


# The gastrointestinal status of healthy adults: a *post hoc* assessment of the impact of three distinct probiotics

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## Abstract

There is a growing awareness that supplementation with probiotic bacteria can impart beneficial effects during gastrointestinal disease, but less is known about the impact of probiotics on healthy subjects. Here, we report the outcomes of a *post hoc* analysis of recorded daily gastrointestinal events and bowel habits completed by healthy adults participating in a placebo-controlled, single-centre, randomised, double-blind, quadruple-arm probiotic tolerability study. Extensive screening ensured the healthy status of subjects entering the study and during a 2-week pre-intervention run-in period, a burden of gastrointestinal events (stomach pains, indigestion, acid reflux, stomach tightening, nausea and vomiting, stomach rumbling, bloating, belching and flatulence) was identified suggesting GI discomfort within the population. In the subsequent 12-week intervention period with 3 distinct probiotic formulations and a matched-placebo, reductions in the incidence rates of bloating, borborygmus, stomach pains, slow faecal transit and incomplete defecations were observed in the probiotic groups compared to the placebo. These results highlighted differing responses among the probiotic formulations tested and indicated potential anti-constipation effects. Product specific modulations in circulating interleukin-6 levels and in the composition of the gut microbiota were also detected. Together, these data suggest a role for probiotic supplementation to exert beneficial effects on the gastrointestinal functioning of healthy subjects and highlight the need for further longer-term studies in healthy populations to gain a greater understanding of the impact of probiotics.

**Keywords:** probiotic, constipation, interleukin-6, microbiota

## 1. Introduction

The trillions of organisms residing in the human gastrointestinal (GI) tract, collectively known as the gut microbiota, play a vital role in maintaining human health (Fassarella *et al.*, 2021). It is becoming very evident that modulation of the gut microbiota can lead to improvements in host health and there is substantial interest in the potential of supplementation with probiotic bacteria (Sanders *et al.*, 2019).

Probiotics are defined as ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ (FAO/WHO, 2006) and much of the research

has focused on diseased populations (Dronkers *et al.*, 2020), with over 60% of the studies targeting gastrointestinal/digestive diseases and infections such as irritable bowel syndrome (Zhang *et al.*, 2022) and constipation (Dimidi *et al.*, 2020). Much less is known regarding the impact of probiotics in overtly healthy populations, particularly in relation to gastrointestinal health (Khalesi *et al.*, 2019), and that is exacerbated by the lack of consensus on what constitutes a normal/healthy microbiota (McBurney *et al.*, 2019; Sharon *et al.*, 2022). However, it is estimated that the biggest consumers of probiotic supplements are non-diseased ‘healthy’ individuals who have an expectation of ‘gastrointestinal benefits’ (Yilmaz-Ersan *et al.*, 2020). Gastrointestinal events such as bloating, abdominal pains

and constipation have been observed in the 'healthy control' groups in observational studies (Azpiroz *et al.*, 2015; Del Piano *et al.*, 2010; Laurikka *et al.*, 2016) and in epidemiological studies with 'general populations' (Avramidou *et al.*, 2018; Sezgin *et al.*, 2019; Tielemans *et al.*, 2013) implying that, for many, a degree of GI discomfort is considered normal. It has been found that even mild GI disturbances can be associated with anxiety/depression (Vivier *et al.*, 2020) and impairments in quality-of-life (Tielemans *et al.*, 2013) highlighting the necessity to better understand the GI characteristics of healthy populations and the potential impacts of probiotic supplementation.

We have performed a double-blind, randomised, placebo-controlled study with a cohort of free-living adults receiving one of three different probiotic formulations: Probiotic-1 comprising two strains of *Lactobacillus acidophilus* and two strains of bifidobacteria, Probiotic-2 comprising the components of Probiotic-1 together with 9 strains of lactobacilli, 2 bifidobacteria, *Streptococcus thermophilus* and *Pediococcus pentosaceus* (a total of 17 strains), or Probiotic-3 comprising two *Lactobacillus* spp. and one strain of *Bifidobacterium*. The primary objective of the study was to confirm the tolerability and safety of the 3 probiotic interventions.

Here we present a *post hoc* analysis of the data gathered as part of a safety and tolerability study. Daily gastrointestinal health questionnaires and bowel habit diaries were completed during the 2-week pre-intervention run-in and 12-week intervention periods and we have assessed the impact of three probiotic consortia and a control on the gastrointestinal functioning in an overtly healthy adult cohort.

## 2. Materials and methods

### Study approval

This study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with ICH Guidelines for Good Clinical Practice (Current Step 4 Version dated June 10, 1996). Ethical approval was granted by the ethics boards of the Natural Health Product Directorate (NHPD), Health Canada (Submission No: 230832, Approval date: 08/08/2017).

### Study design

This was a single-centre, 4-arm, double-blind, randomised and placebo-controlled trial intended to evaluate the safety and tolerability of 3 distinct probiotic interventions against a placebo.

### Recruitment and consent

Adults aged 18 to 64 were recruited between 14/12/2017 and 18/01/2018 (London, ON, Canada). The participants had a body mass index (BMI) between 18.5 and 29.9 kg/m<sup>2</sup>; were in good general health (determined by medical history, haematological and serological analysis and physical examination during pre-enrolment screening), non-smokers without illicit drug use and excessive alcohol intake (>14 units per week) and were willing to provide faecal and blood samples. Females were not pregnant (determined by urine test) and agreed to use a medically approved method of birth control. Participants were asked to avoid the consumption of antibiotics, prebiotics and any other probiotics (Supplementary Table S1) and maintain their normal diet and lifestyle during the study. Participants were not eligible if they had a history of gastrointestinal disorders, cardiovascular disease or systemic diseases (cancer, dementia and/or organ failure) nor if they had taken antibiotics, anti-inflammatories or dietary supplements containing probiotic bacteria or prebiotics in the 30 days leading up to the trial. A detailed description of the inclusion and exclusion are shown in Supplementary Table S2. On the basis of previous probiotic tolerability studies (Clinicaltrial.gov identifier: NCT02155972, NCT01048567, NCT02176889), a sample size of 24 participants per group (including 15% attrition rate) was selected. Informed consent was obtained from each participant prior to performing any study-related activities.

### Randomisation

Eligible participants were assigned a unique randomisation number by a blinded investigator according to a randomisation list generated using [www.randomization.com](http://www.randomization.com). Participants were assigned to the four study-arms, Placebo, Probiotic-1 (P1), Probiotic-2 (P2) and Probiotic-3 (P3), at a ratio of 1:1:1:1. Participant allocations were not available to any member of the investigational team until study completion but were held at the trial site in sealed envelopes in case of emergency.

### Intervention

After randomisation the participants received daily two capsules of the appropriate intervention for 84 days. The composition and dose of each intervention are shown in Table 1. All capsules and packaging were identical to ensure blinding. Participants were provided with product at visits 2 and 3 and any unused capsules were collected at visits 3 and 4 to monitor compliance. The participants were instructed to take the capsules with water at the first meal of the day and store the intervention in a refrigerator.

Table 1. Study interventions and daily dose.

Group	Intervention	Daily dose
Placebo	Potato maltodextrin	2 capsules
Probiotic-1 (P1)	<i>Lactobacillus acidophilus</i> CUL-60 + <i>L. acidophilus</i> CUL-21 and <i>Bifidobacterium bifidum</i> CUL-20 + <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> CUL-34	2 capsules delivering a total of $2.7 \times 10^{11}$ cfu
Probiotic-2 (P2)	<i>L. acidophilus</i> CUL-60 + <i>L. acidophilus</i> CUL-21, <i>B. bifidum</i> CUL-20 + <i>B. animalis</i> subsp. <i>lactis</i> CUL-34, <i>Ligilactobacillus salivarius</i> CUL-61, <i>Lacticaseibacillus paracasei</i> CUL-08, <i>Lactiplantibacillus plantarum</i> CUL-66, <i>Lacticaseibacillus casei</i> CUL-06, <i>Limosilactobacillus fermentum</i> CUL-67, <i>Lactobacillus gasseri</i> CUL-09, <i>Pediococcus pentosaceus</i> CUL-15, <i>Bifidobacterium breve</i> CUL-74, <i>Streptococcus thermophilus</i> CUL-68, <i>Lacticaseibacillus rhamnosus</i> CUL-63, <i>Limosilactobacillus reuteri</i> JBD301, <i>B. bifidum</i> CUL-73, <i>Lactobacillus helveticus</i> CUL-76	2 capsules delivering a total of $2.7 \times 10^{11}$ cfu
Probiotic-3 (P3)	<i>L. rhamnosus</i> GG, <i>L. rhamnosus</i> HN001, <i>B. animalis</i> subsp. <i>lactis</i> HN019	2 capsules delivering a total of $2.7 \times 10^{11}$ cfu

## Outcomes

Changes in (1) gastrointestinal health (monitored with daily gastrointestinal symptoms rating scale (GSRS) and bowel habit diaries), (2) plasma biomarkers (interleukin-6 (IL-6) concentration) and (3) faecal microbiota composition (by traditional microbial culture).

## Data and sample collection

The schedule of data and sample collection is shown in Figure 1. Participants completed a daily Gastrointestinal Symptoms Rating Scale (GSRS) questionnaire and a bowel habit diary throughout the run-in and intervention periods. Overnight fasted blood samples and faecal samples were taken at visit 2 (day 0) and visit 4 (day 84).

## Assessment of upper gastrointestinal events using the GSRS

GSRS is a validated, self-assessed and disease-specific scale recording the occurrence of gastrointestinal (GI) events (Svedlund *et al.*, 1988) (Supplementary Figure S1). On a daily basis, each item was scored as follows: 0 (no event) to 3 indicating increasing severity. The upper GI events assessed were stomach pains (abdominal pains), indigestion (heartburn), acid reflux (acid regurgitation), stomach tightening (sucking sensation at the epigastrium), nausea and vomiting, stomach rumbling (borborygmus), bloating (abdominal distension), belching (eructation) and flatulence. Event incidences were calculated for both the individual and total number of events per month (30 days). Event onset was defined as the number of days until the first report of a GI event from the start of the intervention period.

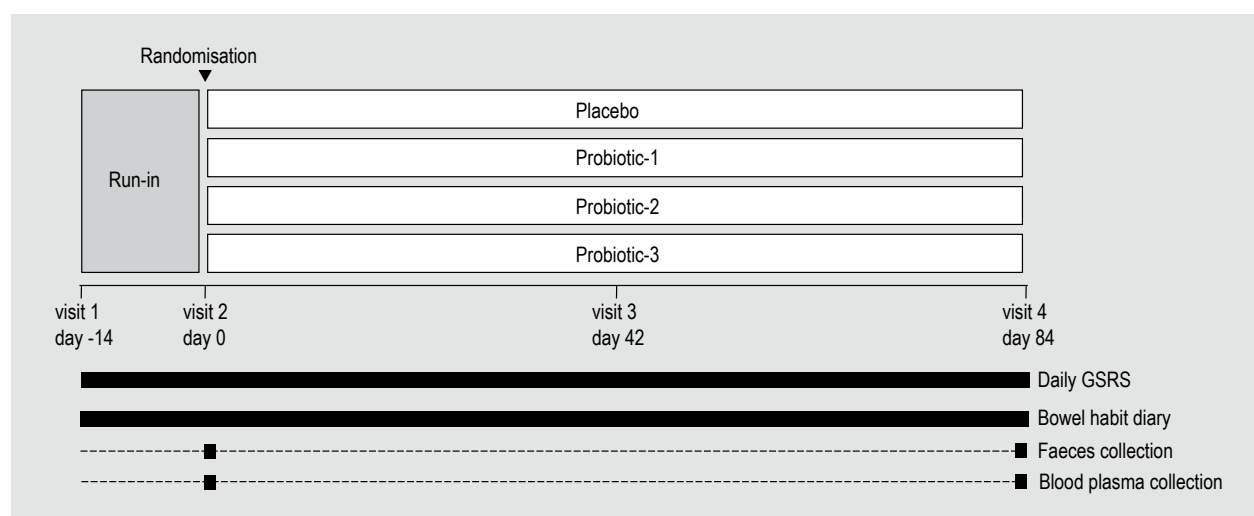


Figure 1. Study design and scheme of sample/data collection. GSRS = gastrointestinal symptom rating scale.

## Assessment of lower GI-related events using bowel habit diaries

The bowel habit diary comprised a 7-point questionnaire (Supplementary Figure S2) detailing (1) the date and time of defecation, (2) whether straining to start defecation, (3) whether straining to stop defecation, (4) whether experiencing feeling of incomplete defecation, (5) the Bristol Stool Score (BSS) rating, (6) usage of laxative, enema or suppository prior to defecation, and (7) the effect of menses on the defecation. Water consumption rates on the day of defecation were also recorded. Faecal transit times were based on the BSS: scores of 1-2 indicate slow transit, 3-5 indicate normal transit, and 6-7 indicate fast transit times (Lewis and Heaton, 1997; Mitelmão *et al.*, 2021).

A participant was defined as constipated if reporting two or more of the following: (1) straining for >25% of defecations, (2) lumpy or hard stools (BSS 1 or 2, slow transit) for >25% of defecations, (3) the sensation of incomplete evacuation for >25% of defecations or (4) an average of <3 spontaneous defecations per week (based on the Rome IV criteria for the diagnosis of functional constipation (Supplementary Table S3)).

## Processing, storage and analysis of blood plasma samples

12 h fasted bloods were collected into sodium heparinised tubes and the plasma was separated by centrifugation (2,000×g, 10 min), aliquoted and stored at -80 °C until required. IL-6 was quantified using the human IL-6 immunoassay (Abcam, Cambridge, UK) according to the manufacturer's instructions.

## Storage, processing and analysis of faecal samples

### Storage

Faecal samples were frozen (-20 °C) immediately after collection and transported to the trial centre within 2 days where they were stored at -80 °C pending analysis.

### Faecal moisture

Faecal samples (~0.2 g) were vacuum-dried at -40 °C and 0.01 mbar pressure for 1 h followed by 20 °C and 0.01 mbar pressure for 5 days (Modulyo Freeze Dryer; Edwards, UK). Dried samples were re-weighed and the percentage moisture content was calculated as: (weight before drying - weight after drying)/(weight before drying) × 100.

## Bacterial enumeration

Faecal samples were assessed for viable bacterial numbers using a modified version of the Miles Misra (1938) plate count technique. Decimal dilution series were set up in Maximum Recovery Diluent (MRD) and appropriate dilutions were plated onto selective media (Supplementary Table S4). Bacterial identification involved Gram staining, colony morphology, agglutination (*Staph* latex test; Microgen Bioproducts, Camberley, UK) and by Analytical Profile Index (API; BioMerieux, Marcy-l'Étoile, France). Viable bacterial cell counts were expressed as log<sub>10</sub> of the number of colony forming unit (cfu)/g sample dry weight.

## Statistical analysis

Analysis of study outcomes was performed on an intention-to-treat basis defined as participants who took at least one dose of intervention and had recorded any post-randomisation data. The incidences of GI events and bowel habits were analysed using a generalised linear model (GLM) that included treatment as the only predictor from which the treatment effect in terms of mean difference and incidence rate ratio with 95% CI and *P*-values were calculated (performed using SAS® version 9.4; SAS Institute Inc., Cary, NC, USA). Time-to-event for the first GI event was analysed with the Kaplan-Meier method and its significance was assessed by the log-rank Mantel-Cox test. For plasma IL-6 levels, differences between baseline and post-treatment were evaluated by Wilcoxon matched-pairs signed rank test and differences between groups were evaluated using the Kruskal-Wallis test with Dunn's *post hoc* analysis. The number of viable organisms in faeces were compared using one-way ANOVA with Tukey's *post hoc* test. All statistical analysis was performed in GraphPad Prism, version 8.2.2 (La Jolla, CA, USA) unless otherwise stated and values of *P*<0.05 were considered significant.

## 3. Results

### Recruitment, compliance and demographics

The flow diagram of study enrolment, allocation, follow-up and analysis is shown in Figure 2. 230 participants were screened for eligibility; 134 participants did not meet the inclusion criteria post screening and 96 healthy participants were enrolled into the study. A total of 7 participants were lost to follow-up; 5 withdrew consent and 2 were excluded due to protocol deviations. Compliance to the intervention was 91.48% for the placebo group, 98.30% for the P1 group, 98.38% for the P2 group and 95.43% for the P3 group.

The demographics of study participants are presented in Table 2; the participants were between 19 and 64 years of age with a BMI of 18.99 to 29.90 kg/m<sup>2</sup> and 69.9% of the

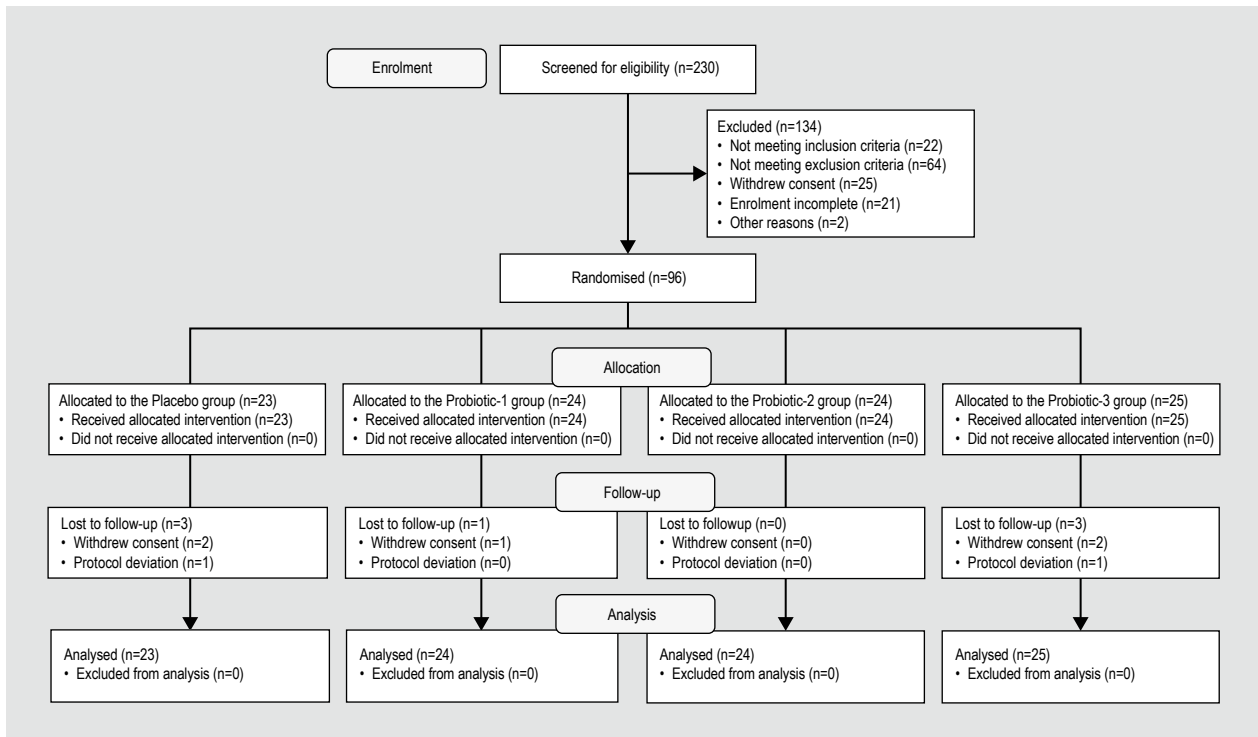


Figure 2. Flow diagram of study.

study population was female. Physiological, haematological and serological parameters were within normal ranges.

### Gastrointestinal events reported during the pre-intervention run-in period

GI events recorded using the gastrointestinal GSRS were assessed in the total population during the 2-week pre-randomisation run-in period; a total of 1229 GI events were recorded equivalent to  $27.43 \pm 38.90$  events/participant/month. Since 92.2% of events reported by the total population were categorised as mild, incidence rates were calculated irrespective of events severity. During the run-in period the incidences of upper GI events (Figure 3A) were: flatulence ( $7.59 \pm 9.25$  days/participant/month), bloating ( $6.18 \pm 8.50$  days/participant/month), stomach rumbling ( $4.69 \pm 7.43$  days/participant/month), belching ( $3.24 \pm 6.39$  days/participant/month), stomach pain ( $1.65 \pm 4.99$  days/participant/month), indigestion ( $1.50 \pm 4.17$  days/participant/month), acid reflux ( $1.16 \pm 3.92$  days/participant/month), stomach tightening ( $0.89 \pm 3.67$  days/participant/month) and nausea/vomiting ( $0.54 \pm 2.04$  days/participant/month).

The bowel habit diaries provided an assessment of lower GI events and the average number of defecations during the run-in period was  $1.43 \pm 0.57$  per day with an average BSS of  $3.64 \pm 0.75$ . Approximately 15% of defecations were reported with difficulty starting and 15% with incomplete evacuation (Figure 3B). Faecal transit times were calculated

from BSS (Lewis and Heaton, 1997) and  $16.8 \pm 21.02\%$  were slow,  $76.04 \pm 21.12\%$  were normal and  $7.16 \pm 11.08\%$  were fast (Figure 3B). Participants, 21/96 (21.9%), were defined as constipated and they reported higher incidence rates of the upper GI events than the non-constipated participants (Supplementary Figure S3).

### Impact of probiotics on the incidence of self-reported gastrointestinal events.

Participants in the placebo group reported  $30.03 \pm 32.02$  events per month (similar to run-in) with  $10.24 \pm 12.01$  in P1,  $30.44 \pm 54.29$  in P2 and  $18.70 \pm 18.75$  in P3. The P1 and P3 groups were significantly below the placebo ( $-19.79$  events/participant/month, 95% CI:  $-38.44$  to  $-1.13$ ,  $P=0.0376$ ;  $-20.20$  events/participant/month, 95% CI:  $-38.65$  to  $-1.74$ ,  $P=0.0319$ , respectively).

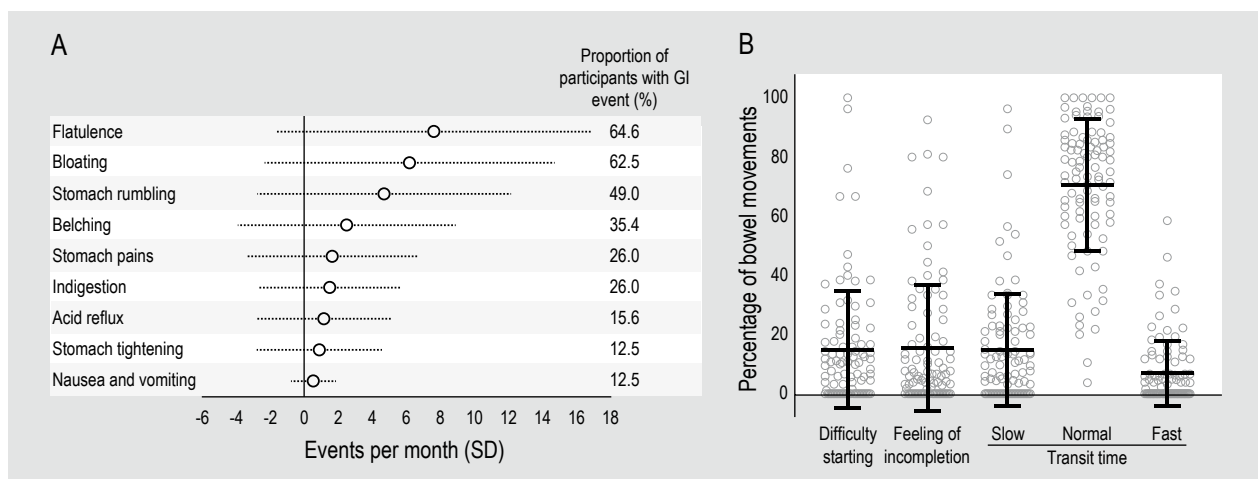
The average number of days per month for the individual GI events are shown in Figure 4 and compared to the placebo. Those receiving the P1 probiotic showed a reduction in all events with stomach pain reduced by 88.2% ( $-2.92$  days, 95% CI:  $-5.50$  to  $-0.34$ ,  $P=0.0267$ ), stomach rumbling reduced by 79.5% ( $-3.66$  days, 95% CI:  $-6.47$  to  $-0.8$ ,  $P=0.0106$ ) and bloating reduced by 67.7% ( $-4.09$  days, 95% CI:  $-7.57$  to  $-0.61$ ,  $P=0.0212$ ). Supplementation with P2 had little impact on any GI event and for P3 there was a 58.3% reduction in stomach rumbling compared to the placebo ( $-2.69$  days, 95% CI:  $-5.47$  to  $0.09$ ,  $P=0.0582$ ).

**Table 2. Demographic and baseline characteristics of the study population.<sup>1,2</sup>**

	Placebo (n=23)	P1 (n=24)	P2 (n=24)	P3 (n=25)
Female/male (n (%))	19/4 (82.6/17.4)	15/9 (62.5/37.5)	15/9 (62.5/37.5)	18/7 (72.0/28.0)
Age (years)	42.74 (14.52)	45.58 (13.93)	49.79 (7.71)	44.56 (13.58)
Ethnicity (n (%))				
Hispanic or Latino	1 (4.3)	1 (4.2)	4 (16.7)	0 (0.0)
Western European White	19 (82.6)	13 (54.2)	13 (54.2)	19 (76.0)
Eastern European White	2 (8.7)	4 (16.7)	6 (25.0)	2 (8.0)
Black or African American	0 (0.0)	0 (0.0)	0 (0.0)	2 (8.0)
Asian	0 (0.0)	1 (4.2)	0 (0.0)	1 (4.0)
Middle Eastern	0 (0.0)	2 (8.3)	1 (4.2)	1 (4.0)
South American	1 (4.3)	3 (12.5)	0 (0.0)	0 (0.0)
Physiology				
BMI (kg/m <sup>2</sup> )	24.34 (2.91)	25.12 (2.36)	24.98 (3.18)	24.36 (3.10)
SBP (mmHg)	114.67 (11.11)	116.15 (9.67)	119.10 (9.05)	115.98 (13.51)
DBP (mmHg)	71.67 (8.07)	71.58 (6.94)	74.42 (6.83)	72.72 (9.57)
Heart rate (bpm)	72.02 (9.41)	67.56 (7.99)	69.40 (9.58)	70.32 (11.18)
Body temperature (°C)	36.42 (0.36)	36.45 (0.31)	36.34 (0.30)	36.37 (0.31)
Haematology				
Total WBC (×10 <sup>9</sup> /l)	5.45 (1.32)	5.36 (1.57)	5.68 (1.67)	5.05 (0.99)
Haemoglobin (g/l)	138.43 (11.37)	140.38 (11.24)	139.29 (11.20)	139.36 (14.31)
Platelets (×10 <sup>9</sup> /l)	252.09 (51.37)	248.63 (46.30)	252.88 (55.00)	243.80 (62.41)
Serum biochemistry				
Total cholesterol (mmol/l)	5.09 (1.00)	5.16 (1.24)	5.13 (0.97)	5.32 (1.55)
Triglycerides (mmol/l)	0.97 (0.49)	0.98 (0.53)	1.01 (0.59)	1.03 (0.51)
Glucose (mmol/l)	5.02 (0.38)	5.00 (0.32)	5.04 (0.41)	4.91 (0.42)
ALT (U/l)	19.13 (5.99)	22.83 (10.64)	19.50 (7.96)	19.08 (9.69)
AST (U/l)	19.00 (3.97)	21.92 (7.74)	18.83 (4.45)	21.20 (5.50)
Creatinine (mmol/l)	73.70 (9.21)	76.75 (13.26)	72.21 (15.89)	76.64 (16.40)

<sup>1</sup> The data represents the mean ± standard deviation of the assigned number(n) participants in each group. The number of male or female participants in each group are expressed as a percentage of the total group size.

<sup>2</sup> BMI = body mass index; bpm = beats per minute; SBP = systolic blood pressure; DBP = diastolic blood pressure; ALT = alanine transaminase; AST = aspartate transaminase; WBC = white blood cells.



**Figure 3. Gastrointestinal (GI) events during the run-in period. (A) Gastrointestinal event incidence presented as days per month with event ± standard deviation (SD) of 96 participants. (B) The proportion of bowel motions with difficulty starting, feeling of incompleteness, slow transit, normal transit or fast transit presented as mean ± SD of 96 participants.**



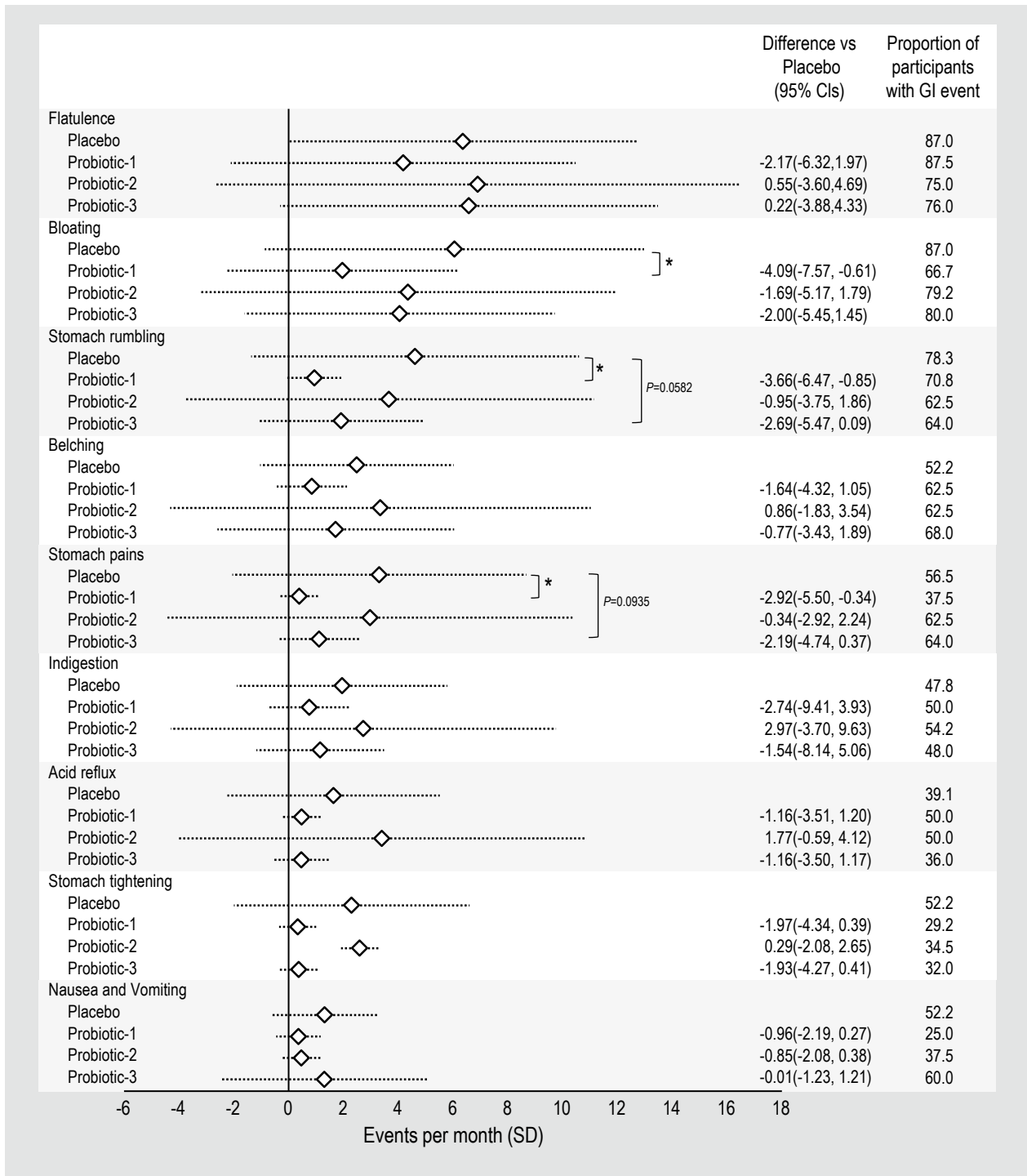
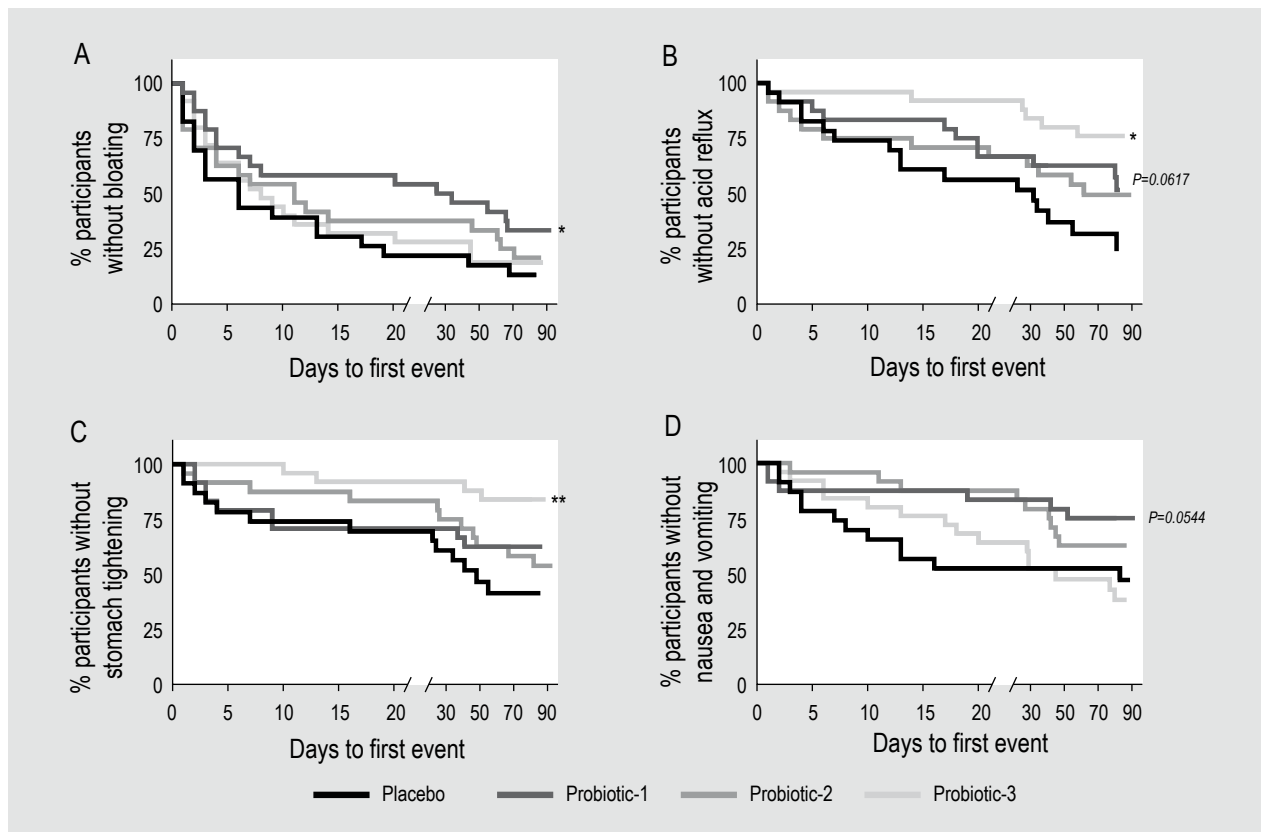


Figure 4. The impact of probiotic supplementation on the incidence of upper gastrointestinal events in healthy adults. The incidence of upper gastrointestinal events in the placebo group versus the P1, P2 and P3 groups presented as days per month with event  $\pm$  standard deviation. *P*-values were calculated using GLM.

Between group differences are shown in Supplementary Table S5 and significantly lower incidences of stomach pain ( $P=0.0267$ ) and acid reflux ( $P=0.0139$ ) were observed in P1 compared to P2 with no significant differences between P1 and P3. The incidence of acid reflux in the P3 group was significantly lower than that of P2 ( $P=0.0129$ ).

### Probiotics can delay the onset of self-reported upper gastrointestinal events

The dynamics of GI event onset during the intervention period were assessed by measuring the number of days to the first reported event (Figure 5). Compared to the



**Figure 5.** The impact of probiotic supplementation on the onset of upper gastrointestinal events in healthy adults. Kaplan-Meier plots of the time to the first report of (A) bloating, (B) acid reflux, (C) stomach tightening and (D) nausea and vomiting during the intervention period. *P*-values compared to the placebo were calculated using the log-rank Mantel-Cox test where \*  $P < 0.05$  or as stated.

placebo group there was a significant delay in the first report of bloating ( $\chi^2=3.98$ ,  $P=0.0461$ , Figure 5A) in the P1 group along with delays in acid reflux ( $\chi^2=3.49$ ,  $P=0.0617$ , Figure 5B) and nausea and vomiting ( $\chi^2=3.70$ ,  $P=0.0544$ , Figure 5D). For P3, the first reports of acid reflux ( $\chi^2=12.41$ ,  $P=0.0004$ , Figure 5B) and stomach tightening ( $\chi^2=9.88$ ,  $P=0.0017$ , Figure 5C) were delayed compared to the placebo. It is worthy of note that differences in timings to first event between P1 and the placebo (Figure 5A) and P3 and the placebo (Figures 5B and 5C) were observable even within the first week of supplementation. Time to first event plots for all other GI events are shown in Supplementary Figure S4 and no statistically significant changes were observed.

### Probiotic supplementation can modulate bowel habits

The bowel habits of the participants during the intervention period are shown in Table 3. The mean number of defecations per participant per day was  $1.14 \pm 0.32$  in the placebo group,  $1.40 \pm 0.62$  in P1,  $1.42 \pm 0.67$  in P2 and  $1.27 \pm 0.47$  in P3. There were no significant differences among the groups for mean BSS. Faecal moisture content increased in P1 ( $2.73 \pm 7.63\%$ ) and P2 ( $1.09 \pm 6.59\%$ ) and decreased in the placebo ( $-1.5 \pm 8.61\%$ ) and P3 ( $-0.74 \pm 8.68\%$ ). Faecal

transit rates (based on BSS) for P1 showed less defecations with slow transit time compared to the placebo ( $-9.15\%$ , 95% CI:  $-18.50$  to  $0.20$ ,  $P=0.0551$ ) and the difficulty starting was lower in P1 ( $8.06 \pm 11.0\%$ ) and P2 ( $10.33 \pm 21.52\%$ ) than for the placebo ( $12.01 \pm 11.21\%$ ) and P3 ( $13.85 \pm 17.90\%$ ). For the feeling of incompleteness, compared to the placebo P1 trended towards a significant reduction ( $-10.94\%$ , 95% CI:  $-22.45$  to  $0.58$ ,  $P=0.0624$ ). Water consumption rates on the day of defecation were comparable for all groups and laxatives, enemas, or suppository use and links with menses were low for all groups (data not shown).

### Probiotic supplementation may impart anti-constipation effects

In the placebo group during the intervention period, 21.7% (Table 3) participants were constipated (consistent with 21.9% reported for the total population during run-in) compared to 8.3% in P1, 12.5% in P2 and 16% in the P3 group.



**Table 3. The impact of probiotic supplementation on bowel habits.<sup>1,2</sup>**

	Placebo (n=23)	P1 (n=24)	P2 (n=24)	P3 (n=25)
Number of defecations per day (mean (SD))	1.14 (0.32)	1.40 (0.62)	1.42 (0.67)	1.27 (0.47)
Bristol stool score (mean (SD))	3.59 (0.15)	3.92 (0.11)	3.64 (0.14)	3.72 (0.12)
Change in faecal moisture content, V4 vs V2 (% (SD))	-1.50 (8.61)	2.73 (7.63)	1.09 (6.59)	-0.74 (8.68)
Proportion of defecations (% (SD)) with:				
slow faecal transit time	17.34 (16.49)	8.19 (12.56) <sup>#</sup>	11.60 (19.17)	14.76 (15.68)
normal faecal transit time	75.01 (18.38)	83.32 (14.55)	83.62 (19.10)	76.92 (16.00)
fast faecal transit time	7.64 (14.22)	8.49 (8.24)	4.79 (6.59)	8.32 (10.95)
difficulty starting	12.01 (11.21)	8.06 (11.06)	10.33 (21.52)	13.85 (17.90)
difficulty stopping	0.06 (0.16)	0.04 (0.10)	0.03 (0.09)	0.03 (0.03)
the feeling of incomplete evacuation	18.15 (21.95)	7.22 (14.95) <sup>##</sup>	13.03 (21.01)	14.51 (20.85)
Participants with constipation (%)	21.7	8.3	12.5	16

<sup>1</sup> P-values were calculated using a GLM and are stated versus the placebo: <sup>#</sup>  $P=0.0551$ ; <sup>##</sup>  $P=0.0624$ .

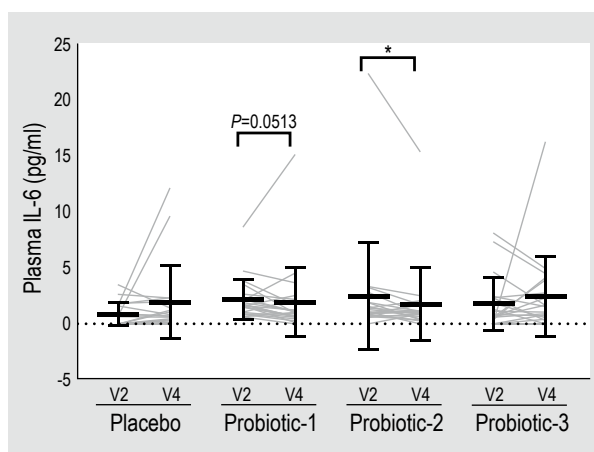
<sup>2</sup> SD = standard deviation.

### Daily supplementation with probiotics can reduce plasma interleukin-6 concentrations

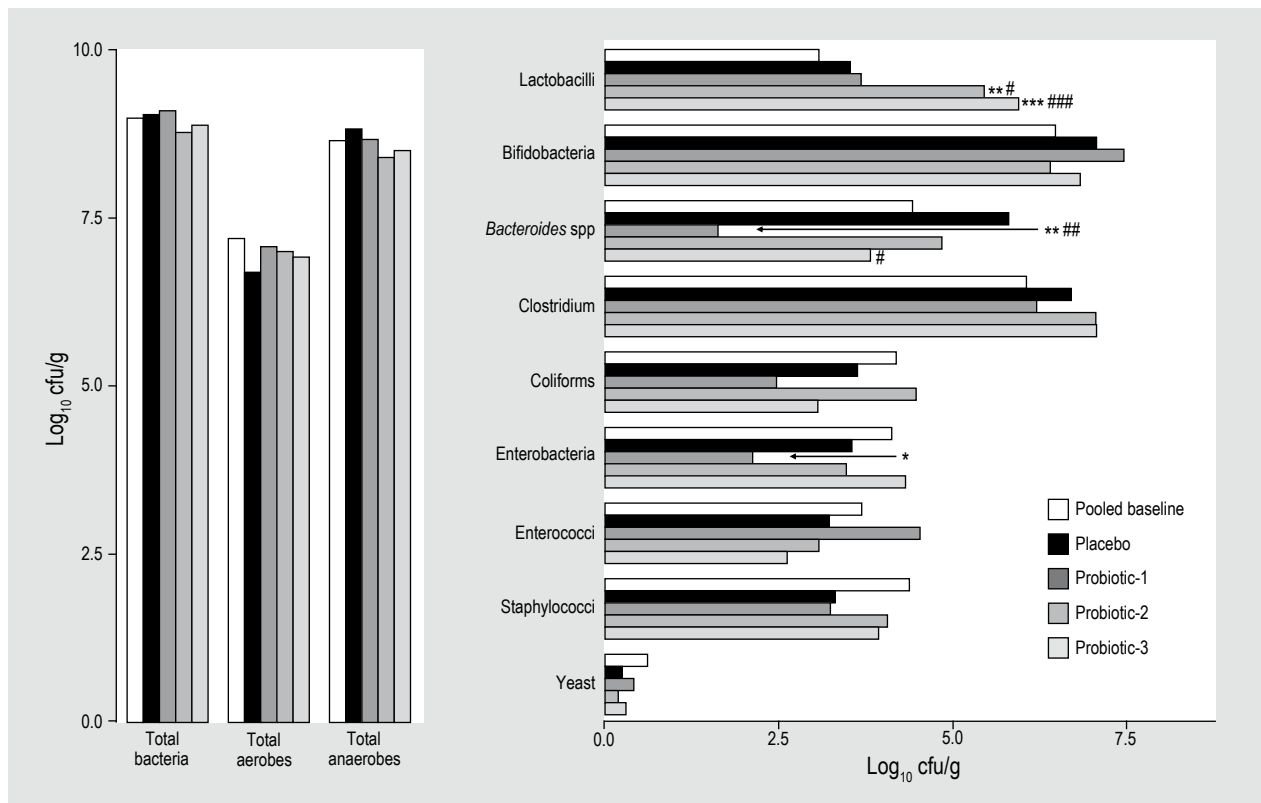
No between-group differences in plasma concentration of IL-6 were detected (Figure 6) but, compared to baseline, levels were significantly reduced in the P2 group (41.2%,  $P=0.0473$ ), trended towards a significant reduction in P1 (12.6%,  $P=0.0513$ ) and were unchanged in the placebo and P3.

### The impact of supplementation on the composition of the faecal microbiota

Faecal samples were collected at baseline (V2, n=82) and endpoint (V4, n=85) and a 'Pooled baseline' was generated by combining the V2 data from all participants to provide a robust representation of the pre-intervention microbiota (Figure 7). At the end of intervention, between group microbial population differences were detected for P1 where the *Bacteroides* spp. numbers were significantly lower ( $P=0.0012$ ) than for the placebo group. For the P2 group, the *Lactobacillus* spp. numbers were significantly higher ( $P=0.0139$ ) than those in the placebo and for P3, the *Bacteroides* spp. numbers were significantly lower ( $P=0.0399$ ) and the *Lactobacillus* spp. numbers were significantly higher ( $P>0.0001$ ) than those in the placebo. Changes from baseline for P1 indicated that the numbers of enterobacteria ( $P=0.0259$ ) and *Bacteroides* spp. ( $P=0.0055$ ) decreased significantly whilst for groups P2 and P3, significant increases in the numbers of lactobacilli were observed ( $P=0.0012$  and  $P>0.0001$ , respectively).



**Figure 6. Changes in plasma interleukin-6 (IL-6) over the duration of the intervention period. The data is presented as individual values at baseline (V2) and the study endpoint (V4) overlaid with the group mean  $\pm$  standard deviation. P-values were calculated using the Wilcoxon matched pairs signed ranked test comparing V2 vs V4 in the same group or the Kruskal-Wallis test with Dunn's *post hoc* analysis comparing variations between groups where \*  $P<0.05$  or as stated. Participant numbers in the Placebo, P1, P2 and P3 groups were 20, 23, 23 and 21, respectively.**



**Figure 7.** The composition of the faecal microbiota. Viable numbers of common gut organisms and total bacteria in faeces samples taken at the start (pooled baseline) and the endpoint of the study. Data is presented as the mean cfu/g of dry weight faeces. *P*-values were calculated using one-way ANOVA with Tukey's *post hoc* test where \* *P*<0.05, \*\* *P*<0.01 and \*\*\* *P*<0.001 compared to the Pooled baseline or # *P*<0.05, ## *P*<0.01 and ### *P*<0.001 compared to the Placebo. Participant numbers were 82 in the pooled baseline and 20, 22, 22 and 21 in the Placebo, Probiotic-1, Probiotic-2 and Probiotic-3 groups, respectively.

#### 4. Discussion

As part of a more extensive safety study, the GI status and bowel habits of a free-living healthy adult population were monitored daily using participant completed questionnaires. A *post hoc* analysis of the incidence of gastrointestinal events indicated a positive response to probiotic supplementation. An extensive screening protocol ensured the healthy status of the 96 subjects entering the study and during the pre-randomisation run-in period, participants reported an average of 27 gastrointestinal events per month that were dominated by flatulence, bloating (distension) and stomach rumbling (borborygmus). Participants also reported high proportions of defecations occurring with difficulty starting, with a feeling of incompleteness and with slow faecal transit times. Nearly 22% of the study population conformed with the Rome IV definition for functional constipation. GI discomfort and constipation are associated with significant impairments of day-to-day quality-of-life (Bovenschen *et al.*, 2004; Tielemans *et al.*, 2013) and probiotic supplementation is gaining recognition as a strategy to improve gastrointestinal health (Sanders *et al.*, 2019).

At the end of the 3-month intervention period, the incidence rate of GI events for the placebo group (~30 events/month) was comparable to that recorded in the pre-intervention run-in period (~27 events/month) and each of the probiotic consortia reduced the incidence of these events. In the P1 group there was significantly decreased incidences of stomach pain, stomach rumbling and bloating and the onset of bloating was significantly delayed compared to the placebo group with an indication of a fairly rapid response to the supplementation (within one week). These findings agree with the outcomes of a study by Del Piano and colleagues showing reduced bloating in healthy adults receiving probiotics (Del Piano *et al.*, 2010). The P1 formulation has also been shown to significantly reduce stomach pain amongst irritable bowel syndrome (IBS) sufferers (Williams *et al.*, 2009). For P3, there were significant delays in the reporting of acid reflux and bloating and improvements in GI event incidence that highlight differences in response to different probiotic consortia. The P3 consortium has also been shown to improve GI symptomatology and severity during IBS trials (Bonfrate *et al.*, 2020; Pedersen *et al.*, 2014). The P2 consortium comprised the organisms in the P1 formulation but with the inclusion

of 13 additional organisms (17 in total). It is interesting that this more complex formulation appeared to be less effective than the simpler P1 consortium. These differing responses might reflect a dilution of the activity of the more effective strains and/or antagonism among the complex of different organisms (McFarland, 2021; Ouwehand *et al.*, 2018).

The P1 group moved towards more 'normal' bowel habits (reduced proportion of slow transit defecations /increased defecation rates/reductions in defecations with difficulty starting or incompleteness) without any changes in water consumption or laxative usage and a similar but less extensive response was observed for P2. Data collected during the run-in period suggested that 22% of the total population met the Rome IV criteria for the diagnosis of functional constipation (FC). During the study, the constipated proportion of the placebo group was unchanged but the proportions for P1 dropped to 8.3% with rates of 12.5 and 16% for P2 and P3 respectively. P1 has been shown to improve bowel movement satisfaction in IBS sufferers (Williams *et al.*, 2009) and probiotics have been shown to improve defecation frequency and stool form in healthy adults (Del Piano *et al.*, 2010; Higashikawa *et al.*, 2010; Sakai *et al.*, 2011).

Probiotic-mediated improvements in gastrointestinal health are strongly linked with alleviation of inflammation (Cristofori *et al.*, 2021) and IL-6 is considered to be a pro-inflammatory cytokine (Guo *et al.*, 2021). Neyrinck *et al.* (2021) observed reductions in circulating IL-6 levels alongside improvements in gastrointestinal symptoms in constipated adults receiving a synbiotic formulation (Neyrinck *et al.*, 2021). Plasma levels of IL-6 were reduced from baseline in the P1 and P2 groups (the groups with fewest constipated participants), but not in the placebo or P3 groups which could be considered to represent an anti-inflammatory response. *In vitro* studies with lipopolysaccharide challenged peripheral blood mononuclear cells (PBMC) extracted from healthy adults receiving the P1 consortium showed reduced IL-6 production (Hepburn *et al.*, 2013) and P1 supplemented Wistar rats had lower circulating IL-6 levels than the control population (Webberley *et al.*, 2021). *Lactocaseibacillus paracasei* CUL08 (present in P2) has been shown to impair IL-6 secretion by lipopolysaccharide challenged human PBMC (Sun *et al.*, 2017). Numerous strains of lactobacilli and bifidobacteria have been shown to impact IL-6, particularly in murine models of colitis (Cristofori *et al.*, 2021).

Although the intake of probiotics has been linked with improvements in the prognosis and management of gastrointestinal disorders and may be linked with modulation of the gut microbiota (Kim *et al.*, 2019) there has been uncertainty whether this has any impact upon the microbiota of healthy populations (Kristensen *et al.*, 2016).

We enumerated the viable faecal populations and observed significant decreases in the numbers of *Bacteroides* spp. and enterobacteria in the P1 group, increased numbers of lactobacilli with P2 and increased lactobacilli but decreased *Bacteroides* spp. numbers in the P3 group. Our findings suggest that probiotics impact upon the healthy microbiota but that the response appears to be related to the composition of the probiotic consortium.

The study has a number of strengths and limitations that require consideration. A clear strength of the study includes the use of healthy, free-living adults to compare 3 different probiotic consortia at equivalent dosage levels within the framework of a single study. The detection of probiotic-mediated changes to the viable bacterial numbers in the study population provides strong support for additional metagenomic analysis. Limitations are the exploratory, unpowered nature of the study along with the female dominated cohort.

In summary, *post hoc* analysis of the data from the safety study focusing on the impact of probiotic supplementation on the incidence of GI events and bowel habits identified the potential for probiotic consortia to impact on GI status. The impacts vary according to the probiotic used, and there were indications of reduced systemic inflammation and modulations in the composition of the gut microbiota.

## Supplementary material

Supplementary material can be found online at <https://doi.org/10.3920/bm2022.0092>.

**Table S1.** Prohibited concomitant medications and supplements.

**Table S2.** Study inclusion and exclusion criteria.

**Table S3.** Rome IV criteria for the diagnosis of functional constipation.

**Table S4.** Selective microbial culture conditions.

**Table S5.** Changes in incidence of gastrointestinal events over the duration of the study.

**Figure S1.** Daily gastrointestinal symptoms rating scale.

**Figure S2.** Bowel habits diary.

**Figure S3.** Gastrointestinal event incidence in constipated and non-constipated participants during the run-in period.

**Figure S4.** The impact of probiotic supplementation on the onset of upper gastrointestinal events in healthy adults.

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## Conflicts of interest

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## References

- Avramidou, M., Angst, F., Angst, J., Aeschlimann, A., Rossler, W. and Schnyder, U., 2018. Epidemiology of gastrointestinal symptoms in young and middle-aged Swiss adults: prevalences and comorbidities in a longitudinal population cohort over 28 years. *BMC Gastroenterology* 18: 21. <https://doi.org/10.1186/s12876-018-0749-3>
- Azpiroz, F., Guyonnet, D., Donazzolo, Y., Gendre, D., Tanguy, J. and Guarner, F., 2015. Digestive symptoms in healthy people and subjects with irritable bowel syndrome: validation of symptom frequency questionnaire. *Journal of Clinical Gastroenterology* 49: e64-70. <https://doi.org/10.1097/mcg.000000000000178>
- Bonfrate, L., Di Palo, D.M., Celano, G., Albert, A., Vitellio, P., De Angelis, M., Gobetti, M. and Portincasa, P., 2020. Effects of *Bifidobacterium longum* BB536 and *Lactobacillus rhamnosus* HN001 in IBS patients. *European Journal of Clinical Nutrition* 50: e13201. <https://doi.org/10.1111/eci.13201>
- Bovenschen, H.J., Laheij, R.J., Tan, A.C., Witteman, E.M., Rossum, L.G. and Jansen, J.B., 2004. Health-related quality of life of patients with gastrointestinal symptoms. *Alimentary Pharmacology and Therapeutics* 20: 311-319. <https://doi.org/10.1111/j.1365-2036.2004.02076.x>
- Cristofori, F., Dargenio, V.N., Dargenio, C., Miniello, V.L., Barone, M. and Francavilla, R., 2021. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. *Frontiers in Immunology* 12: 578386. <https://doi.org/10.3389/fimmu.2021.578386>
- Del Piano, M., Carmagnola, S., Anderloni, A., Andorno, S., Ballarè, M., Balzarini, M., Montino, E., Orsello, M., Pagliarulo, M., Sartori, M., Tari, R., Sforza, F. and Capurso, L., 2010. The use of probiotics in healthy volunteers with evacuation disorders and hard stools: a double-blind, randomized, placebo-controlled study. *Journal of Clinical Gastroenterology* 44 Suppl. 1: S30-34. <https://doi.org/10.1097/MCG.0b013e3181ee31c3>
- Dimidi, E., Mark Scott, S. and Whelan, K., 2020. Probiotics and constipation: mechanisms of action, evidence for effectiveness and utilisation by patients and healthcare professionals. *Proceedings of the Nutrition Society* 79: 147-157. <https://doi.org/10.1017/S0029665119000934>
- Dronkers, T.M.G., Ouwehand, A.C. and Rijkers, G.T., 2020. Global analysis of clinical trials with probiotics. *Heliyon* 6: e04467. <https://doi.org/10.1016/j.heliyon.2020.e04467>
- Food and Agriculture Organization of the United Nations/World Health Organization (FAO/WHO), 2006. Probiotics in food. Health and nutritional properties and guidelines for evaluation. FAO Food and Nutritional paper no. 85. FAO, Rome, Italy. Available at: <https://www.fao.org/3/a0512e/a0512e.pdf>.
- Fassarella, M., Blaak, E.E., Penders, J., Nauta, A., Smidt, H. and Zoetendal, E.G., 2021. Gut microbiome stability and resilience: elucidating the response to perturbations in order to modulate gut health. *Gut* 70: 595-605. <https://doi.org/10.1136/gutjnl-2020-321747>
- Guo, Y., Wang, B., Wang, T., Gao, L., Yang, Z.J., Wang, F.F., Shang, H.W., Hua, R. and Xu, J.D., 2021. Biological characteristics of IL-6 and related intestinal diseases. *International Journal of Biological Sciences* 17: 204-219. <https://doi.org/10.7150/ijbs.51362>
- Hepburn, N.J., Garaiova, I., Williams, E.A., Michael, D.R. and Plummer, S., 2013. Probiotic supplement consumption alters cytokine production from peripheral blood mononuclear cells: a preliminary study using healthy individuals. *Beneficial Microbes* 4: 313-317. <https://doi.org/10.3920/BM2013.0012>
- Higashikawa, F., Noda, M., Awaya, T., Nomura, K., Oku, H. and Sugiyama, M., 2010. Improvement of constipation and liver function by plant-derived lactic acid bacteria: a double-blind, randomized trial. *Nutrition* 26: 367-374. <https://doi.org/10.1016/j.nut.2009.05.008>
- Khalesi, S., Bellissimo, N., Vandelanotte, C., Williams, S., Stanley, D. and Irwin, C., 2019. A review of probiotic supplementation in healthy adults: helpful or hype? *European Journal of Clinical Nutrition* 73: 24-37. <https://doi.org/10.1038/s41430-018-0135-9>
- Kim, S.K., Guevarra, R.B., Kim, Y.T., Kwon, J., Kim, H., Cho, J.H., Kim, H.B. and Lee, J.H., 2019. Role of probiotics in human gut microbiome-associated diseases. *Journal of Microbiology and Biotechnology* 29: 1335-1340. <https://doi.org/10.4014/jmb.1906.06064>
- Kristensen, N.B., Bryrup, T., Allin, K.H., Nielsen, T., Hansen, T.H. and Pedersen, O., 2016. Alterations in fecal microbiota composition by probiotic supplementation in healthy adults: a systematic review of randomized controlled trials. *Genome Medicine* 8: 52. <https://doi.org/10.1186/s13073-016-0300-5>
- Laurikka, P., Salmi, T., Collin, P., Huhtala, H., Maki, M., Kaukinen, K. and Kurppa, K., 2016. Gastrointestinal symptoms in celiac disease patients on a long-term gluten-free diet. *Nutrients* 8: 429. <https://doi.org/10.3390/nu8070429>
- Lewis, S.J. and Heaton, K.W., 1997. Stool form scale as a useful guide to intestinal transit time. *Scandinavian Journal of Gastroenterology* 32: 920-924. <https://doi.org/10.3109/00365529709011203>



- McBurney, M.I., Davis, C., Fraser, C.M., Schneeman, B.O., Huttenhower, C., Verbeke, K., Walter, J. and Latulippe, M.E., 2019. Establishing what constitutes a healthy human gut microbiome: state of the science, regulatory considerations, and future directions. *Journal of Nutrition* 149: 1882-1895. <https://doi.org/10.1093/jn/nxz154>
- McFarland, L.V., 2021. Efficacy of single-strain probiotics versus multi-strain mixtures: systematic review of strain and disease specificity. *Digestive Diseases and Sciences* 66: 694-704. <https://doi.org/10.1007/s10620-020-06244-z>
- Mitelmão, F.C.R., De Cassia Bergamaschi, C., Gerenutti, M., Hächel, K., Silva, M.T., Balcão, V.M. and Vila, M.M.D.C., 2021. The effect of probiotics on functional constipation in adults. *Medicine* 100: e24938. <https://doi.org/10.1097/md.00000000000024938>
- Neyrinck, A.M., Rodriguez, J., Taminiau, B., Amadiou, C., Herpin, F., Allaert, F.A., Cani, P.D., Daube, G., Bindels, L.B. and Delzenne, N.M., 2021. Improvement of gastrointestinal discomfort and inflammatory status by a synbiotic in middle-aged adults: a double-blind randomized placebo-controlled trial. *Scientific Reports* 11: 2627. <https://doi.org/10.1038/s41598-020-80947-1>
- Ouwehand, A.C., Invernici, M.M., Furlaneto, F.A.C. and Messori, M.R., 2018. Effectiveness of multistrain versus single-strain probiotics: current status and recommendations for the future. *Journal of Clinical Gastroenterology* 52 Suppl. 1: S35-S40. <https://doi.org/10.1097/MCG.0000000000001052>
- Pedersen, N., Andersen, N.N., Végh, Z., Jensen, L., Ankersen, D.V., Felding, M., Simonsen, M.H., Burisch, J. and Munkholm, P., 2014. Ehealth: low FODMAP diet vs *Lactobacillus rhamnosus* GG in irritable bowel syndrome. *World Journal of Gastroenterology* 20: 16215-16226. <https://doi.org/10.3748/wjg.v20.i43.16215>
- Sakai, T., Makino, H., Ishikawa, E., Oishi, K. and Kushiro, A., 2011. Fermented milk containing *Lactobacillus casei* strain Shirota reduces incidence of hard or lumpy stools in healthy population. *International Journal of Food Sciences and Nutrition* 62: 423-430. <https://doi.org/10.3109/09637486.2010.542408>
- Sanders, M.E., Merenstein, D.J., Reid, G., Gibson, G.R. and Rastall, R.A., 2019. Probiotics and prebiotics in intestinal health and disease: from biology to the clinic. *Nature Reviews Gastroenterology and Hepatology* 16: 605-616. <https://doi.org/10.1038/s41575-019-0173-3>
- Sezgin, O., Akpinar, H., Ozer, B., Toruner, M., Bal, K. and Bor, S., 2019. Population-based assessment of gastrointestinal symptoms and diseases: Cappadocia Cohort, Turkey. *Turkish Journal of Gastroenterology* 30: 1009-1020. <https://doi.org/10.5152/tjg.2019.19882>
- Sharon, I., Quijada, N.M., Pasolli, E., Fabbri, M., Vitali, F., Agamennone, V., Dotsch, A., Selberherr, E., Grau, J.H., Meixner, M., Liere, K., Ercolini, D., De Filippo, C., Caderni, G., Brigidi, P. and Turroni, S., 2022. The core human microbiome: does it exist and how can we find it? A critical review of the concept. *Nutrients* 14: 2872. <https://doi.org/10.3390/nu14142872>
- Sun, K.-Y., Xu, D.-H., Xie, C., Plummer, S., Tang, J., Yang, X.F. and Ji, X.H., 2017. *Lactobacillus paracasei* modulates LPS-induced inflammatory cytokine release by monocyte-macrophages via the up-regulation of negative regulators of NF-kappaB signaling in a TLR2-dependent manner. *Cytokine* 92: 1-11.
- Svedlund, J., Sjödin, I. and Dotevall, G., 1988. GSRS – a clinical rating scale for gastrointestinal symptoms in patients with irritable bowel syndrome and peptic ulcer disease. *Digestive Diseases and Sciences* 33: 129-134. <https://doi.org/10.1007/bf01535722>
- Tielemans, M.M., Jaspers Focks, J., van Rossum, L.G.M., Eikendal, T., Jansen, J.B.M.J., Laheij, R.J.F. and van Oijen, M.G.H., 2013. Gastrointestinal symptoms are still prevalent and negatively impact health-related quality of life: a large cross-sectional population based study in the Netherlands. *PLoS ONE* 8: e69876. <https://doi.org/10.1371/journal.pone.0069876>
- Vivier, H., Ross, E.J. and Cassisi, J.E., 2020. Classification of gastrointestinal symptom patterns in young adults. *BMC Gastroenterology* 20: 326. <https://doi.org/10.1186/s12876-020-01478-7>
- Webberley, T.S., Masetti, G., Baker, L.M., Dally, J., Hughes, T.R., Marchesi, J.R., Jack, A.A., Plummer, S.F., Ramanathan, G., Facey, P.D. and Michael, D.R., 2021. The impact of Lab4 probiotic supplementation in a 90-day study in Wistar rats. *Frontiers in Nutrition* 8: 778289. <https://doi.org/10.3389/fnut.2021.778289>
- Williams, E.A., Stimpson, J., Wang, D., Plummer, S., Garaiova, I., Barker, M.E. and Corfe, B.M., 2009. Clinical trial: a multistrain probiotic preparation significantly reduces symptoms of irritable bowel syndrome in a double-blind placebo-controlled study. *Alimentary Pharmacology and Therapeutics* 29: 97-103.
- Yilmaz-Ersan, L., Ozcan, T. and Akpinar-Bayazit, A., 2020. Assessment of socio-demographic factors, health status and the knowledge on probiotic dairy products. *Food Science and Human Wellness* 9: 272-279. <https://doi.org/10.1016/j.fshw.2020.05.004>
- Zhang, T., Zhang, C., Zhang, J., Sun, F. and Duan, L., 2022. Efficacy of probiotics for irritable bowel syndrome: a systematic review and network meta-analysis. *Frontiers in Cellular and Infection Microbiology* 12: 859967. <https://doi.org/10.3389/fcimb.2022.859967>