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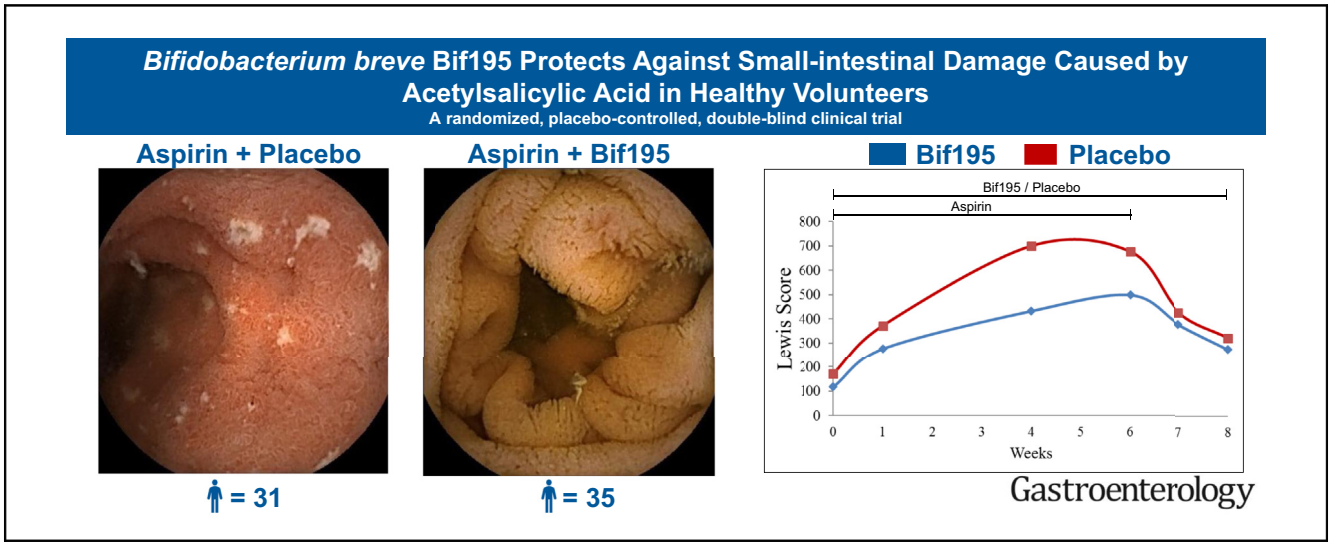
CLINICAL—ALIMENTARY TRACT

Bifidobacterium breve Bif195 Protects Against Small-Intestinal Damage Caused by Acetylsalicylic Acid in Healthy Volunteers

Brynjulf Mortensen,[1] Clodagh Murphy,[2,3] John O'Grady,[2,3] Mary Lucey,[4] Gafer Elsafi,[3] Lillian Barry,[4] Vibeke Westphal,[1] Anja Wellejus,[1] Oksana Lukjancenko,[5] Aron C. Eklund,[5] Henrik Bjørn Nielsen,[5] Adam Baker,[1] Anders Damholt,[1] Johan E. T. van Hylckama Vlieg,[1] Fergus Shanahan,[2,3] and Martin Buckley[2,3,4]

[1]Chr. Hansen A/S, Human Health Innovation, Hoersholm, Denmark; [2]APC Microbiome Ireland, Cork, Ireland; [3]Department of Medicine, University College Cork, National University of Ireland, Cork, Ireland; [4]Centre for Gastroenterology, Mercy University Hospital, Cork, Ireland; and [5]Clinical Microbiomics A/S, Copenhagen, Denmark



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BACKGROUND & AIMS: Enteropathy and small-intestinal ulcers are common adverse effects of nonsteroidal anti-inflammatory drugs such as acetylsalicylic acid (ASA). Safe, cytoprotective strategies are needed to reduce this risk. Specific bifidobacteria might have cytoprotective activities, but little is known about these effects in humans. We used serial video capsule endoscopy (VCE) to assess the efficacy of a specific *Bifidobacterium* strain in healthy volunteers exposed to ASA. **METHODS:** We performed a single-site, double-blind, parallel-group, proof-of-concept analysis of 75 heathy volunteers given ASA (300 mg) daily for 6 weeks, from July 31 through October 24, 2017. The participants were randomly assigned (1:1) to groups given oral capsules of *Bifidobacterium breve* (Bif195) ($\geq 5 \times 10^{10}$ colony-forming units) or placebo daily for 8 weeks. Small-intestinal damage was analyzed by serial VCE at 6 visits. The area under the curve (AUC) for intestinal damage (Lewis score) and the AUC value for ulcers were the primary and first-ranked secondary end points of the trial, respectively. **RESULTS:** Efficacy data were obtained from 35 participants given Bif195 and 31 given placebo. The AUC for Lewis score was significantly lower in the Bif195 group (3040 ± 1340 arbitrary units) than the placebo group (4351 ± 3195) ($P = .0376$). The AUC for ulcer number was significantly lower in the Bif195 group (50.4 ± 53.1 arbitrary units) than in the placebo group (75.2 ± 85.3 arbitrary units) ($P = .0258$). Twelve adverse events were reported from the Bif195 group and 20 from the placebo group. None of the events was determined to be related to Bif195 intake. **CONCLUSIONS:** In a randomized, double-blind trial of healthy volunteers, we found oral Bif195 to safely reduce the risk of small-intestinal enteropathy caused by ASA. ClinicalTrials.gov no: NCT03228589.

Keywords: Aspirin; Bacteria; Microbiota; Bleeding.

Abbreviations used in this paper: **au, arbitrary unit; AUC, area under the curve; ASA, acetylsalicylic acid; COX, cyclooxygenase; CVD, cardiovascular disease; ELISA, enzyme-linked immunosorbent assay; GI, gastrointestinal; GLP, good laboratory practice; GSRS, Gastrointestinal Symptoms Rating Score; I-FABP, intestinal fatty acid–binding protein; NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E2; PPI, proton pump inhibitor; TXB2, thromboxane B2; VCE, video capsule endoscopy.**

Most current article

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Nonsteroidal anti-inflammatory drugs (NSAIDs) are used worldwide both as prescription and over-the-counter products for their analgesic, anti-inflammatory, and cardiovascular disease (CVD) risk-reduction properties, and they are among the most used pharmaceuticals in the world today.[1] Chronic, low-dose use (commonly defined as 75–325 mg daily) of the NSAID acetylsalicylic acid (ASA) is widely recommended for both primary and secondary prevention of CVD. More than 30% of the US population aged above 40 years are estimated to be taking chronic, daily, low-dose ASA for that reason alone.[2] However, chronic use of ASA is also associated with adverse effects, including small-intestinal mucosal lesions and ulcers, perforations, major hemorrhage, and, in rare instances, death.[3–5] A recent review and meta-analysis addressing both the efficacy of ASA in prevention of CVD and also bleeding-related adverse effects concluded that a balanced, cautious approach should be taken in the case of primary CVD prevention because of these adverse effects,[6] highlighting the unmet need to reduce the risk of adverse effects associated with chronic ASA use.

For decades, endoscopists have acknowledged the vulnerability of the gastroduodenal mucosa to NSAID-induced enteropathy. Complications include ulceration, blood loss, protein loss, perforation, and occasional strictures. The pathogenesis of tissue injury at the gastric and small-intestinal sites appears to differ[7,8]; therefore, distinct and separate preventative strategies are probably required to combat enteropathy and gastropathy. For example, the risk of gastropathy can be offset by acid suppression, usually with proton pump inhibitors (PPIs). However, the pathogenesis of NSAID-induced damage in the small bowel seems to be much more complex and has been shown to involve microbiota composition, bile, and enterohepatic circulation of certain NSAIDs.[8] Moreover, there is evidence to suggest that PPIs may actually increase the risk of NSAID-associated small bowel injury,[9] possibly by disturbing the composition of the small bowel microbiota.[10] The importance of the microbiota is emphasized by the facts that administration of NSAIDs to germ-free animals is associated with minimal damage to the small-intestinal mucosa and that coadministration of antibiotics reduces NSAID-induced injury.[7,8] Besides the well-established inhibitory effect of cyclooxygenase (COX), ASA specifically has been recognized to compromise the phospholipid layer in mucus,[11] increasing access to luminal aggressors like lipopolysaccharide and bile, as well as disrupting intestinal permeability and causing inflammation.[12] Given that deleterious compositional changes to the microbiota, in addition to direct effects on mucus and epithelial tissue, may increase the risk of NSAID enteropathy, we hypothesized that an intervention targeting microbiome–host interactions may offer an attractive preventative strategy. Our strain selection was based on the anti-inflammatory properties of certain bifidobacteria[13,14] and experimental preclinical evidence for a role of bifidobacteria in NSAID-associated ulceration,[15–17] as well as unpublished preclinical screening data suggesting a particular potential of efficacy for the specific strain belonging to this genus. In addition, another strain of *Bifidobacterium breve* has been shown to express a pilus-associated protein (Tad E) in vivo, but not in vitro, which promotes colonic epithelial proliferation.[18]

Here, we describe the development of a clinical model to assess the quantitative and time-resolved induction of small-intestinal injury upon ASA administration. Using this model, we addressed whether oral coadministration of a single bacterial strain of *B breve*, Bif195, can reduce the risk of low-dose ASA-induced intestinal ulceration in humans in a randomized, placebo-controlled, parallel-group, double-blind trial using serial VCE as a rigorous demonstration of efficacy.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Enteropathy and small-intestinal ulcers are common side effects of NSAID such as ASA. There is an unmet need for safe, cytoprotective strategies to reduce this risk.

NEW FINDINGS

Daily, oral intake of Bifidobacteria Bif195 is safe and confers a significant reduced risk of small-intestinal enteropathy caused by acetylsalicylic acid in humans.

LIMITATIONS

Longer intervention clinical trials are needed to truly confirm if Bif195 has long-term clinical efficacy in a larger population of chronic users of low-dose acetylsalicylic acid.

IMPACT

This finding reveals new possibilities to reduce the risk of side effects from acetylsalicylic acid use and make the clinical effect vs side-effect evaluation of low-dose acetylsalicylic acid use in cardiovascular disease prevention more favorable.

Methods

Study Design

This clinical trial was a single-site, randomized, double-blind, placebo-controlled, parallel-group, proof-of-concept trial. The trial was conducted at the contract research organization Atlantia Food Clinical Trials (Cork, Ireland). The trial was conducted in accordance with the ethical principles set forth in the current version of the Declaration of Helsinki and the International Conference on Harmonisation E6 Good Clinical Practice. The trial was approved by the Clinical Research Ethics Committee of the Cork Teaching Hospitals (Cork, Ireland) before trial was conducted. The trial period was from July through December 2017. The trial was registered at ClinicalTrials.gov under identification number NCT03228589.

Participants

All participants were carefully informed about the trial before they signed the informed consent form and were screened for participation criteria. The main inclusion criteria

were age between 18 and 40 years, healthy and without gastrointestinal (GI) symptoms, sedentary lifestyle, and willingness to refrain from other bacterial products and medications known to alter GI function throughout trial participation.

The main exclusion criteria were history of abdominal surgery (except appendectomy and cholecystectomy), history of peptic ulcers, known bleeding disorders, known allergy to ASA, history of diseases related to *Helicobacter pylori* infection, diastolic blood pressure $\geq$ 90 mm Hg, systolic blood pressure $\geq$ 140 mm Hg, body mass index $>$ 27 kg/m^2, smoking or use of other nicotine products, lactose intolerance, pregnancy, lactation and regular use of probiotics, systemic antibiotics, steroids (except contraceptives), NSAIDs, laxatives, anti-diarrheal medications, PPIs, and/or immunosuppressant drugs before screening. After inclusion, participants went through a 2-week run-in period before baseline data were obtained at visit 2, with randomization performed at the very end of visit 2.

Randomization and Masking

Before the trial was conducted, the allocation of participants in a 1:1 ratio to Bif195 or placebo intervention was planned according to randomization lists. The randomization procedure was stratified by sex, and the lists were drawn up to n = 50 for each strata using the proc plan procedure in SAS (SAS Institute, Cary, NC). Randomization blocks of n = 6 were used throughout, and the trial site and sponsor were kept blinded to the use of randomization blocks. The randomization list and unblinding list were produced by a third party not otherwise involved in the trial.

At screening, participants were assigned a 4-digit screening number according to their chronological entry into the trial. If an individual was found eligible and enrolled for trial participation, he or she received a randomization number by blinded trial staff after all baseline assessments performed at visit 2. Randomization numbers included the stratification number and was allocated sequentially by trial staff in the order in which the participants completed visit 2.

The test and placebo products were produced by the sponsor to be similar in smell, taste, and appearance. All trial product was packaged in identical packs with identical labeling, except for the randomization number. All trial participants, the clinical team, statisticians, and the sponsor were blinded during the entire trial until database lock and signature of the request for the unblinding document.

An emergency unblinding procedure with emergency code-break opaque sealed envelopes was established to allow the investigator the option of disclosing the product assignment for any individual participant if clinical circumstances required such unblinding. This option was not required in the conduct of this trial. The randomization list and production of emergency code-break envelopes were performed by a third party not otherwise involved in the trial. The labeling of product vials, based on the randomization list, was also performed by a third party not otherwise involved in the trial.

Procedures

Bif195 or placebo were administered in a 1:1 ratio daily to 75 randomly assigned participants for 8 weeks. To induce damage to the small intestine, all participants were cotreated daily with 300 mg of ASA for the first 6 weeks of the 8-week Bif195/placebo intervention period.

To document small intestine damage, we performed VCE at 6 visits during the 8-week intervention period (Supplementary Figures 1 and 2). The time course kinetics of ASA-induced damage, as well as a potentially protective effect by Bif195, were expressed as area under the curve (AUC) for the 8-week intervention period for all data sets obtained.

All participants were given 2 hypromellose capsules daily with or without *B breve* Bif195 starting the day after visit 2 for a duration of 8 weeks. The product stability was monitored in parallel to trial conduct and showed at least 5×10^{10} colony-forming units of Bif195 per daily dose during the trial period. Detailed trial product and placebo descriptions are provided in Supplementary Table 1.

All randomly assigned participants were also given 300 mg of ASA (Alliance Pharmaceuticals, Dublin, Ireland) to induce small intestine damage. This dose was taken daily from the day after visit 2 for a duration of 6 weeks.

VCE is the widely accepted reference standard for assessment of occult gastrointestinal bleeding. Current uses include exploration and surveillance of bowel pathology such as in Crohn's disease, polyps, small bowel malignancy, and drug-induced mucosal injury.[19] To standardize the findings from VCE, we used a reproducible clinical scoring system to categorize small intestinal mucosal damage, the Lewis score. The Lewis score is a validated tool that evaluates villous edema, ulcers, and stenosis to quantify small bowel inflammatory.[20] This scoring system uses specific definitions for each of the recorded parameters to reduce interreviewer variability. In addition, we also counted red spots as observed during VCE.

For all VCE analyses (visits 2–7), data were recorded using the SB3 Pillcam video-recording capsule (Medtronic, Dublin, Ireland). For all visits, participants were fasting in the morning, and the Pillcam capsule was swallowed with water. Video images were recorded for a total of 8 hours during each visit, after which the capsule was verified in the video to have passed from the small intestine.

Four experienced gastroenterologists, blinded to the intervention and not allowed to communicate internally regarding obtained VCE data, reviewed the video material retrieved from the capsules using Pillcam Reader Software, version 9.0, from Medtronic. The VCE video material from all 6 VCE visits for each of the participants was evaluated by 2 randomly assigned reviewers, and the mean values for each participant's visit were calculated. In cases in which the number of ulcers from a specific visit differed by 4 or more, a third reviewer would review the VCE data set. The mean value of all 3 data sets was then calculated and used as the final data point for that specific visit. All VCE reviews were performed before database lock and unmasking of the randomization key. Representative pictures of the VCE material obtained are shown in Supplementary Figure 2.

Fecal samples and blood samples were obtained during all visits from visit 2 through visit 7 for secondary and exploratory analyses.

At all visits, participants completed the GI Symptoms Rating Score (GSRS) questionnaire to assess GI symptoms.[21]

Intestinal fatty acid–binding protein (I-FABP) was measured by Nordic BioSite (Tampere, Finland) in triplicate

heparin plasma samples by using the HK406 human I-FABP enzyme-linked immunosorbent assay (ELISA) kit from Hycult Biotech (Uden, The Netherlands) under good laboratory practice (GLP) conditions. Serum calprotectin was measured in duplicate serum samples under GLP conditions by Nordic BioSite using the HK379 Human Calprotectin ELISA kit from Hycult Biotech. Fecal calprotectin was measured in duplicate under GLP conditions by Synlab, Lucerne, Switzerland, using an ELISA kit from Immundiagnostik (Bensheim, Germany).

Outcomes

The primary outcome of this trial was the effect of the Bif195 intervention on the AUC Lewis score obtained by VCE from visit 2 (randomization) to visit 7 (end of treatment). As the first secondary end point, the effect of the Bif195 intervention on the AUC number of ulcers obtained by VCE from visit 2 to visit 7 was tested. Other secondary end points were, in hierarchic order: AUC of the pain module from the GSRS questionnaire, AUC of the total score from the GSRS questionnaire, AUC of blood I-FABP, AUC of red spots from the VCE procedure, AUC of fecal calprotectin, and AUC of blood calprotectin.

As exploratory end points, data stratified into tertiles (small intestine divided into thirds) on the effects of the Bif195 intervention on ulcerations observed by VCE were analyzed, and further post hoc analyses on intervention effects on prostaglandin E2 (PGE_2) and thromboxane B2 (TXB2) in serum samples downstream of COX were studied.

Safety was assessed by means of adverse events. A complete list of adverse events is provided in Table 1.

Statistical Analysis

For all data obtained, AUCs were calculated to evaluate the intervention effects by comparing the AUC in the Bif195 arm vs the placebo arm. For this purpose, the kinetics of the Lewis score for each participant over the 6 VCE visits was fitted to a third-degree polynomial, and the total AUC was calculated by computing the integral. This approach was taken for all VCE-obtained data.

Statistical tests were predefined and agreed in the statistical analysis plan finalized and signed before unblinding of the randomization key. The randomization list was made, and the labeling of the trial product was performed by third parties not otherwise involved in the trial. No imputation of data was carried out in cases of missing data, but all available data were used.

Participant characteristics and all efficacy data presented are based on the full analysis set population. Criteria for

Table 1. Overview of Trial Adverse Events

	Bif195	Placebo	Total
Treatment	n (%), E	n (%), E	n (%), E
Number of participants	38	37	75
All adverse events	8 (21.1), 12	14 (37.8), 20	22 (29.3), 32
Back pain	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Blocked sinuses	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Chest infection	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Cold and flu	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Cold/flu symptoms	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Cold/flu symptoms including a nosebleed	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Cough, nasal congestion	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Cramping in the stomach	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Cystitis	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Headache	1 (2.6), 1	2 (5.4), 2	1 (1.3), 1
Headache, sore throat, rhinorrhea	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Heartburn	0 (0), 0	2 (5.4), 2	2 (2.7), 2
Inflammation in kidneys due to kidney stones	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Lower abdominal pain	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Nasal congestion	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Nausea	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Nausea, vomiting, headache, fatigue	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Pain and discomfort in the stomach and gut region	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Pain and discomfort in the stomach/gut region	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Pain/discomfort in the stomach and gut region	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Painful headache that caused vomiting, thigh and back pain	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Participant became pregnant	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Participant was physically assaulted and suffered facial injuries	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Sore throat and flu symptoms	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Stomach cramps	0 (0), 0	1 (2.7), 1	1 (1.3), 1
Stomach cramps and loose stools	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Stomach discomfort	1 (2.6), 1	0 (0), 0	1 (1.3), 1
Vomiting bug	0 (0), 0	2 (5.4), 2	2 (2.7), 2

E, number of events in total in the group; n, number of subjects in the group having the event.

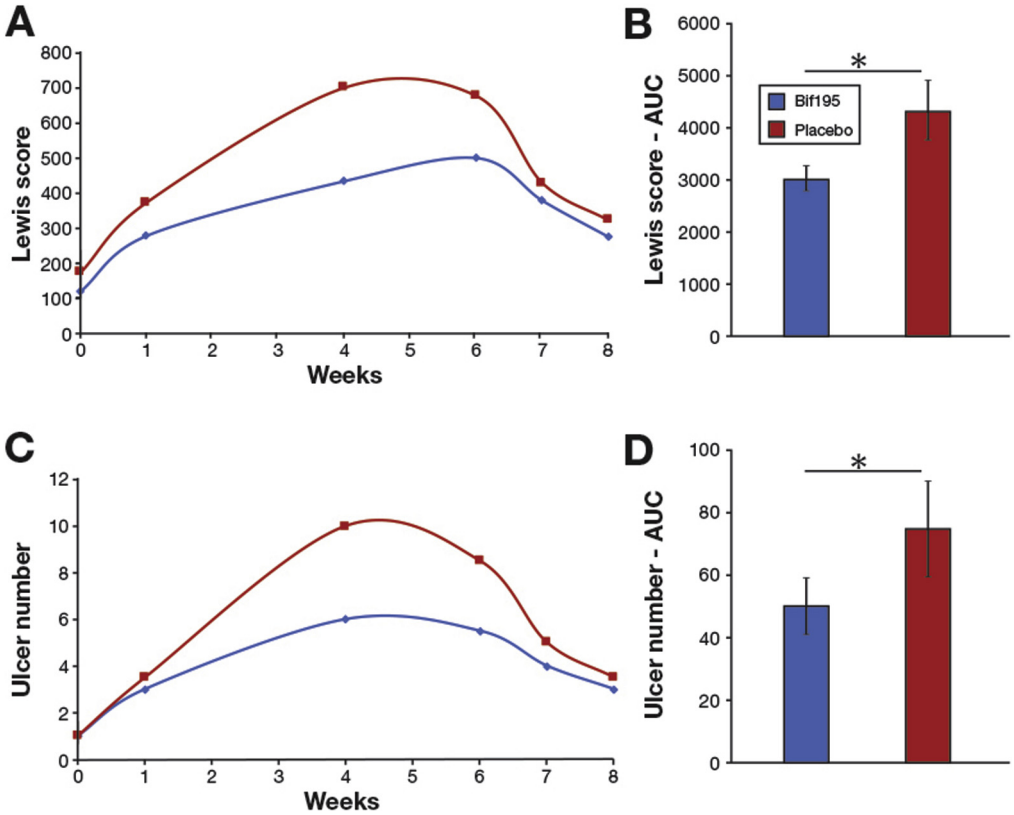

Figure 1. Primary and secondary end points. (*A*) Mean Lewis score per visit. (*B*) The primary end point mean Lewis score AUC ± standard error of the mean per treatment arm. (*C*) Median number of ulcers per visit. (*D*) The secondary end point ulcer number AUC ± standard error of the mean per treatment arm. *$P < .05$. Effects sizes were (*B*) 30% lower AUC in the Bif195 arm and (*D*) 33% lower AUC in the Bif195 arm.

inclusion in the full analysis set was defined as a maximum of 1 missing visit between the randomization visit (visit 2) and end of the trial (visit 7). The safety reporting by the listing of adverse events included all participants who were randomly assigned to intervention groups (n = 75).

A sample size calculation was performed before trial initiation based on the primary end point of the trial. The curve shapes were assumed to fit with a third-degree polynomial. We considered a 30% lower AUC after treatment of Bif195 compared with placebo to be clinically relevant and aimed for a trial design that would have 80% power in detecting an intervention effect of this size as statistically significant. To our knowledge, there is no previous information on AUC values and standard deviations. Sample size calculation was therefore performed on the percent difference of AUC between 2 normalized curves (active vs placebo) as an approximation. We assumed similar standard deviations in each arm and planned for 2-sided testing with a significance level of 5%. Given these assumptions, the number of participants needed in each arm was 30. To account for potential participant dropout, we aimed to include a total of 75 participants. Participants who withdrew within 1 week of randomization were replaced by standby participants.

In general, data sets were modeled as the dependent variable in a linear mixed model. The model included the baseline value as the covariate and sex and Bif195/placebo intervention as factors. Model check was always assessed for all data sets using Q–Q residual plots together with the Kolmogorov–Smirnov test for normality. In cases in which data sets did not meet a normal distribution, a log transformation was performed, and a check for normality performed again. In cases in which a normal distribution was still not obtained, the data set was tested for intervention effects using a nonparametric Mann–Whitney test. The curves in Figures 1–3 are shown as mean values or medians, depending on normality. The bars in Figures 1–4 are shown as mean ± standard error of the mean.

All authors had access to the study data and reviewed and approved the final manuscript.

Results

Between July 31, 2017 and October 24, 2017, 109 participants were screened for eligibility, of whom 75 were enrolled and randomly assigned to the intervention groups. Among the 75 randomly assigned participants, 9 participants discontinued during the intervention (n = 3 active and n = 6 placebo) and, therefore, efficacy data were obtained in a total of 66 participants, the analysis population (n = 35 active arm and n = 31 placebo) (Figures 1–4).

In general, the arms were similar in their baseline parameters as shown in Table 2, including sex distribution, age, body mass index, and blood pressure. Accountability of both ASA and trial product were generally very high in both arms (Table 2).

This clinical trial met its primary end point with a statistically significantly ($P = .0376$) lower AUC Lewis score, as captured by VCE, during the 8-week intervention in the Bif195 arm vs the placebo arm (3040 ± 1340 arbitrary units [au] in the Bif195 arm vs 4351 ± 3195 au in the placebo arm) (Figure 1*A* and *B*). In addition, the trial met its

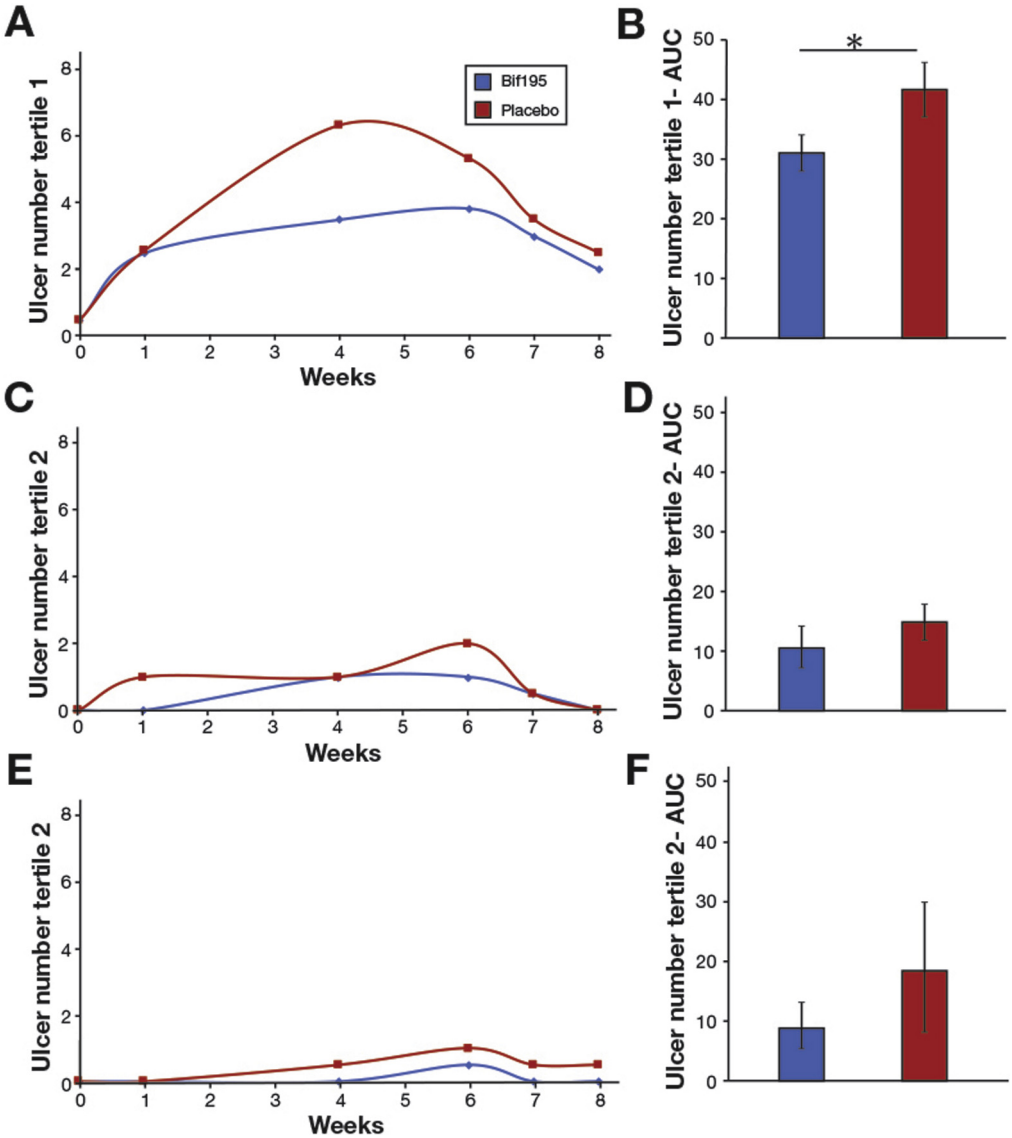

Figure 2. Tertile stratification of ulceration. (*A*, *C*, and *E*) Median ulcer numbers per visit and (*B*, *D*, and *F*) mean ulcer number AUC ± standard error of the mean from VCE stratified on small intestine tertiles (thirds of small intestine). *$P < .05$.

secondary end point with a significantly ($P = .0258$) lower AUC ulcer number, as captured with VCE during the intervention in Bif195 participants vs those in the placebo group (50.4 ± 53.1 au in the Bif195 arm vs 75.2 ± 85.3 au in the placebo arm) (Figure 1*C* and *D*).

An exploratory tertile stratification of VCE data showed that the damage induced by ASA occurs primarily in the first tertile (Figure 2), where a significant Bif195 protective effect ($P = .03$) was also observed (31.0 ± 16.8 au in the Bif195 arm vs 41.6 ± 25.2 au in the placebo arm) (Figure 2*A* and *B*).

The other secondary end points, GSRS pain AUC, GSRA total score AUC, plasma I-FABP AUC, red spots from VCE AUC, and serum calprotectin AUC, were not statistically significant (Figure 4), whereas fecal calprotectin AUC was significantly lower ($P = .0347$) in the Bif195 arm compared with the placebo arm (Figure 4*E*).

ASA and trial product were both generally well tolerated by the participants. In total, 32 adverse events were registered from 22 different participants included in the safety analysis set (n = 75). Twelve of these adverse events were reported from the Bif195 arm and 20 from the placebo arm (Table 1). None of the adverse events was related to Bif195 intake, and 10 of them were assumed to be related to ASA intake, as assessed by the principal investigator. The number of adverse events related to ASA did not differ between the 2 intervention arms (4 and 6 in the Bif195 and placebo arm, respectively).

DNA sequencing of all fecal samples obtained showed an increase after randomization in the abundance of *B breve* in fecal samples obtained from participants in the Bif195 arm compared with the placebo arm, confirming trial product compliance (Supplementary Figure 3). The Bif195 intervention was not associated with significant changes in the abundance of specific microbial taxa or in the changes of the overall microbiome composition (as shown by Bray-Curtis dissimilarity index) (Supplementary Figure 4).

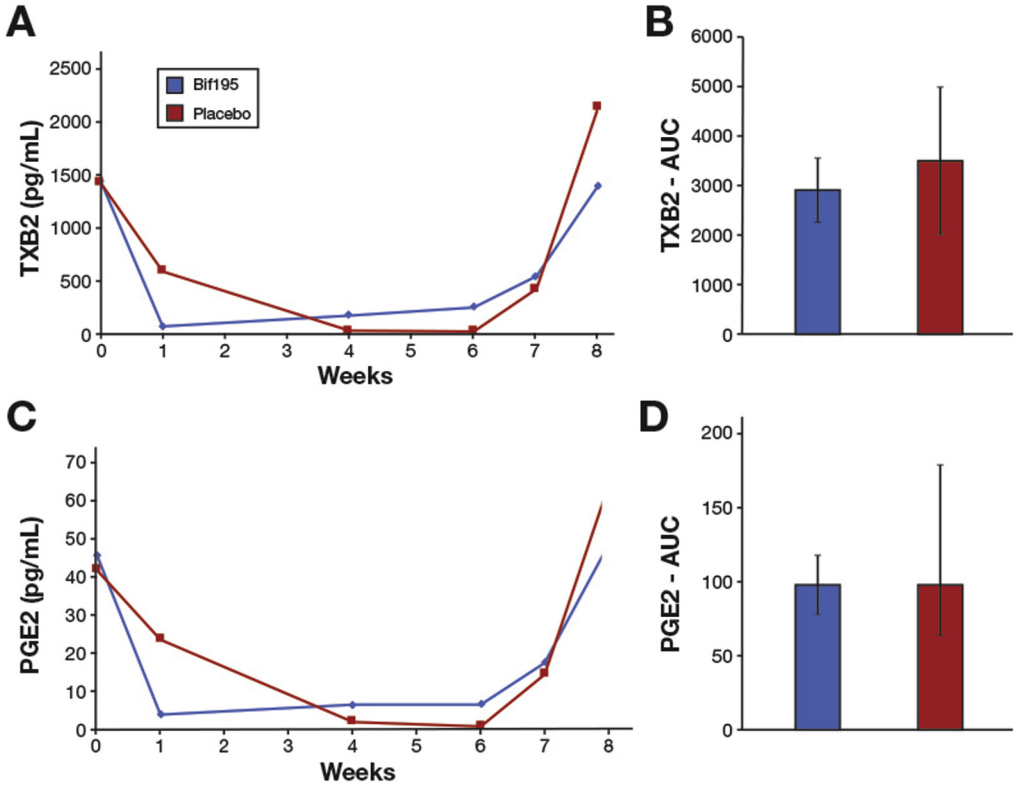

Figure 3. (*A*) Mean serum concentrations of PGE_2 per visit and (*B*) AUC ± standard error of the mean. Mean serum concentrations of (*C*) TXB2 per visit and (*D*) AUC ± standard error of the mean.

Serum PGE_2 and TXB2 concentrations showed a robust decline during ASA intake and a reversal to baseline levels during the final 2-week recovery period. The Bif195 intervention did not have significant effects on these data sets (Figure 3).

Discussion

The trial results indicate that *B breve* Bif195 confers significant and objectively verifiable protection against small-intestinal damage caused by a 6-week ASA challenge in healthy volunteers. The primary and first secondary efficacy criteria for the trial were met, thereby highlighting the potential of Bif195 cotreatment in future prevention strategies for a growing population experiencing silent or overt small-intestine enteropathy from chronic ASA use. Although prior studies have described gastric damage from NSAIDs, this is, to the best of our knowledge, the first trial to record the detailed time-resolved kinetics, and reversal, of ASA-induced small-intestine damage. This data set shows a gradual increase in the damage observed with VCE during the 6 weeks of daily ASA intake and a partial reversal toward baseline levels over a 2-week recovery period. Furthermore, the small-intestine tertile stratification clearly shows that ASA-induced enteropathy is mainly a duodenal phenomenon. This site coincides with localization of the main effect of the Bif195 intervention on ulceration, further highlighting the potential of protective intervention with this strain. The strategy of performing serial capsule endoscopies in this trial enabled us to obtain the sensitivity needed to observe a significant effect in a dynamic environment where damage formation and healing coexist. Thus, it represents a superior and more sensitive form of assessment than the more usually adopted before/after intervention trial design.

The efficacy of Bif195 in NSAID-associated small-intestine injury may be partly explained by the difference in pathogenesis between NSAID-associated small-intestine injury and NSAID-associated gastropathy. Whereas acid and pepsin are the principal luminal aggressors in NSAID-associated gastropathy, bile and, indeed, bacteria are the luminal factors in NSAID-associated enteropathy.[22] Although preclinical studies in animals have been encouraging, previous trials in humans of putative probiotics in NSAID enteropathy have been inconsistent. Certain strains of bifidobacteria are known to strengthen the intestinal epithelium layer, modulate the local immunoinflammatory response, and compete with potential bacterial aggressors. The molecular details of bifidobacterial-mediated protection against small-intestine epithelial injury are currently under investigation, but one candidate is the pilus-associated protein Tad E, which exerts a proliferative effect on host colonic epithelium after oral consumption of *B breve*.[18] This appears to be a characteristic of all *B breve* strains and supports our choice of the strain used in this trial. Interestingly, fecal microbiome analysis showed that changes were limited to a marked increase in the total *B breve* population in the Bif195 arm. These data provide further evidence that microbial intervention strategies targeting the microbiome can be clinically efficacious without inducing major alterations in the overall microbial population structure.

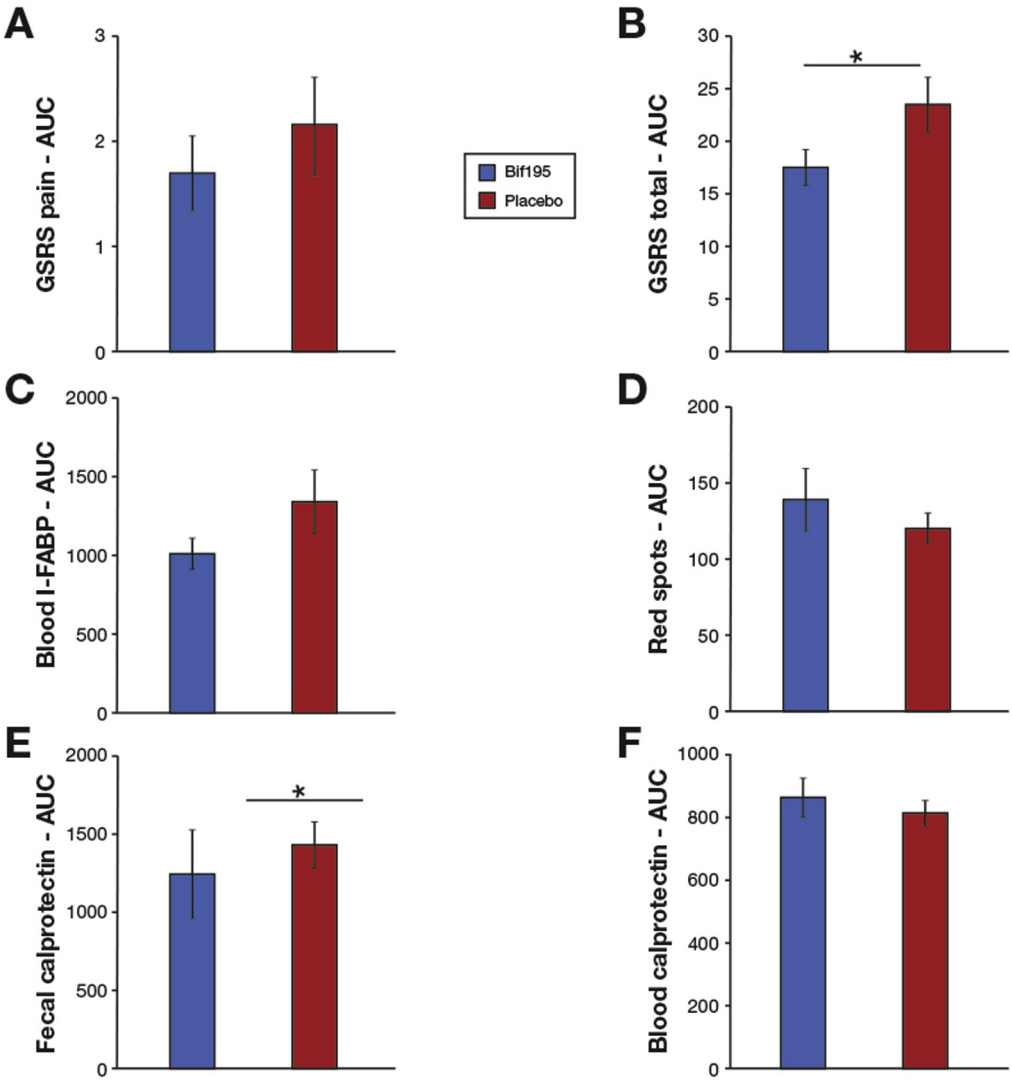

Figure 4. Other secondary end points measured in the trial. AUC ± standard error of the mean (SEM) of the (*A*) pain module and (*B*) total score from in the GSRS questionnaire. (*C*) AUC ± SEM of blood I-FABP, (*D*) AUC ± SEM of red spots from VCE, and (*E*, *F*) AUC ± SEM of (*E*) fecal and (*F*) blood calprotectin. *$P < .05$.

Our 6-week ASA challenge model yielded minor responses in the GSRS questionnaire and in the biomarkers of damage, I-FABP in blood and calprotectin in blood and feces. Although trends were observed for I-FABP, only the fecal calprotectin end point reached statistical significance, indicating a modest Bif195 protective effect. Our data suggest that VCE is the method of choice when conducting human challenges with mild induction of small-intestine damage by NSAIDs over a limited time period.

Although encouraging, the present clinical trial has limitations in terms of translation to a real-life clinical setting. The relatively short-term challenge in healthy volunteers, for proof of concept, used a higher dose of ASA than is most commonly prescribed for primary CVD prevention. However, it is a dose that is readily available for over-the-counter use. In addition, a recent report suggested that the current cardioprotective dosage of ASA may be insufficient and recommended doses based on a mg/kg basis.[23]

Because of our AUC approach based on a polynomic curve fitted to data points obtained from 6 different visits, data imputation is not feasible for participants who dropped out for whom only baseline data are available. Therefore, we acknowledge that long-term–intervention clinical trials will be needed to determine whether Bif195 has long-term clinical efficacy in a larger intention-to-treat population of chronic users of ASA taking lower doses for CVD prevention.

In addition, we acknowledge that the division of the small intestine into tertiles by VCE is based on assumptions and that tertile-specific data are an approximation.

As expected, the ASA intake was associated with robust inhibition downstream of the COX enzyme on serum PGE_2 and TXB2 concentrations. The Bif195 intervention did not alter these well-described ASA-induced changes in metabolites downstream of COX.[24,25] This suggests that the small-intestinal protective actions of Bif195 are unlikely to interfere with the specific cardiovascular-protective properties of ASA. Close monitoring of adverse events during this trial suggests that daily oral intake of Bif195 is safe and without adverse effects. Further clinical trials are required to test whether the strain has clinical efficacy in

Table 2. Participant Baseline Characteristics and Trial Compliance of the Analysis Population

Characteristics	Bif195	Placebo
N	35	31
Age, *y*	30.5 ± 6.8	31.2 ± 6.4
Sex, *male/female*	16/19	14/17
Nonwhite race, n	2	0
Height, *cm*	172.2 ± 12.1	173.4 ± 10.2
Weight, *kg*	73.5 ± 12.5	72.0 ± 11.4
Body mass index,[a] *kg/m²*	24.6 ± 2.1	23.8 ± 2.2
Systolic blood pressure, *mm Hg*	124.1 ± 7.8	121.6 ± 10.2
Diastolic blood pressure, *mm Hg*	78.7 ± 6.9	77.1 ± 7.6
Alcohol consumption, *drinks/wk*	5.1 ± 3.2	5.5 ± 3.7
Compliance with ASA intake,[b] %	98.7 ± 2.4	99.1 ± 1.9
Compliance with trial product,[b] %	98.6 ± 2.4	99.0 ± 1.9

NOTE. Values are reported as mean ± standard deviation unless noted otherwise.
[a]Body mass index is the weight in kilograms divided by the square of height in meters.
[b]100% = amount of product participant should have taken during trial.

other settings and populations, that is, in chronic users of ASA.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2019.05.008.

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Reprint requests
Address requests for reprints to: Fergus Shanahan, MD, APC Microbiome Ireland, Cork, Ireland. e-mail: f.shanhan@ucc.ie.

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Author contributions: Brynjulf Mortensen contributed to the trial conception and trial design, wrote the trial protocol, prepared and managed the trial on behalf of the sponsor, drafted the article, and contributed to the final version. Clodagh Murphy reviewed the VCE data and contributed to the final version. John O'Grady reviewed the VCE data and contributed to the final version. Mary Lucey reviewed the VCE data and contributed to the final version. Gafer Elsafi reviewed the VCE data and contributed to the final version. Lillian Barry oversaw the VCE procedure, reviewed the VCE data, and contributed to the final version. Vibeke Westphal contributed to the trial conception, trial design, and final version. Anja Wellejus contributed to the trial conception, trial design, and final version. Oksana Lukjancenko performed analysis and statistics on microbiome data obtained from fecal samples and contributed to the final version. Aron C. Eklund performed analysis and statistics on microbiome data obtained from fecal samples and contributed to the final version. Henrik Bjørn Nielsen performed analysis and statistics on microbiome data obtained from fecal samples, drafted parts of the article, and contributed to the final version. Adam Baker contributed to the trial conception, trial design, and final version. Anders Damholt contributed to the trial conception, trial design, and final version. Johan E.T. van Hylckama Vlieg contributed to the trial conception, trial design, and final version. Fergus Shanahan contributed to the trial conception and trial design, was co–principal investigator during the trial, oversaw the VCE procedure, reviewed the VCE data, wrote in part the manuscript, and contributed to the final version. Martin Buckley contributed to the trial conception and trial design, was principal investigator during the trial, oversaw the VCE procedure, reviewed the VCE data, and contributed to the final version.

Conflicts of interest
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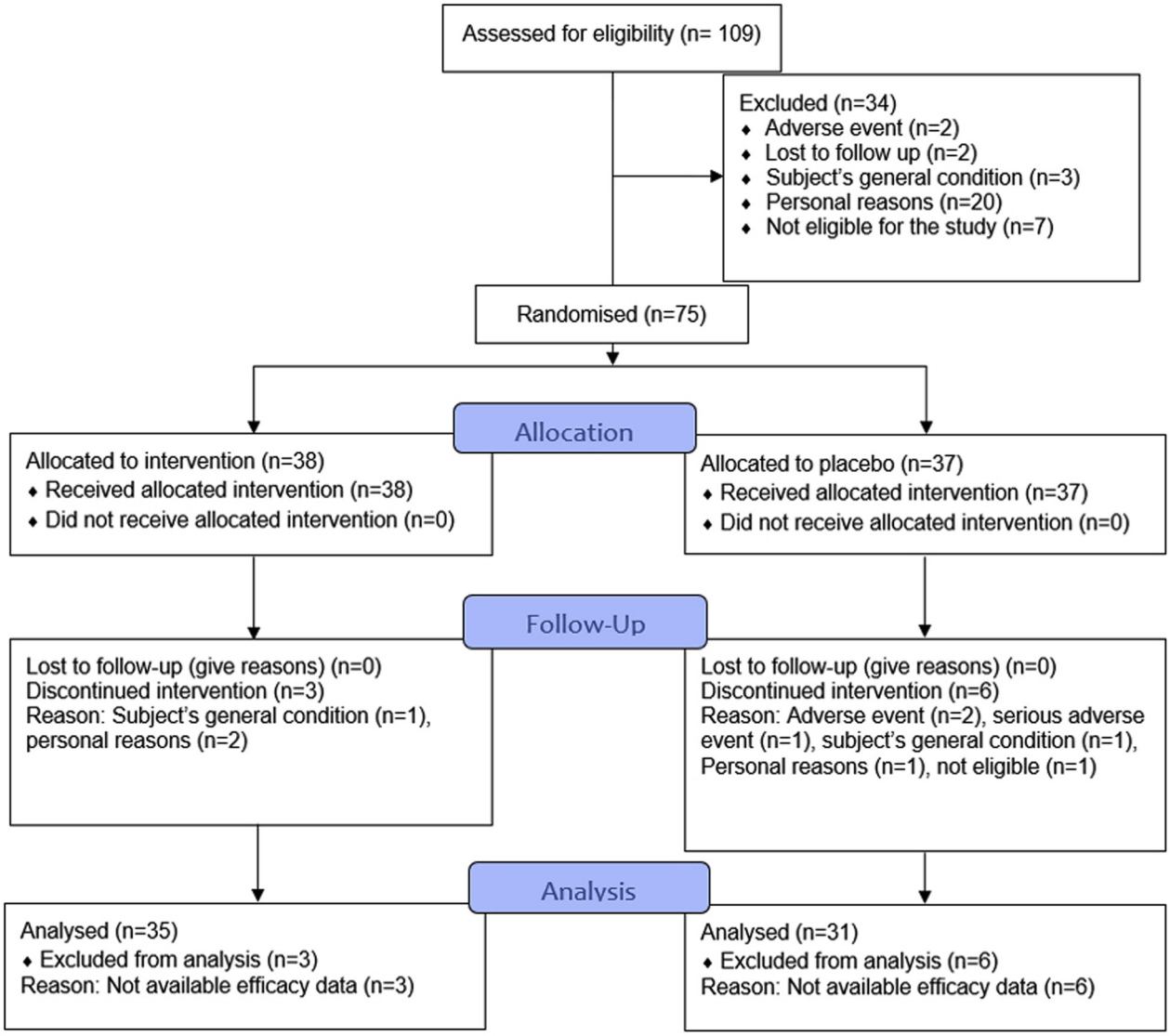
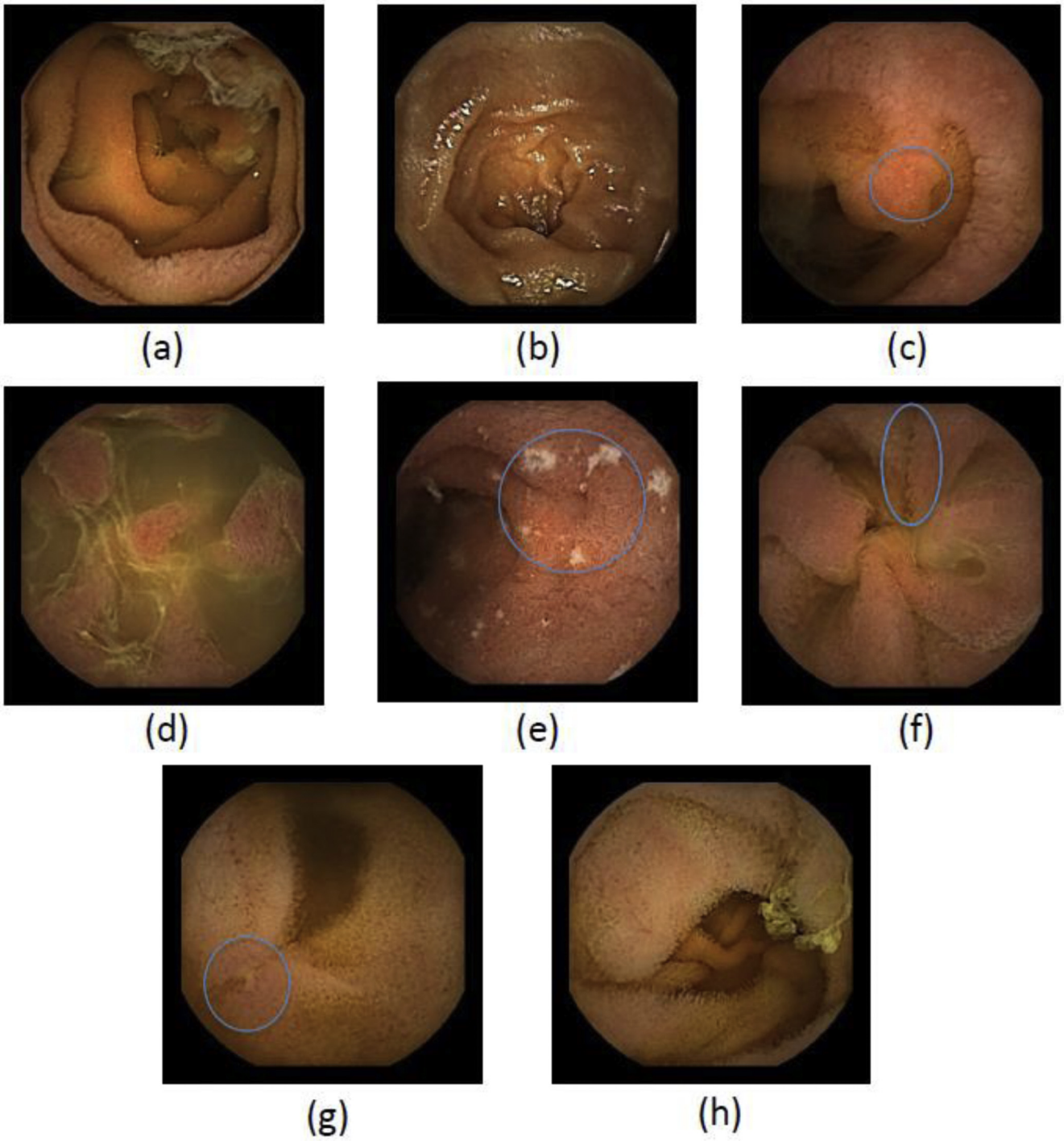
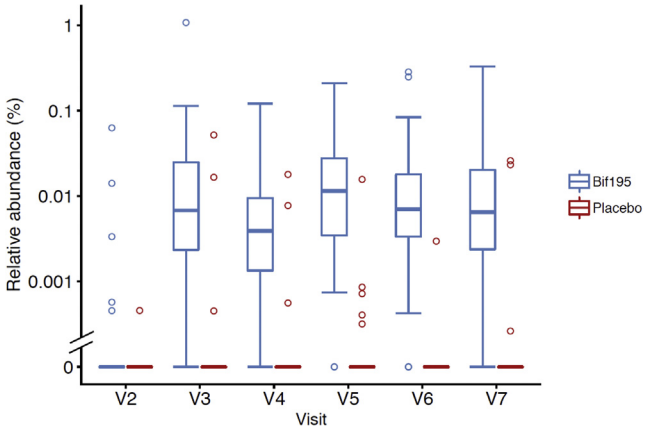


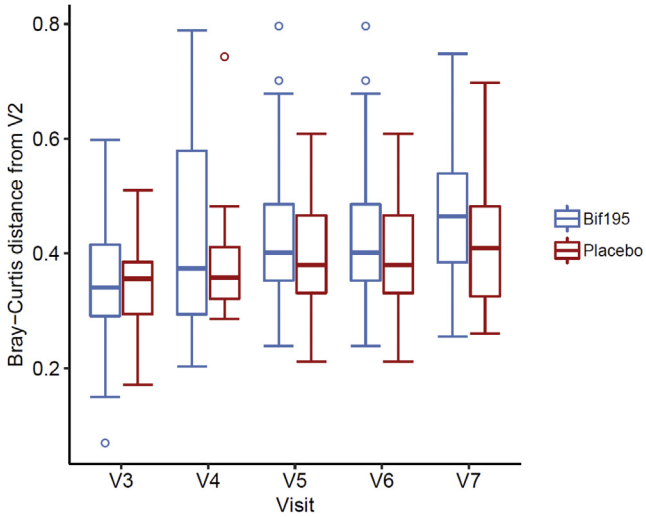
Supplementary Figure 1. Enrollment and randomization of subjects according to the CONSORT Flow Diagram.



Supplementary Figure 2. Representative VCE data. Representative images obtained by VCE from 1 participant throughout the intervention period. All images were obtained from the first tertile of the small intestine. The pictures show (*A*) visit 2 with normal intestinal mucosa, (*B*) visit 3 with normal intestinal mucosa, (*C*) visit 4 with ulcer highlighted by blue circle, (*D*) visit 4 with villous edema, (*E*) visit 5 with ulcer highlighted by blue circle, (*F*) visit 5 with villous edema highlighted by blue circle, (*G*) visit 6 with ulcer highlighted by blue circle, and (*H*) visit 7 with normal intestinal mucosa.



Supplementary Figure 3. Boxplot showing the relative abundances of *B breve* in stool at visits 2 through 7. The box extends from the first quartile (Q1) to the third quartile (Q3), and the line within the box shows the median value. The lower whisker extends to the smallest value within Q1 − 1.5 × interquartile range (IQR), and the upper whisker extends to the largest value within Q3 + 1.5 × IQR. Values outside the whiskers are shown as circles. After unblinding, a post hoc laboratory and bioinformatic analysis was performed on DNA extracted from all obtained fecal samples by using a NucleoSpin 96 Soil kit (Macherey-Nagel, Düren, Germany) and randomly sheared into 350–base pair fragments. Libraries were constructed using NEBNext Ultra Library Prep Kit for Illumina (New England Biolabs, Ipswich, MA) and sequenced to at least 30 million read pairs per sample (2 × 150–base pair paired-end Illumina sequencing). Sequencing reads were filtered to remove human and low-quality reads, mapped to the Clinical Microbiomics Human Gut 22M gene catalog, and summarized as a taxonomic relative abundance table, as described previously.[21] The involved parties were kept blinded to the intervention during analyses. Changes in relative abundances of taxa between visit 2 and the integral of later time points was tested using the Wilcoxon rank-sum test and corrected for multiple comparison using a Bonferroni correction. Similarly, the Bray–Curtis distance between visit 2 and later time points were compared between the 2 arms (*t* test). V, visit.



Supplementary Figure 4. Boxplot showing the Bray–Curtis dissimilarity of stool microbial composition, comparing visit 2 with later visits (V3–V7). The box extends from the first quartile (Q1) to the third quartile (Q3), and the line within the box shows the median value. The lower whisker extends to the smallest value within Q1 − 1.5 × inter-quartile range (IQR), and the upper whisker extends to the largest value within Q3 + 1.5 × IQR. Values outside the whiskers are shown as circles. V, visit.

Supplementary Table 1. Trial Product Composition

Product details	Placebo capsules	Probiotic capsules *Bifidobacterium breve*
Manufacturing	Chr. Hansen A/S, Denmark	Chr. Hansen A/S, Denmark
Brief description	Capsules with excipients only	Capsules containing *B breve* and excipients
Capsules	Size 1 HPMC capsules	Size 1 HPMC capsules
Capsule shell	73.6 mg hypomellose 1.4 mg titanium dioxide	73.6 mg hypomellose 1.4 mg titanium dioxide
Active ingredients	None	*B breve* Bif195
Excipients	Microcrystalline cellulose 6 mg per capsule Magnesium stearate 1.5 mg per capsule Maltodextrin 277.8 mg per capsule Sodium ascorbate 14.7 mg per capsule	
Supplied as	CSP Activ-Vials containing 24 capsules in each vial	
Storage conditions	Store at 2°C–8°C	

HPMC, hydroxyprolyl methyl cellulose.

Supplementary Table 2. Overview of Participants Who Did Not Complete Study

Participant ID	Reason for trial discontinuation	Bif195/placebo	Trial product taken	Aspirin taken	Baseline Lewis score
1013	Participant became pregnant—contraindicates continuation	Placebo	Yes	Yes	0
1026	Withdrawal for personal reasons	Placebo	Yes	Yes	184
1029	Baseline VCE capsule did not reach cecum—contraindicates continuation	Bif195	No	No	—
1042	Baseline VCE capsule retained in stomach—contraindicates continuation	Placebo	No	No	—
1048	Withdrawal for personal reasons	Bif195	Yes	Yes	0
1077	Baseline VCE capsule retained in stomach—contraindicates continuation	Placebo	No	No	—
1083	Baseline VCE capsule did not reach cecum—contraindicates continuation	Placebo	No	No	—
1089	SAE due to prolonged hospitalization (back pain); event unrelated to intake of trial product	Placebo	Yes	Yes	67.5
1108	Withdrawal for personal reasons	Bif195	Yes	Yes	67.5

ID, identification; SAE, serious adverse event.