 11, 2020, of 93 patients assessed for eligibility, 68 were included and randomly assigned (51 [75%] women, mean age 40·1 years [SD 14·4], 34 on proton-pump inhibitors; figure 1). 32 participants were randomly assigned to probiotics and 36 to placebo. Baseline characteristics are shown in table 1. Mean duration of proton-pump inhibitor therapy in patients on proton-pump inhibitors was 3·14 (SD 5·21) years. During the first 8 weeks (randomised controlled trial phase), there was one discontinuation in the probiotic group (due to an adverse event) and seven discontinuations in the placebo group (four patients had adverse events, three withdrew consent; figure 1). LPDS scores were missing for an additional four patients on probiotics and one patient on placebo due to non-adherence to study guidelines.

The primary endpoint of proportion of clinical responders (decrease in PDS score ≥0·7) was higher for probiotics (12 [48%] of 25) than placebo (six [20%] of 30; RR 1·95 [95% CI 1·07–4·11]; p=0·028) in the intention-to-treat analysis (seven patients randomly assigned to probiotics and six to placebo with baseline PDS score of less than 1 were not included; table 2, figure 2). When including individuals with low PDS scores (baseline PDS score <1) as non-responders, efficacy of probiotics (12 [38%] of 32) was greater than placebo (six [17%] of 36; RR 1·80 [1·00–3·79]). Responses with probiotics were not significantly higher in patients with functional dyspepsia on proton-pump inhibitors (six [46%] of 13 *vs* two [13%] of 15; RR 2·60 [95% CI 0·98–9·36]) or off proton-pump inhibitors (six [50%] of 12 *vs* four [27%] of 15; RR 1·62 [0·78–4·05]). Results for the per-protocol analysis were similar, indicating higher efficacy of probiotics in all randomly assigned participants and those completing the study (appendix p 2).

Minimal clinical response (decrease in PDS score ≥0·5) was higher with probiotics than placebo (14 [56%] of 25 *vs* eight [27%] of 30; RR 1·83 [95% CI 1·07–3·50]; table 2, figure 2). Weekly responder-rates (decrease in PDS score ≥0·7) were higher with probiotics than placebo at week 7 (RR 1·88 [0·99–4·24]) and week 8, whereas minimal responder-rates (decrease in PDS score ≥0·5)

were higher with probiotics than placebo at week 3 (RR 1·56 [0·93–2·86]), week 4 (RR 1·79 [0·99–3·77]), and week 6 (RR 1·70 [0·99–3·24]; figure 2).

When assessing changes in PDS scores from baseline, the decrease with probiotics was significantly higher than with placebo after 8 weeks (β –0·30 [95% CI –0·95 to 0·00]). The decrease in EPS scores was significant with probiotics but not placebo after 8 weeks (β –0·28 [–0·55 to 0·00]; appendix p 3).

The decrease in PAGI-SYM and increase in PAGI-QOL from baseline after 8 weeks was similar in both groups (table 2). Open-label probiotic treatment decreased symptoms in the original placebo group, with clinical effects maintained after 16 weeks in the original probiotics group (appendix p 3). Thus, probiotics improved PDS scores and EPS scores after 8 weeks, and this effect was maintained during the open-label extension phase.

No within-group or between-group differences were found for high-sensitivity C-reactive protein or lipopolysaccharide-binding protein in the first 8 weeks (table 2). Based on the clinical efficacy of probiotics during the open-label extension phase, changes in systemic cytokines and stimulated $CD4^+$ T cells were also assessed after 16 weeks of probiotics and there was a significant decrease in IL-17A (appendix p 8). Although amounts of circulating regulatory T cells (Tregs) decreased after 8 weeks with probiotics and not placebo, effects on $CD4^+$ T cells were more apparent after 16 weeks with probiotics, including significantly decreased amounts of T-helper (Th)17 cells (appendix p 3). Thus, effects of probiotics included decreased Th17 signalling with an additional decrease in Th2 signalling and gut-homing T cells in patients with functional dyspepsia on proton-pump inhibitors (appendix p 3).

No within-group or between-group differences were found for α-diversity (ie, Richness, Shannon, Inverse Simpson) after 8 weeks and 16 weeks (table 2). Partial redundancy analyses showed that spore-forming probiotics did not significantly contribute to the conditional variation (on participant) in relative or quantitative microbial community composition when combining samples after 8 weeks and 16 weeks of probiotics in both groups over the entire study period (appendix p 3). Nevertheless, a proportional but not quantitative increase in *Faecalibacterium* with increased abundances of *Roseburia* and the family Leuconostocaceae were found with probiotics (after 8 weeks and 16 weeks of probiotics) versus control samples (at baseline and after 8 weeks of placebo; appendix p 3). The proportion of positive breath tests on proton-pump inhibitors with probiotics versus placebo was similar at baseline (three [18%] of 17 *vs* four [25%] of 16; RR 0·80 [95% CI 0·28–1·66]) but significantly lower after 8 weeks (one [7%] of 15 *vs* five [38%] of 13; RR 0·26 [0·05–0·96]), suggesting a reduction of small intestinal bacterial overgrowth with spore-forming probiotics.

On the basis of the decreased Tregs or Th17 signalling and proportionally increased *Faecalibacterium* or *Roseburia* with probiotics, changes in these biological endpoints were entered into the models of PDS symptoms within the probiotics and placebo group (randomised controlled trial phase). Although no association was found for Tregs, the decrease in PDS scores was only significant in the case of mean or greater reductions in IL-17A or Th17 cells with probiotics and not placebo (appendix p 8). In addition, decreased PDS scores were only found with mean or greater increases in *Faecalibacterium* but not *Roseburia* with probiotics and not placebo treatment (appendix p 3). Thus, changes in Th17 signalling and *Faecalibacterium* were associated with efficacy of probiotics.

Treatment with probiotics was safe compared with placebo, with a similar incidence of all adverse events (five [16%] of 32 *vs* 12 [33%] of 36) and gastrointestinal-specific adverse events (one [3%] of 32 *vs* five [14%] of 36; table 3). In addition to the single adverse event (skin infection) that led to discontinuation with probiotics during the randomised controlled trial phase, four adverse events led to discontinuations with placebo (diarrhoea in two patients, skin or lung infection in two patients; table 3). Two serious adverse events occurred during the open-label extension phase (appendicitis and syncope in two separate patients), which were assessed by the investigators as unlikely to be related to the study product. There were no treatment-related deaths.

| | Probiotic group (n=32) | Placebo group (n=36) |
|---|---|---|
| Number of patients with adverse events | 5 (16%) | 12 (33%) |
| Cardiac disorders | | |
| Palpitations | 0 | 1 (3%)* |
| Gastrointestinal disorders | | |
| Diarrhoea | 0 | 2 (6%)†‡ |
| Gastritis | 1 (3%)† | 2 (6%)† |
| Vomiting | 0 | 1 (3%)† |
| General disorders | | |
| Fever | 0 | 1 (3%)* |
| Influenza-like symptoms | 2 (6%)* | 1 (3%)* |
| Infections and infestations | | |
| Skin infection | 1 (3%)*‡ | 1 (3%)*‡ |
| Lung infection | 0 | 1 (3%)*‡ |
| Renal or urinary disorders | | |
| Renal colic | 0 | 1 (3%)* |
| Respiratory or thoracic disorders | | |
| Allergic rhinitis | 0 | 1 (3%)* |
| Skin or subcutaneous tissue | | |
| Maculopapular rash | 1 (3%)* | 0 |

Data are n (%) for the full analysis set. *Unlikely to be related to study product. †Possibly related to study product. All adverse events in the first 8 weeks were mild (grade 1) or moderate (grade 2). ‡Denotes adverse events leading to discontinuation.

***Table 3:*** **Adverse events per system organ class in the first 8 weeks**

## Discussion

In this exploratory study, we showed the efficacy and safety of *B coagulans* MY01 and *B subtilis* MY02 spore-forming probiotics in patients with functional dyspepsia compared with placebo. Reduced PDS scores were noted for patients with functional dyspepsia with probiotics versus placebo (randomised controlled trial phase). The effects of probiotics on PDS and EPS symptoms were corroborated for the key individual symptoms of the daily diary compared with placebo. The beneficial effects were also maintained with probiotics during the open-label extension phase. Despite the absence of between-group differences in systemic immune activation at 8 weeks, changes in T cells were evident after longer-term probiotic treatment with decreased Th17 signalling, which was associated with clinical efficacy. Despite the absence of major shifts in relative or quantitative faecal microbiota community composition, the proportional increase in *Faecalibacterium* was also associated with probiotic efficacy. Moreover, spore-forming probiotics reduced the proportion of positive glycocholic acid breath tests in patients with functional dyspepsia on proton-pump inhibitors, suggesting a reduction of small intestinal bacterial overgrowth. Finally, treatment with spore-forming probiotics was well tolerated.

Despite the high prevalence of functional dyspepsia (approximately 10% of the adult population fulfils symptom-based criteria for Rome IV functional dyspepsia[7]), treatment options are limited in efficacy or safety due to potential side-effects.[2] Although we have shown that routine or short-term proton-pump inhibitor therapy reduces eosinophils, mast cells, and intestinal permeability in functional dyspepsia, luminal effects of proton-pump inhibitors could also provoke similar duodenal alterations in long-term users of such drugs.[5] As these changes are not fully reversible during proton-pump inhibitor withdrawal, this factor would justify the search for alternative treatments as reflected by the absence of consensus for effective functional dyspepsia therapies.[14] Although a Japanese randomised controlled trial showed similar overall efficacy with some improvement of postprandial fullness with daily intake of *Lactobacillus gasseri* OLL2716 (LG21) in uninvestigated dyspepsia,[13] the combination of *B coagulans* MY01 and *B subtilis* MY02 strains in the current study was efficacious for PDS, EPS, and key individual symptoms. Analyses in patients with functional dyspepsia on and off proton-pump inhibitors also require replication in larger and multicentre studies. Despite several discontinuations due to adverse events (most of which were unlikely to be related to study drug) and a small number of withdrawals of consent, positive outcomes from the intention-to-treat analyses were corroborated in the per-protocol analysis, with the 20–30% improvement compared with placebo being higher than the suggested 10–15% considered as a clinically meaningful outcome.[19] Weekly responder rates were highest at the end of the randomised controlled trial phase and the absence of a significant effect of probiotics on PAGI-SYM and PAGI-QOL scores at 8 weeks could partly be explained by the 2-week recall period, which would not capture the highest efficacy of probiotics in the final week of the randomised controlled trial phase. A statistically significant effect of probiotics was evident using the PAGI-SYM and PAGI-QOL scores at 16 weeks.

Several studies have reported immune activation in functional dyspepsia.[2] On one hand, increased IL-5 and IL-13 production after stimulation of PBMCs from patients with functional dyspepsia suggested a shift from a Th1-type to Th2-type inflammation.[20] On the other hand, increased IL-1β-production of cultured PBMCs suggested that a Th17 response and production of granulocyte-macrophage colony-stimulating factor from Th17 cells could drive mucosal eosinophil recruitment in functional dyspepsia,[21] which was significantly increased in the duodenum of patients with the condition.[5,22] In our study, there was decreased Th17 signalling after 16 weeks of treatment with probiotics. In patients with functional dyspepsia on proton-pump inhibitors, there was an additional decrease in $Th2^+$ T cells and gut-homing T cells after ex-vivo stimulation of PBMCs. $CD4^+$ T cells co-expressing integrin α4β7 and CCR9, indicative of small intestinal mucosal migration, were previously reported to be upregulated in functional dyspepsia.[6] Similar to the inverse association between intestinal and systemic gut-homing T cells in inflammatory small bowel diseases,[6] the decrease in Tregs after 8 weeks of probiotics could be related to increased intestinal recruitment of Tregs. Increased $CD45RA^+$ Tregs were detected after 16 weeks of probiotics. Although CD45RA is not exclusively expressed on naive T cells, this finding might point to immunoregulatory properties of these probiotics, which could be more pronounced in patients on proton-pump inhibitors due to microbiome-related side-effects of proton-pump inhibitors.[11] However, only the decreased IL-17A or Th17, and not Tregs, were associated with efficacy of probiotics and not placebo during the randomised controlled trial phase.

Although a previous study reported beneficial changes in individual genera with multispecies probiotics (including *B coagulans* and *B subtilis*) in long-term users of proton-pump inhibitors, no changes were found in α-diversity or overall faecal microbiota composition.[11] Similarly, spore-forming probiotics had only minor and non-significant effects on the relative and quantitative community composition on the genus level, but with a relative increase of *Faecalibacterium* and *Roseburia* compared with control samples. Although the lower concordance between relative and absolute abundances of *Faecalibacterium* is known, increased enumeration of *F prausnitzii* was also found and possibly related to increased antigen-stimulated production of IL-10 by PBMCs with intake of *B coagulans*.[23] Although both commensal bacteria have anti-inflammatory activity with decreased Th17 signalling,[24,25] only the increased

*Faecalibacterium* was associated with probiotic efficacy in the current study. Proportional increases in the family Leuconostocaceae have also been reported after treatment with anti-inflammatory proteins of *Lactobacillus plantarum* or *Lactobacillus paracasei* LC-37, with effects on the gut barrier and inflammation or metabolites.[26,27] In functional dyspepsia, the reduced abundance of intestinal-like bacteria in the gastric fluid suggested a reduction of bacterial overgrowth with LG21.[12] Although concomitant intake of *Lactobacillus reuteri* DSM 17938 reduced bacterial overgrowth after 3 months of proton-pump inhibitor treatment,[28] the systematic prevention of proton-pump inhibitor-related side-effects is not recommended. In the present study, reduction of bacterial overgrowth as evaluated by the glycocholic acid breath test was also found in long-term users of proton-pump inhibitors after 8 weeks, pointing to additional benefits of spore-forming probiotics.

The limitations of this exploratory study include the limited duration and generalisability of a single centre and tertiary care study, although baseline characteristics and distribution of functional dyspepsia subtypes were similar to the general population.[7] As we did not select patients on the basis of PDS severity, numbers of eligible patients for the analysis of minimal clinical responders were lower but probiotic efficacy was also corroborated when assessing changes in PDS and EPS scores from baseline. Although the LPDS diary is mainly used for PDS (the primary outcome), it was one of the most promising outcome measures for symptom evaluation in clinical trials in functional dyspepsia.[18] Confirmation of our preliminary findings is needed, especially for EPS as coexisting or predominant symptom or subgroup. We studied systemic and not local immune activation; thus, providing only indirect evidence for changes in duodenal inflammation in functional dyspepsia.[2] Dietary intake was not accounted for and although changes in the faecal microbiota are not representative of the small bowel microbiome, similarities exist between both and in particular for *Faecalibacterium*.[29] Finally, in addition to common limitations inherent to all non-invasive breath tests, substrate availability and low amounts of radiation limit the use of $^{14}$C-glycocholic acid breath tests.

The strengths of this study include the rigorous study design with additional information on longer-term efficacy and safety from the open-label extension phase. We included patients who were clinically well characterised with functional dyspepsia (Rome IV criteria) with strict inclusion and exclusion criteria and assessment by a single study physician, reducing other potential sources of variability. Spore-forming probiotics offer the advantage of high stability and long shelf life, similar to heat-inactivated but non-viable strains.[30] The use of the validated daily diary was more comprehensive for clinical endpoints than the questionnaires and we did a detailed immune and faecal microbial characterisation, including co-expression of markers for small bowel homing and relative and quantitative microbiota profiling. As changes in the microbiome were more prominent with proton-pump inhibitors than antibiotics or other commonly used drugs in previous population-based studies,[9] the potential for a reduction in bacterial overgrowth with spore-forming probiotics in patients with functional dyspepsia who cannot be weaned off proton-pump inhibitors warrants further study.

In conclusion, the current combination of *B coagulans* MY01 and *B subtilis* MY02 spore-forming probiotics was effective and safe in patients with functional dyspepsia. Both a decreased Th17 signalling and an increased *Faecalibacterium* relative abundance were associated with probiotic efficacy. Although spore-forming probiotics could be considered as monotherapy, changes in immune activation were more pronounced with probiotics in patients with functional dyspepsia on proton-pump inhibitors, suggesting additional beneficial effects on chronic alterations with proton-pump inhibitor therapy. This pilot study underscores the potential role of microbiota in functional dyspepsia and provides effect sizes, which are informative to design larger and multicentre trials. Future studies should strengthen this preliminary evidence for spore-forming probiotics in different populations and functional dyspepsia subtypes, including immune activation and the microbiome as possible underlying mechanisms, which will help to establish the positions of probiotics as an add-on to proton-pump inhibitors or monotherapy in functional dyspepsia.

#### Contributors

LW was responsible for study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, and drafting of the manuscript. HS, KDP, MC, and SW were responsible for acquisition of data, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content. KG and JTo were responsible for acquisition of data and critical revision of the manuscript for important intellectual content. WT and RD provided technical support. DW, EB, KV, JTa, TVdW, and NH were responsible for technical support, analysis and interpretation of data, and critical revision of the manuscript for important intellectual content. TV was responsible for study concept and design, analysis and interpretation of data, critical revision of the manuscript for important intellectual content, and study supervision. EB, NH, TVdW, and TV verified the data. All authors except WT and RD had full access to all the data in the study. All authors have reviewed and approved the final manuscript. The corresponding author oversaw collection and analysis of the data and had final responsibility for the decision to submit for publication.

#### Declaration of interests

LW and TV have received speakers' fees from MY HEALTH. RD and WT are employees of MY HEALTH and MY RESEARCH, respectively, and have applied for an international PCT patent, number PCT/EP2020/058850 (international publication date 08/10/2020; international publication number: WO 2020/201153 A1; title: *Bacillus coagulans* and *Bacillus subtilis* for the prevention and treatment of functional gastrointestinal disorders). All other authors declare no competing interests.

#### Data sharing

All available data are provided in the Article and in the appendix.

#### Acknowledgments

This study was investigator-initiated, funded by an unrestricted research grant from MY HEALTH (Kermt, Belgium). The company provided feedback on the protocol, which was drafted by the first and last author. The company provided the spore-forming probiotics and placebo control

products as well as information about the probiotics. This study was also supported by the Interreg Euregio Meuse-Rine Healthy Aging project grant (EMR51), funded by the European Fund for Regional Development of the EU, supporting innovation in this region. LW and TV are supported by Flanders Research Foundation through a doctoral fellowship (1190619N) and senior clinical research mandate (1830517N). KDP is recipient of an EOS Flanders Research Foundation grant (30770923). JTa is supported by a Methusalem grant of KU Leuven (EZX-C9725-METH/14/05). The authors thank Tim Lacoere, Greet Vandermeulen, Laura Dusaer, and Christel Bocken for technical assistance. The human biological material for PBMCs was stored in and provided by the University Biobank Limburg (Hasselt, Belgium).

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