ORIGINAL RESEARCH

A Randomized, Double-blind, Placebo-controlled, Parallel Study Evaluating the Efficacy of Bacillus subtilis MB40 to Reduce Abdominal Discomfort, Gas, and Bloating

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ABSTRACT

Introduction • Bloating is a common yet poorly managed complaint among healthy people, with a complex etiology that impacts health and general well-being. The study intended to evaluate the efficacy and safety of supplementation with a probiotic, Bacillus subtilis MB40 (MB40), on bloating, abdominal discomfort, and gas in healthy participants.

Methods • In this multi-center, double-blind, placebo-controlled, parallel trial, 100 participants were randomized to receive either MB40 at 5×10^9 colony forming units (CFU; n = 50) or a placebo (n = 50) once daily for 4-weeks. Participants completed 3 questionnaires daily: a modified Abdominal Discomfort, Gas, and Bloating (mADGB) questionnaire, a modified Gastrointestinal Symptoms Rating Scale (mGSRS), and a Bowel Habits Diary (BHD). Participants' responses to each question were combined into weekly averages.

Results • At the end of 4-weeks, there were no significant differences in average weekly change in daily bloating intensity, number of days with and duration of bloating, abdominal discomfort and gas between MB40 and placebo groups. However, the male sub-group on MB40 achieved clinical thresholds with a greater decrease over placebo in the intensity of (1.38) and number of days with (1.32) bloating, the number of days (1.06) and duration (86-minutes) of gas, the number of days with abdominal discomfort (1.32) and diarrhea symptom score (1.02). Role limitation (physical; $P = .026$), vitality ($P = .034$) and social functioning ($P = .037$) were significantly improved from baseline to week 4 in the MB40 group. At 2-weeks, physical functioning ($P = .017$) significantly improved in the MB40 group versus placebo.

Conclusions • Although MB40 supplementation did not significantly improve bloating across all populations, the male sub-group demonstrated clinically significant reductions in bloating intensity, number of days with abdominal discomfort, gas, bloating, and duration of gas, compared to placebo. Additionally, the male sub-group receiving MB40 had a 10% improvement in general health score. MB40 supplementation at a dose of 5×10^9 CFU daily for 4-weeks was also safe and well-tolerated as all biometric, vital, and hematological measures remained within normal laboratory ranges (Clinical Trials NCT02950012). (*Altern Ther Health Med.* 2021;27(S1):146-157).

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INTRODUCTION

Bloating is a subjective abdominal discomfort that is associated with abdominal inflation due to the accumulation of excessive gas (flatulence); and may not necessarily be accompanied by abdominal distension.[1] Unexplained bloating symptoms are very commonly experienced by healthy persons (20-30%) across different populations[1]; and are more frequent in females.[2] Bloating symptoms are generally subjective in nature and therefore assigning an objective score is fraught with inter- and intra-subject variations. The severity of bloating-related abdominal discomfort experienced by individuals can range from mild to severe[3] and the discomfort can negatively impact health and general well-being and is reported to increase the

frequency of missed work and leisure day.[4,5] Bloating symptoms tend to worsen postprandially, but no association with the size of the consumed meal has been found.[6,7]

The pathophysiology and mechanisms underlying bloating are poorly understood. Several studies have shown that bloating is linked with an altered gut microbiota that lead to abnormal bacterial fermentation of undigested food.[8,9] Since the symptoms associated with and the underlying mechanism of bloating are elusive and multi-faceted in nature, it remains a very common yet difficult to manage problem and current therapeutic strategies such as antibiotics and anti-depressants tend to be inconsistently successful or potentially contraindicated for long-term use.[9-13]

Probiotics are live microorganisms, which when ingested in appropriate numbers may restore the gut microflora and help alleviate GI problems.[14,15] Although exact mechanisms by which probiotics confer these health benefits have not been completely elucidated, different groups have proposed that probiotics may displace gas-producing bacterial species, alter the inflammatory cytokine profiles and produce antimicrobial compounds that suppress the growth of harmful bacteria hence modulating the health of the GI tract 16. Few research groups have demonstrated the beneficial effects of lactobacilli or bifidobacterial alone or in combination to alleviate GI tract problems, especially irritable bowel syndrome (IBS).[17] However relatively few studies defining the efficacy of specific types of probiotics in relieving bloating problems have been published.[14] Since the effects of probiotics are known to be strain specific, and clinical studies are needed to identify not only beneficial strains, but also the magnitude of their effect on controlling bloating problems.

Certain species of Bacillus (e.g. *B. subtilis*) that are present in baked and fermented food products have a long and safe history of use in humans[18-21] and are safe up to 10×10^9 CFU per day.[22] One previous study has demonstrated the effectiveness of *B. subtilis* in controlling intestinal hydrogen production[23] and supplementation is reported to promote regularity of bowel movements.[24] *B. subtilis* produces spores that remains viable in a wide temperature and pH range making it an easy supplement for improving gut health. A recent clinical study found that a formulation containing *B. subtilis* in combination with *Streptococcus faecium* could significantly reduce the frequency and severity of abdominal bloating pain.[25] However, well designed randomized placebo-controlled studies demonstrating its efficacy in healthy populations are lacking.

Restoration of abdominal gases to normal levels with a reliable change in symptoms are primary indexes of clinical significance. Interestingly, previous research has defined a specified clinical difference threshold of '1' to be acceptable for comparison of between-group significance when using questionnaires designed with a rating scale.[22] Most bloating related studies use questionnaires for the assessment of study-related outcomes and lend themselves to the measurement of clinical significance. Other studies have previously reported clinically significant reductions in bloating.[26,27] The current study investigated the efficacy and safety of *B. subtilis* MB40 (MB40) on abdominal bloating in a healthy population over a 4-week supplementation period and examined the clinical improvements associated with the supplementation.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board Services, Aurora, ON, Canada on September 27, 2016 and reviewed by the Therapeutic Products Directorate (TPD) and the Natural and Non-prescription Health Products Directorate (NHPD) of Canada where approvals were obtained on November 08, 2016. This study was conducted in accordance with the ethical principles in Declaration of Helsinki and its subsequent amendments. The study was adequately explained and voluntary written and informed consent to participate in the study was obtained prior to any study related procedures.

Participants

Healthy adults, 18-75 years of age, were recruited from the regions of Southwestern ON, Canada, Irvine, CA, USA and Orlando, FL, USA using local electronic and physical advertisements from within and outside of KGK Science's internal participant database. Inclusion criteria were: average daily bloating score of ≥5 and/or bloating for >7 days during the run-in period prior to the start of supplementation; body mass index (BMI) between 17.5 and 30.9 kg/m^2 (inclusive); healthy determined by physical exam, clinical chemistry, liver and kidney functions tests as assessed by the medical director; non-consumption of probiotics for 1-week and fiber for 2-weeks; maintenance of current diet and exercise patterns. Exclusion criteria: pregnant and breast feeding females, history of chronic inflammation; structural abnormality of the digestive tract; gastrointestinal disorders or structural abnormalities of the digestive tract such as inflammatory bowel disease (IBD), duodenal or gastric ulcers, intestinal ulcers, intestinal obstruction or symptomatic cholelithiasis; nocturnal or progressive abdominal pain; colorectal cancer, anal abscess, anal fistula, anal fissure, anal stenosis, gastric retention or obstruction, bowel resection, rectocele or colostomy; adenomatous polyposis, irritable bowel syndrome (IBS), chronic gastritis, functional dyspepsia, Crohn's disease, celiac disease; gastrointestinal bleeding or acute infection; recent significant weight loss; type I and II diabetes mellitus; chronic gastrointestinal disorders; renal or hepatic insufficiency; immunodeficiency; and any condition which may have adversely affected the volunteer's ability to complete the study or which posed significant risk to the volunteer.

Study design

This was a multi-center, randomized, double-blind, placebo-controlled, 2-arm parallel group study consisting of a 2-week run-in and a 4-week supplementation period. The study was conducted at KGK Science clinical sites located in

London, ON, Canada, Orlando, FL, USA and Irvine, CA, USA between November 2016 and March 2017. During the run-in period, participants were categorized as "high bloaters" as defined by an average bloating score of ≥ 5 on question 3 of the Modified Daily Abdominal, Gas and Bloating (mADGB) Questionnaire or "high frequency bloaters" as defined by greater than 7 dyads of high bloating. Participants also completed a 3-day food record, and questionnaires as outline below during the run-in period. After the run-in period, participants returned for the baseline visit. After confirming eligibility, participants were randomized to receive MB40 or a placebo once daily for 4-weeks. Subsequent clinic visits were scheduled at 2- and 4-weeks to collect outcome and compliance data. Study questionnaires included a modified Abdominal Discomfort, Gas, and Bloating (mADGB questionnaire, a modified Gastrointestinal Symptoms Rating Scale (mGSRS), a Bowel Habits Diary (BHD), and a modified RAND SF-36 (mRAND SF-36). Participants completed the mADGB, the mGSRS, and the BHD daily whereas the mRAND SF-36 questionnaire was administered at baseline, weeks 2 and 4. For the BHD and mADGB questionnaire, average baseline symptom or domain scores for each participant were calculated for the two weeks prior to randomization. Counts were standardized to seven days. For the GSRS questionnaire, average baseline domain scores for each participant were calculated using three pre-randomization scores. For the mRAND SF-36 questionnaire, average baseline domain scores were calculated for the week prior to baseline. Weekly averages were calculated post-baseline for the respective symptom or domain scores for the questionnaires mentioned above. At screening, 3-day food records were dispensed and volunteers were instructed on completing 3-day food records on two weekdays and one weekend day prior to their baseline visit. Volunteers were instructed to maintain their current physical activity levels and reminded to maintain a controlled diet throughout the study and to complete a food diary for 3-days prior to their baseline visit. In addition, nutritional counselling regarding foods to avoid was provided with meal ideas.

Participants recorded any adverse events that occurred between their visits to the clinic in a diary, and bloodwork was collected at the screening and end of study visit. At screening, participants' medical history, concomitant medications, health status, were assessed and urine pregnancy test performed. Inclusion and exclusion criteria were reviewed and participant BMI, resting blood pressure, and heart rate were determined. Abdominal bloating, questionnaire was applied and blood levels of electrolytes (sodium, potassium, chloride ions), HbA_{1c}, creatinine, AST, ALT and bilirubin were determined.

Intervention

The investigational products (OPTI-BIOME® and placebo) were provided by BIO-CAT Microbials LLC Shakopee, MN, USA as identical, white, and oblong capsules where no differences in size, color, texture, or packaging were detectable. The study products were provided in bottles labelled with a treatment code by a KGK staff member who was not involved in conducting the study. Participants were instructed to consume 1 capsule each morning for 4-weeks.

Prior to giving them to participants, the study products were stored in a dry location, not exposed to direct sunlight, and in a lockable room with controlled temperature and humidity. OPTI-BIOME® (Batch No. 1616703) contained the probiotic *B. subtilis* MB40 at 5×10^9 CFU with excipients maltodextrin, magnesium stearate, gelatin, and silicon dioxide. The placebo (Batch No. 1616704) contained only the excipients. Investigational products were labelled according to requirements of the International Conference on Harmonization Good Clinical Practice Guidelines, applicable local regulatory guidelines, and the label included the applicable randomization number.

Outcome Measures

The primary outcome of this study was the change from baseline in the bloating score as assessed by the mADGB questionnaire which consisted of 18 questions grouped equally into 3 symptoms (abdominal discomfort, gas, and bloating) rated on a scale from 0 (none) to 10 (most severe). Secondary outcomes included the differences in the weekly mean of the abdominal gas and discomfort intensity scores, the weekly mean of the daily mGSRS, the weekly mean of the daily BHD, and the change from baseline to end of study in the Short-Form 36 Questions from the RAND Corporation (mRAND SF-36). The mGSRS questionnaire evaluated the severity of five symptoms, diarrhea, constipation, abdominal discomfort, indigestion, and reflux symptom scores, based on 13 questions that utilized a rating scale of 1 (least severe) to 7 (most severe). The BHD assessed 4 stool related measures including the Bristol Stool Scale (BSS) for stool consistency, (2) time of bowel movement (3) straining to start and stop defecation and (4) feeling of incomplete defecation.[27] The Modified RAND SF-36 is a 36-Item Health Survey developed at RAND as part of the Medical Outcomes Study. The modified RAND SF-36 uses one multi-item scale that assesses eight health concepts from the participants point of view: limitations in physical functioning due to health problems, limitations in social activities due to physical or emotional problems, limitations in usual role activities due to physical health problems, bodily pain, general mental health, limitations in usual role activities due to emotional problems, vitality, and general health perceptions. The scores range from 0 to 100 where higher scores indicate a better state of health.

A product questionnaire assessed tolerability the end of study.

Safety outcomes included vital signs (resting heart rate and blood pressure), BMI, complete blood counts, electrolytes, creatinine, aspartate aminotransferase, alanine aminotransferase, total bilirubin, as well as the incidence of any adverse events. Blood samples were analyzed at Life Labs (London, ON, Canada) and LabCorp (Irvine, CA, USA; Orlando, FL, USA). Participants completed the questionnaires,

reported adverse events as defined by the Medical Dictionary for Regulatory Activities version 17.0, and listed use of concomitant medications in a study diary.

Randomization and Blinding

A block randomization schedule was prepared by an un-blinded person at the study site who was not involved in study assessment. Within each block of 4 consecutively enrolled participants, 2 received OPTI-BIOME® and 2 received a placebo in a random order generated using randomization.com. Upon enrollment, every participant was assigned a unique randomization number based on the randomization schedule. Treatment allocation was implemented using 6-digit randomization codes, with the list generated by an un-blinded KGK staff member who was not involved in conducting the study. The same individual also prepared sealed opaque envelopes labelled with the randomization number that contained the associated treatment for a participant in the case of a reported serious adverse event that required the randomization code to be broken for that individual.

Statistical Analysis

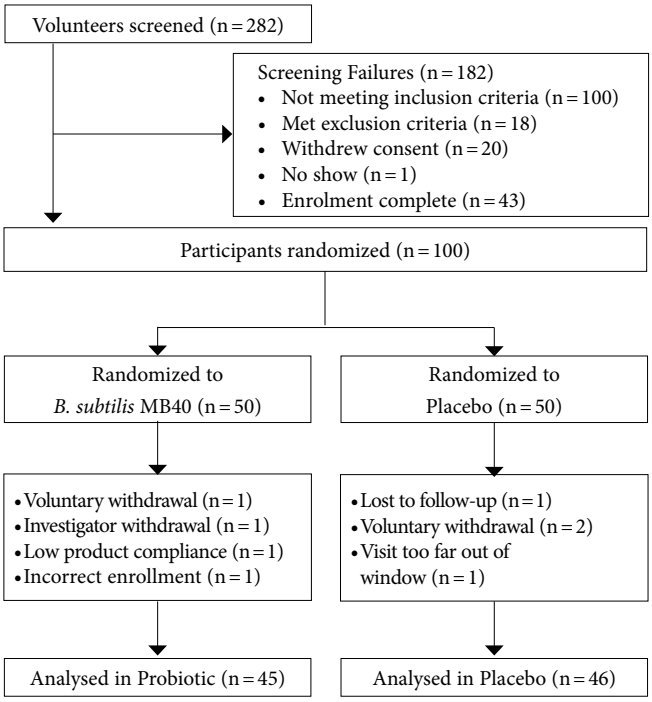
The sample size for this study was 100 enrolled participants with a high bloating score but otherwise healthy, which were randomized equally into 2 groups of 50 participants each in a double-blinded manner. The sample size calculation was based on a previous 4-week study performed by KGK Science that compared the average daily bloating score between a probiotic and a placebo. A standard deviation of 1.85, significance level of 5% (2-sided α), 80% power, 20% attrition, and a 1.18 detectable between-group difference were used in the calculation.

An efficacy analysis based on both Intent-to-treat (ITT) as well as Per-Protocol (PP) population was performed. The ITT population consisted of all participants who received either product and on whom any post-randomization efficacy information was available. The Per Protocol (PP) population consisted of all participants who consumed at least 80% of probiotic or placebo doses, did not have any major protocol violations, and completed all study visits and procedures connected with measurement of the primary variable. All efficacy outcomes analyzed in the PP population are described and discussed in detail, along with remarks about the results obtained for the ITT population. All safety outcomes are described for the ITT population.

All hypotheses testing was conducted using 2-sided tests and a type I error rate of $\alpha = 0.05$. All outcomes were tested by comparing treatments within each period and testing within treatments across time. Stool consistency was analyzed with the BSS as both a continuous and categorical value where the latter was defined as hard (BSS<3), optimal (BSS ≥3 to ≤5), and loose (BSS>5).

As per previously validated literature, the mADGB, mGSRS, and mRAND SF-36 questionnaires were compared with a predetermined specified clinical difference threshold of 1 (Hanifi et al[22]). All statistical analysis was completed using the R Statistical Software Package Version 3.2.3 (R Core Team 2015, Vienna, Austria). Data are presented as mean ± the standard error of the mean (SEM). Compliance was assessed by counting all returned study product and calculated by determining the number of dosage units consumed divided by the number of dosage units expected to have been consumed and multiplied by 100. If a discrepancy between the number of reported versus returned units was found, calculations were based on the amount of product returned unless an explanation was provided. Participants with a study product compliance <80% at the 2-week visit were counselled and an overall study-product compliance of <80% was considered non-compliant which lead to the participant being removed from the PP analysis. A planned subgroup analysis on sex was conducted on the PP population and an efficacy analysis was performed in a similar manner.

Figure 1. Study Participants



RESULTS

Baseline Characteristics of Participants

A total of 282 volunteers were screened and 100 eligible participants were enrolled into this study and randomized (Figure 1). Participants were well matched for sex, ethnicity, race, alcohol use, smoking status, age, weight, height, BMI, and the average bloating intensity and number of days with bloating during the run-in (Table 1). All participants were deemed healthy by physical examination and screening laboratory parameters. Overall compliance for both groups was 100%. One participant needed to be un-blinded during the study on the advice of the qualified investigator (QI) however, the integrity of the study was not compromised.

The ITT population behaved similar to the PP population except in the following outcomes: number of bowel movements and emotional well-being score. At week 4, within the ITT population, there was a significant within group decrease ($P = .047$) in the number of bowel movements in the MB40 group, while no such difference was observed for the Placebo group (data not shown). The increase in emotional well-being was significantly greater for the ITT MB40 group compared to that at baseline ($P = .023$) (data not shown). The results of the PP population are described below in detail.

Modified Abdominal Discomfort, Gas, and Bloating Questionnaire

While participants in both groups reported significant improvements in bloating (intensity ($P < .05$), number of days with ($P < .05$), and duration ($P < .05$)), abdominal discomfort

Table 1. Demographics and characteristics of all participants at screening (N = 99)[a]

Measure	MB40 (n = 50)		Placebo (n = 49)[a]		*P* Value
	n	%	n	%	
Sex					
Female	36	72	38	78	.645
Male	14	28	11	22	
	Mean	SEM	Mean	SEM	*P* value
Age (years)	41.6	2.2	39.6	2.0	.188
BMI (kg/m^2)	26.1	0.4	25.6	0.5	.398
Days with bloating during run-in	7.0	0.5	6.3	0.4	.258
Bloating intensity at baseline	6.7	0.1	6.7	0.1	.866

[a]One participant was not included in this analysis as there was not post-enrollment information

Table 2. Modified abdominal discomfort, gas and bloating questionnaire

Measure	Per-Protocol (mean ± SEM)		Females (mean ± SEM)		Males(mean ± SEM)	
	MB40 (n = 45)	Placebo (n = 46)	MB40 (n = 34)	Placebo (n = 36)	MB40 (n = 11)	Placebo (n = 10)
Bloating						
Average intensity						
Baseline	6.35 ± 0.15	6.09 ± 0.16	6.26 ± 0.16	6.04 ± 0.19	6.63 ± 0.36	6.27 ± 0.29
Change[a]	-3.06 ± 0.32[b]	-3.03 ± 0.30[b]	-2.84 ± 0.38[b]	-3.22 ± 0.35[b]	-3.71 ± 0.53[b]	-2.33 ± 0.50[b]
Average number of days						
Baseline	6.58 ±0.11	6.59 ±0.10	6.49 ± 0.14	6.57 ± 0.12	6.86 ± 0.07	6.65 ± 0.20
Change[a]	-1.51 ± 0.33[b]	-1.13 ± 0.27[b]	-1.43 ± 0.37[b]	-1.32 ± 0.33[b]	-1.77 ± 0.70b[b]	-0.45 ± 0.29
Average duration (hours)						
Baseline	4.13 ± 0.34	3.74 ± 0.40	4.44 ± 0.39	3.74 ± 0.47	3.17 ± 0.61	3.74 ± 0.70
Change[a]	-1.62 ± 0.31[b]	-0.87 ± 0.32[b]	-1.45 ± 0.36[b]	-0.79 ± 0.39[b]	-2.13 ± 0.62[b]	-1.14 ± 0.48[b]
Abdominal discomfort						
Average intensity						
Baseline	5.39 ± 0.22	5.62 ± 0.21	5.49 ± 0.22	5.52 ± 0.24	5.05 ± 0.57	5.96 ± 0.39
Change[a]	-2.34 ± 0.31[b]	-2.77 ± 0.29[b]	-2.35 ± 0.38[b]	-2.96 ± 0.35[b]	-2.29 ± 0.51[b]	-2.10 ± 0.46[b]
Average number of days						
Baseline	6.40 ± 0.14	6.40 ± 0.16	6.32 ± 0.17	6.35 ± 0.19	6.64 ± 0.24	6.60 ± 0.23
Change[a]	-1.67 ± 0.38[b]	-1.14 ± 0.28[b]	-1.62 ± 0.44[b]	-1.32 ± 0.34[b]	-1.82 ± 0.82[b]	-0.50 ± 0.32
Average duration (hours)						
Baseline	2.98 ± 0.26	3.10 ± 0.31	3.34 ± 0.30	3.03 ± 0.36	1.88 ± 0.37	3.32 ± 0.64[c]
Change[a]	-0.71 ± 0.34[b]	-0.82 ± 0.24[b]	-0.62 ± 0.42[b]	-0.82 ± 0.42[b]	-1.00 ± 0.44[b]	-0.83 ± 0.35[b]
Gas						
Average intensity						
Baseline	4.73 ± 0.31	5.03 ± 0.24	4.48 ± 0.36	4.83 ± 0.29	5.51 ± 0.59	5.76 ± 0.30
Change[a]	-2.24 ± 0.27[b]	-2.12 ± 0.30[c]	-2.13 ± 0.34[b]	-2.26 ± 0.32[b]	-2.58 ± 0.40[b]	-1.61 ± 0.79
Average number of days						
Baseline	5.97 ± 0.25	6.33 ± 0.19	5.76 ± 0.32	6.21 ± 0.23	6.59 ± 0.23	6.75 ± 0.15
Change[a]	-1.57 ± 0.36[b]	-0.89 ± 0.28[c]	-1.62 ± 0.42[b]	-1.04 ± 0.34[b]	-1.41 ± 0.71	-0.35 ± 0.34
Average duration (hours)						
Baseline	3.19 ± 0.44	2.87 ± 0.35	3.44 ± 0.56	2.96 ± 0.44	2.44 ± 0.52	2.56 ± 0.35
Change[a]	-1.20 ± 0.45[b]	-0.45 ± 0.32	-1.23 ± 0.59[b]	-0.70 ± 0.35	-1.11 ± 0.47[b]	0.33 ± 0.71

[a]Change from baseline to Week 4
[b]Within-group significance at week 4 compared to baseline ($P < .05$)
[c]Between-group significance ($P < .05$)

Table 3. Average number of bowel movements and BSS score and the number of participants stool categorized as soft, normal, or hard

Measure	MB40 (n = 45)	Placebo (n = 46)	*P* Value
Number of bowel movements (mean ± SEM)			
Baseline	9.40 ± 0.83	9.80 ± 0.59	.708
Change[a]	-0.90 ± 0.63	-0.60 ± 0.47	.534
Average BSS score (mean ± SEM)			
Baseline	3.88 ± 0.15	3.80 ± 0.13	.654
Change[a]	0.03 ± 0.15	-0.04 ± 0.13	.476
BSS category n (%)			
Baseline			
Soft (BSS >5)	5 (11%)	3 (7%)	.780
Normal (3≤BSS≤5)	32 (71%)	35 (76%)	
Hard (BSS <3)	8 (18%)	8 (17%)	
Week 4			
Soft (BSS >5)	6 (13%)	1 (2%)	.140
Normal (3≤BSS≤5)	34 (76%)	38 (83%)	
Hard (BSS <3)	5 (11%)	7 (15%)	

[a]Change from baseline to Week 4

Table 4. Average modified Gastrointestinal Symptom Rating Scale scores

Measure	Per-Protocol (mean ± SEM)		Females (mean ± SEM)		Males (mean ± SEM)	
	MB40 (n=45)	Placebo (n=46)	MB40 (n=34)	Placebo (n=36)	MB40 (n=11)	Placebo (n=10)
Diarrhea syndrome score						
Baseline	2.83 ± 0.22	2.69 ± 0.18	2.73 ± 0.24	2.69 ± 0.21	3.12 ± 0.52	2.71 ± 0.42
Change[a]	-1.11 ± 0.21[b]	-0.81 ± 0.22[b]	-1.03 ± 0.24[b]	-0.95 ± 0.22[b]	-1.36 ± 0.41[b,c]	-0.34 ± 0.62[c]
Constipation syndrome score						
Baseline	3.56 ± 0.25	3.27 ± 0.20	3.58 ± 0.30	3.32 ± 0.22	3.48 ± 0.48	3.08 ± 0.50
Change[a]	-1.09 ± 0.20[b]	-1.16 ± 0.22[b]	-1.08 ± 0.24[b]	-1.25 ± 0.24[b]	-1.12 ± 0.39[b]	-0.83 ± 0.58
Abdominal discomfort syndrome score						
Baseline	2.39 ± 0.16	2.54 ± 0.19	2.40 ± 0.18	2.53 ± 0.21	2.36 ± 0.36	2.56 ± 0.46
Change[a]	-0.67 ± 0.16[b]	-0.77 ± 0.18[b]	-0.75 ± 0.17[b]	-0.72 ± 0.20[b]	-0.41 ± 0.39	-0.96 ± 0.45
Indigestion syndrome score						
Baseline	3.99 ± 0.15	4.08 ± 0.13	3.99 ± 0.16	3.96 ± 0.15	3.96 ± 0.37	4.50 ± 0.24
Change[a]	-1.48 ± 0.17[b]	-1.58 ± 0.17[b]	-1.51 ± 0.21[b]	-1.61 ± 0.19[b]	-1.39 ± 0.30[b]	-1.47 ± 0.33[b]
Reflux syndrome score						
Baseline	2.05 ± 0.17	2.40 ± 0.22	2.02 ± 0.18	2.30 ± 0.24	2.15 ± 0.43	2.78 ± 0.56
Change[a]	-0.62 ± 0.16[b]	-0.86 ± 0.23[b]	-0.55 ± 0.17[b]	-0.85 ± 0.24[b]	-0.83 ± 0.38[b]	-0.88 ± 0.64

[a]Change from baseline to Week 4
[b]Within-group significance at week 4 compared to baseline ($P < .05$)
[c]Between-group comparisons were made using the ANCOVA F-test adjusting for baseline.

(intensity ($P < .05$), number of days with ($P < .05$), and duration ($P < .05$)), and gas (intensity ($P < .05$) and number of days with($P < .05$)), only those in the MB40 group reported a significant decrease in the duration of gas symptoms from baseline to end-of-study ($P < .05$) (Table 2).

Subgroup analyses indicated that male participants on MB40 alone showed significant improvement in average number of days with: bloating ($P < .05$) and abdominal discomfort ($P < .05$); average intensity of ($P < .05$) and the duration with gas ($P < .05$), at week 4 compared to baseline, however, these measures did not show a significant change between the 2 groups (Table 2). Although male participants in both groups reported significant improvements in bloating intensity on week 4 compared to baseline, males on MB40 also reported a better reduction in bloating intensity (-3.71) compared to the males in Placebo (-2.33) ($P < .107$). Male participants on MB40 group also reported clinical improvements versus placebo with average differences between the 2 groups as follows: 1.32 less days of bloating ($P < .344$), 1.32 less days of abdominal discomfort ($P < .466$), 1.06 less days of gas ($P < .514$), and 86-minutes less of gas per episode ($P < .143$) (Table 2).

Daily Bowel Habits Diary

The number of bowel movements and stool consistency at week 4 remained similar to baseline values in both MB40 and Placebo groups and there were no differences between the 2 groups for these measures (Table 3).

Modified Gastrointestinal Symptoms Rating Scale

Both MB40 and Placebo groups reported significant improvements from baseline to week 4 in the mGSRS for the diarrhea ($P < .05$), constipation ($P < .05$), abdominal discomfort ($P < .05$), indigestion ($P < .05$), and reflux syndrome scores ($P < .05$); however, there were no differences for these measures between the 2 groups (Table 4). Subgroup analysis indicated that males in the MB40 group reported significant reduction in the diarrhea syndrome score (-1.36) ($P = .002$), whereas males in the placebo group did not have a significant reduction in this score (-0.34) ($P = .610$). The average difference in the scores for the diarrhea syndrome score was 1.02 between the 2 groups suggesting a clinical improvement and statistical trend ($P = .099$) (Table 4) Di Stefano et al.[10]

Modified RAND SF-36 (Short-Form 36 Questions from the RAND Corporation)

There were no significant differences between MB40 and Placebo groups in the mRAND SF-36 at 4-weeks for physical functioning, role limitations (physical and emotional), vitality, emotional well-being, social functioning, bodily pain, or general health. There was a trend towards improvement in physical functioning score compared to baseline (Figure 2). However, participants in the MB40 group reported significant improvements from baseline in their role function (physical), vitality, and social functioning score ($P < .05$) occurred for the control group (Table 5). A 10% improvement was reported by males in the MB40 group in their perception of general health compared to the control group and deemed clinically relevant by the QI.[10]

Safety Parameters

A total of 30 adverse events were reported by 22 participants. Of these, 13 adverse events were reported by participants in the MB40 group and 17 were reported by participants in the placebo group. Of the 13 events reported by those in the MB40 group, 8 were classified as possibly related: abdominal discomfort (1), constipation (3), diarrhea (1), dry mouth (1), flatulence (1), and increased appetite (1). Of the 17 events reported by those in the placebo group, 5 were classified as possibly related: abdominal discomfort (1), constipation (2), infrequent bowel movements (1), and paresthesia (1). Adverse event reporting between the 2 groups was similar with 6 of 8 and 4 of 5 possibly related events

Table 5. Average modified RAND SF-36 scores

Measure	Per-Protocol (mean ± SEM)		Females (mean ± SEM)		Males (mean ± SEM)	
	MB40 (n = 45)	Placebo (n = 46)	MB40 (n = 34)	Placebo (n = 36)	MB40 (n = 11)	Placebo (n = 10)
Physical function						
Baseline	84.70 ± 3.03	88.0 ± 3.02	83.0 ± 3.38	92.1 ± 2.67	90.00 ± 6.63	73.50 ± 8.98
Change[a]	5.40 ± 2.80	1.10 ± 3.18	4.60 ± 2.97	-0.30 ± 3.27	7.70 ± 7.18	5.90 ± 9.01
Role limitations (physical)						
Baseline	84.00 ± 4.47	84.00 ± 4.72	85.30 ± 4.61	86.10 ± 4.72	80.00 ± 12.06	75.00 ± 13.28
Change[a]	10.0 ± 4.16[b]	4.90 ± 3.86	6.60 ± 3.86	4.20 ± 3.52	20.00 ± 12.06	8.00 ± 12.97
Role limitations (emotional)						
Baseline	87.40 ± 4.01	90.60 ± 4.10	85.30 ± 4.92	92.60 ± 4.22	93.90 ± 6.06	83.30 ± 11.38
Change[a]	7.00 ± 5.07	6.00 ± 4.72	8.00 ± 6.52	5.00 ± 4.83	6.00 ± 6.03	10.00 ± 14.23
Vitality						
Baseline	61.80 ± 3.16	63.40 ± 2.73	59.30 ± 3.70	63.10 ± 2.92	69.70 ± 5.70	64.50 ± 7.18
Change[a]	7.30 ± 2.88[b]	1.20 ± 2.02	8.80 ± 3.26[b]	1.90 ± 2.02	2.60 ± 6.18	-1.50 ± 5.91
Emotional well-being						
Baseline	77.60 ± 2.77	79.80 ± 2.14	77.10 ± 3.09	79.80 ± 2.32	79.30 ± 6.33	80.00 ± 5.41
Change[a]	6.00 ± 2.50[b]	3.40 ± 1.53[b]	5.20 ± 2.61[b]	4.20 ± 1.73[b]	8.40 ± 6.54	0.40 ± 3.29
Social functioning						
Baseline	91.80 ± 3.77	98.10 ± 3.32	91.20 ± 4.20	100.70 ± 3.68[c]	93.80 ± 8.79	88.80 ± 7.08
Change[a]	9.40 ± 3.80[b]	4.90 ± 3.24	9.90 ± 3.69[b]	2.10 ± 3.65	7.50 ± 11.51	15.00 ± 6.39
Bodily pain						
Baseline	71.70 ± 3.90	74.20 ± 3.05	71.30 ± 4.37	77.20 ± 3.17	73.00 ± 8.86	63.50 ± 7.65
Change[a]	9.40 ± 3.21[b]	6.90 ± 2.82[b]	8.70 ± 3.26[b]	6.70 ± 2.77[b]	11.40 ± 8.62	7.50 ± 8.66
General health						
Baseline	73.10 ± 2.46	72.60 ± 2.57	73.50 ± 2.74	71.90 ± 3.02	71.80 ± 5.61	75.00 ± 4.93
Change[a]	2.10 ± 2.50	1.10 ± 1.58	-0.30 ± 2.85	1.50 ± 1.62	9.50 ± 4.79	-0.50 ± 4.49

[a]Change from baseline to Week 4
[b]Within-group significance compared to baseline ($P < .05$)
[c]Between-group significance ($P < 0.05$)

Figure 2. Significant between-group difference at week 2 compared to baseline was noted ([a]), whereas the placebo group reported a significantly lower physical functioning scale score. Though not significant, there was a trend towards significant increase in physical functioning with OPTI-BIOME® *B. subtilis* MB40 (MB40) compared to baseline.

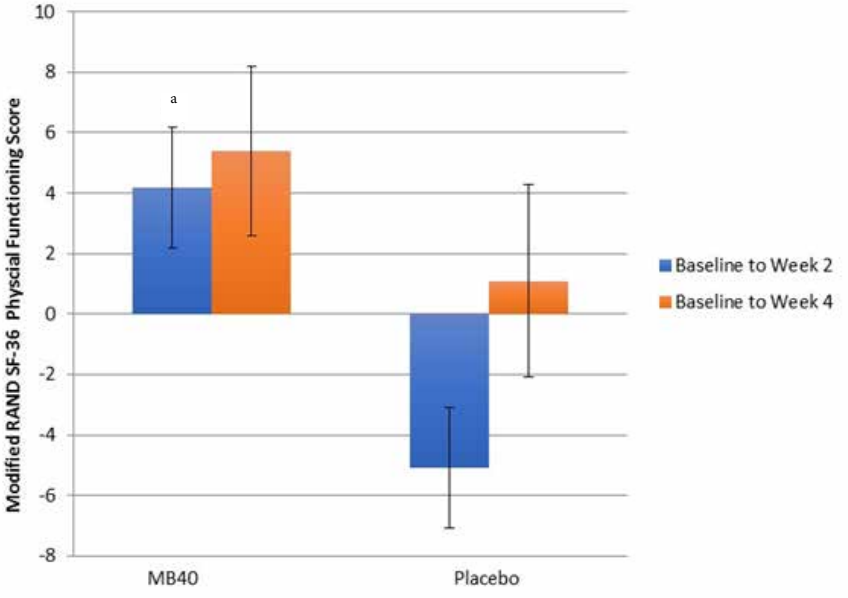


Table 6: Vital signs and anthropometrics

Measure	MB40 Mean ± SEM (n)	Placebo Mean ± SEM (n)	*P* Value
Mean systolic blood pressure (mmHg)			
Baseline	118.30 ± 2.10 (45)	118.0 ± 1.80 (46)	.896
Change[a]	1.70 ± 1.50 (45)	0.20 ± 1.20 (46)	.463
Change[b]	1.10 ± 1.50 (44)	-0.80 ± 1.50 (46)	.379
Mean diastolic blood pressure (mmHg)			
Baseline	74.90 ± 1.40 (45)	76.50 ± 1.30 (46)	.405
Change[a]	1.90 ± 1.10 (45)	-1.30 ± 1.10 (46)	.048
Change[b]	1.10 ± 1.10 (44)	0.20 ± 1.10 (46)	.554
Mean heart rate (bpm)			
Baseline	74.0 ± 1.10 (45)	77.60 ± 1.40 (46)	.050
Change[a]	0.60 ± 1.30 (45)	-3.30 ± 1.20 (46) [δ]	.027
Change[b]	1.70 ± 1.00 (44)	2.50 ± 1.30 (46)	.609
Weight (kg)			
Baseline	73.4 ± 1.90 (45)	70.20 ± 2.00 (46)	.260
Change[a]	0.00 ± 0.20 (45)	1.50 ± 0.70 (46) [δ]	.031
Change[b]	0.20 ± 0.30 (44)	1.20 ± 0.70 (46)	.206
Body Mass Index (kg/m^2)			
Baseline	26.40 ± 0.40 (45)	25.30 ± 0.50 (46)	.081
Change[a]	0.01 ± 0.06 (45)	0.46 ± 0.16 (46)[c]	.012
Change[b]	0.04 ± 0.09 (44)	0.33 ± 0.17 (46)	.132

[a]Change from baseline to Week 2
[b]Change from baseline to Week 4
[c]Within-group significance compared to baseline ($P < .05$)

attributable to gastrointestinal disorders in the MB40 and placebo groups respectively. All other adverse events were assessed as 'unlikely' or 'not related' to the study products. All adverse events were resolved before end-of-study.

In the safety population, all anthropometric, vital, and hematological parameters remained within normal clinical ranges for participants in both groups (Tables 6, 7 and 8). There were no significant between or within group differences in the change from baseline values in any vital parameters. There were no significant between group differences in the change from baseline values in the anthropometric measures at the end of the study (Table 6). Within groups, the participants in the Placebo group showed a significantly greater increase in weight (0.48 ± 1.66 kg) and BMI (0.17 ± 0.50 kg/m^2) ($P = .025$). There were no significant between group differences in the change from baseline values in any of hematology or clinical chemistry parameters (Tables 7 and 8). There were no significant within group differences in any of the hematology or clinical chemistry parameters in the MB40 group. Within groups, the participants in the Placebo group had significant decreases in white blood cell counts ($P = .007$), neutrophil counts ($P = .014$) and monocyte counts ($P = .002$) at week 4 compared to baseline. However, all these changes remained within the clinical ranges and were deemed to be clinically non-relevant by the medical director.

Table 7. Haematology

Measure	MB40 Mean ± SEM (n)	Placebo[a] Mean ± SEM (n)	Between-group *P* Value
Hemoglobin concentration (g/L)			
Screening	137.20 ± 1.60 (45)	134.80 ± 1.80 (46)	.337
Change[b]	-0.70 ± 0.90 (45)	-0.20 ± 0.90 (46)	.722
Hematocrit (L/L)			
Screening	0.408 ± 0.005 (45)	0.405 ± 0.005 (46)	.659
Change[b]	0.0006 ± 0.0028 (45)	0.0007 ± 0.0029 (46)	.968
White blood cell count (x E9/L)			
Screening	6.54 ± 0.28 (45)	6.66 ± 0.25 (46)	.739
Change[b]	-0.27 ± 0.23 (45)	-0.53 ± 0.20 (46)[c]	.401
Red blood cell count (x E12/L)			
Screening	4.58 ± 0.05 (45)	4.61 ± 0.07 (46)	.806[d]
Change[b]	-0.009 ± 0.028 (45)	-0.007 ± 0.031 (46)	.956[d]
Mean corpuscular volume (fL)			
Screening	89.70 ± 0.60 (45)	88.40 ± 0.80 (46)	.182
Change[b]	-0.07 ± 0.24 (45)	0.15 ± 0.25 (46)	.532
Mean corpuscular hemoglobin (pg)			
Screening	30.00 ± 0.21 (45)	29.36 ± 0.30 (46)	.087
Change[b]	-0.11 ± 0.06 (45)	0.00 ± 0.08 (46)	.280
Mean corpuscular hemoglobin concentration (g/L)			
Screening	335.00 ± 0.90 (45)	332.30 ± 1.20 (46)	.117
Change[b]	-1.30 ± 1.00 (45)	-0.80 ± 0.80 (46)	.711
Red cell distribution width (%)			
Screening	13.46 ± 0.09 (45)	13.70 ± 0.16 (46)	.219[d]
Change[b]	-0.03 ± 0.06 (45)	-0.10 ± 0.08 (46)	.484[d]
Platelet count (x E9/L)			
Screening	259.00 ± 8.00 (45)	265.00 ± 8.00 (46)	.565
Change[b]	2.00 ± 3.00 (45)	4.00 ± 6.00 (46)	.839
Neutrophil count (x E9/L)			
Screening	3.94 ± 0.24 (45)	3.90 ± 0.22 (46)	.945[d]
Change[b]	-0.28 ± 0.21 (45)[d]	-0.43 ± 0.17 (46) [c,d]	.429[d]
Lymphocyte count (x E9/L)			
Screening	1.96 ± 0.08 (45)	2.07 ± 0.07 (46)	.268[d]
Change[b]	-0.01 ± 0.05 (45)	-0.03 ± 0.07 (46)	.666[d]
Monocyte count (x E9/L)			
Screening	0.484 ± 0.021 (45)	0.515 ± 0.021 (46)	.299
Change[b]	0.007 ± 0.023 (45)	-0.057 ± 0.017 (46)[c]	.032
Eosinophil count (x E9/L)			
Screening	0.144 ± 0.012 (45)	0.161 ± 0.015 (46)	.405
Change[b]	0.018 ± 0.012 (45)	-0.009 ± 0.014 (46)	.147
Basophil count (x E9/L)			
Screening	0.007 ± 0.004 (45)	0.017 ± 0.006 (46)	.120[e]
Change[b]	0.000 ± 0.004 (45)	0.000 ± 0.006 (46)	1.000[e]

[a]One participant withdrew consent and did not allow blood draw at the early termination visit
[b]Change from baseline to Week 4
[c]Within-group significance compared to baseline ($P < .05$)
[d]Logarithmic transformation was required to achieve normality
[e]Non parametric analysis was done

Table 8. Clinical chemistry, liver and kidney function tests

Measure	MB40 Mean ± SEM (n)	Placebo[a] Mean ± SEM (n)	Between-group *P* Value
Creatinine concentration (μmol/L)			
Screening	68.40 ± 2.40 (45)	65.00 ± 1.90 (46)	.258
Change[b]	1.50 ± 1.10 (45)	2.00 ± 1.40 (46)	.781
Sodium concentration (mmol/L)			
Screening	141.33 ± 0.38 (45)	140.91 ± 0.38 (46)	.440
Change[b]	-0.20 ± 0.42 (45)	0.02 ± 0.36 (46)	.691
Potassium concentration (mmol/L)			
Screening	4.63 ± 0.08 (45)	4.47 ± 0.07 (46)	.124
Change[b]	-0.10 ± 0.08 (45)	0.02 ± 0.08 (46)	.272
Chloride concentration (mmol/L)			
Screening	101.56 ± 0.38 (45)	101.80 ± 0.36 (46)	.633
Change[b]	-0.29 ± 0.43 (45)	-0.33 ± 0.33 (46)	.945
Bilirubin concentration (μmol/L)			
Screening	6.90 ± 0.60 (45)	6.80 ± 0.60 (46)	.487[c]
Change[b]	0.70 ± 0.50 (45)	0.20 ± 0.40 (46)	.900[c]
Aspartate transaminase (U/L)			
Screening	18.50 ± 0.70 (45)	19.40 ± 0.80 (46)	.391[c]
Change[b]	0.20 ± 0.80 (45)	0.30 ± 1.00 (46)	.910[c]
Alanine transaminase (U/L)			
Screening	16.60 ± 0.70 (45)	20.60 ± 1.80 (46)	.126[c]
Change[b]	1.30 ± 1.50 (45)	-1.20 ± 1.30 (46)	.427[c]

[a]One participant withdrew consent and did not allow blood draw at the early termination visit
[b]Change from baseline to Week 4
[c]Logarithmic transformation was required to achieve normality

DISCUSSION

There are limited studies available that examine the efficacy of individual bacterial strains as probiotics to alleviate bloating problems in healthy individuals. This is the first randomized, double-blind, placebo-controlled, parallel study on the efficacy of MB40 in relieving bloating symptoms in healthy persons. This study shows that participants in both MB40 and Placebo experienced significant reductions in the intensity, frequency, and duration of bloating, abdominal discomfort, and gas at week 4 compared to baseline. However, there were no statistical differences between the MB40 and placebo participants in bloating, gas, or abdominal discomfort after the 4-week supplementation. Only participants on MB40 had significant reductions in the duration of gas symptoms at week 4 compared to baseline, however, these findings did not translate into a significant difference between groups.

Of interest to the current study is the definition put forth by Hanifi et al,[22] who defined a between-group difference of 1 as clinically relevant when determining between-group differences with respect to gastrointestinal distress as assessed by daily questionnaires. With this threshold, several clinical improvements, predominantly in males, were observed in the MB40 group over the placebo. Male participants reported clinically relevant reductions in their bloating intensity (1.38 over placebo), number of days with abdominal discomfort, gas, and bloating (1.32, 1.06, and 1.32 days respectively), and duration of gas symptoms by 86 minutes less than placebo respectively. The highly variable bloating symptoms reported in this and previous studies, matched with the transient nature of symptoms in healthy persons likely contributed to the lack of between-group significance.

Several studies have shown that bloating is linked with an altered gut microbiota that lead to abnormal bacterial fermentation of undigested food.[8,9] Molecular characterization of the intestinal microbiota in patients with and without abdominal bloating showed that although differences in bacterial taxa were identified, the relative abundance of major taxonomic groups such as ratio of the Bacteroidetes: Firmicutes: Proteobacteria were not different. Microbiota contribute to the pathophysiology of bloating symptoms in multifactorial ways, including an effect of motility, sensation and fermentation. However, conclusive evidence is lacking due to limitations of these studies, including evaluation of microbiota at a single time point and the effect of diet.[28]

In a previous study, Corazza et al., have shown that following a 15-day *B. subtilis* (6×10^9 CFU) supplementation in persons with long-standing abdominal pain and bloating, 76% of individuals reported improvements, however, no results from a control group were obtained and thus no comparison to a placebo can be drawn.[23] In the current study 93% of participants in both groups reported improvements with a reduced abdominal bloating score at week 4 compared to baseline. Others have reported on the efficacy of *B. subtilis* on abdominal symptoms. Similar to the current study, other studies have reported that there were no between-group differences in gastrointestinal distress or indigestion between healthy participants consuming *B. subtilis* at either 0.1, 1.0, or 10×10^9 CFU or placebo per day for 4-weeks.[22] Interestingly, no improvements were reported at any dose or in the placebo whereas in the current study, significant within-group improvements in bloating, abdominal discomfort and gas were found.[22] A significantly lower number of adults over 45 years who were scheduled to undergo antibiotic treatment and were concomitantly supplemented with a probiotic containing *B. subtilis* indicated abdominal pain and bloating versus those consuming a placebo.[29] It is interesting that the current study found significant within-group improvements in bloating in both the placebo and MB40 participants despite including a healthy population whose symptoms tend to present inconsistently.

The difficulty in demonstrating between-group differences with probiotics in healthy persons also extends to disease populations, such as in those with irritable bowel syndrome (IBS) where the prevalence of bloating has been reported to be as high as 96%.[30,31]

The lack of published research utilizing MB40 as a probiotic in healthy adults warranted its evaluation of its safety and tolerance measures. The current study administered 5.0×10^9 CFU MB40 once a day for 4-weeks. The occurrence

of adverse events related to the study products was a total of 8 in the probiotic and 5 in the placebo group. In participants on MB40 2 of the 8 adverse events were likely related to the product however, neither required treatment. In addition, participants on MB40 reported either no change or a significant reduction of symptoms for bloating, gas, and abdominal discomfort and the product was reported as tolerated. All laboratory measures of complete blood count with differential, hematology, electrolyte count, liver and kidney function tests, and vitals remained within clinically normal levels during this study. The current results support the available evidence for the safe use of MB40 in healthy adults.

The current study modified the GSRS and half-steps were employed to increase sensitivity and 2 questions were removed to better reflect the nature of dietary supplements.[32] The diarrhea, constipation, abdominal discomfort, indigestion, and reflux symptom scores improved significantly at each week in the MB40 and placebo groups however, no significant between-group differences were found. Males in the MB40 group reported a clinical improvement in diarrhea syndrome score (1.02) and a statistical trend ($P = .099$) relative to the placebo group at week 4. These results are supported by other studies where a 4-week administration of *B. coagulans* (2×10^9 CFU/day) significantly improved the GSRS abdominal pain sub score versus placebo in a population of predominantly Hispanic participants where approximately 50% participants were male.[33] In contrast to the current study, a previous study did not find statistical or clinical (defined as >1) differences in mGSRS scores when healthy adults were supplemented with *B. subtilis* 10×10^9 CFU per day for 4-weeks.[22] This may be due to the difference in selection criteria where the current study selected a study population with bloating at baseline, whereas the others did not.[22,33]

Previous research has indicted that treatment strategies addressing the psychological co-morbidities of bloating are likely to be the most effective.[13,34] In the current study, the mRAND SF-36 was modified by reducing the 4-week recall to 2-weeks to match the study visit frequency. When compared to baseline, participants in the MB40 group reported a significant ($P = .017$) improvement over the Placebo group in the physical functioning score at 2-weeks (data not presented) however, this was not maintained at week 4. This can be partly attributed to the high variance seen in the scores for the mRAND SF-36. Significant improvements from baseline were reported in the scores for role limitations (physical), vitality, emotional well-being, social functioning, and bodily pain for the MB40 group and emotional well-being and bodily pain for the placebo. Males in the MB40 group reported a clinically, but not statistically significant improvement of 10% over the placebo group in the general health score. These results support those by Capello et al.[27] who reported a higher number of improvements in mRAND SF-36 domains with a blend of pro and prebiotics (symbiotic) than with a placebo, though they were not significant between-groups. Specifically, participants who consumed the symbiotic reported improvements in 7 of the 8 domains while the placebo group reported improvements in only 3.[27]

In the current study, the MB40 and placebo groups reported improvements in 5 versus 2 measures and female participants reported improvements in 4 versus 2, respectively. Others have found statistical and clinical (>1) significance between groups using a multi-species probiotic containing *B. subtilis* in a 12-week supplementation in persons with Rome II defined functional bowel disorders.[26] Our results support previous research and, when taken together, provide evidence that *B. subtilis* may positively impact an individual's psychological perspective on bloating but further research is needed to quantify these results and any differences based on sex.[13,33]

Clinical studies in the supplement industry face challenges in demonstrating between-group significances due to large placebo effects and small effect sizes and would thus benefit from an alternative to augment statistical differences. The lack of between-group significance in the current study may be attributed to a placebo effect, inclusion criteria, and the study population. High placebo effects are often reported in gastrointestinal studies and participants in the current study reported a placebo effect of 49.8%. Others have reported placebo effects as high as 65.6% after a 4-week supplementation with a symbiotic mixture.[27] Inclusion into the study required a high level of, or frequent, bloating.

As symptoms in any healthy population tend to be transient, the absolute quantification becomes challenging. In the current study, both groups demonstrated large variability in their responses to the questionnaires. Given this challenge, clinical differences have been proposed. Supplements derive efficacy from eliciting multiple effects which is difficult to statistically distinguish with a single endpoint. For instance, much of the previous research as well as the current study found a high number of clinically and low number of statistically significant differences. An alternative representation, such as a global index, that better represents this understanding is needed.

The current study also had some limitations. Although a correlation between BMI and bloating has not been established, excess intra-abdominal fat may contribute to fluctuations in intra-abdominal volume 35. In such cases, measurement of abdominal girth by the participant at the time of experiencing the bloating is useful. However, this study design did not capture this parameter. Although three-day food records were reviewed, and personalized nutritional counselling was provided, this study did not account for the bloating induced by FODMAPs (fermentable oligosaccharides, disaccharides, monosaccharides and polyols).

The duration of probiotic administration is a major factor in determining the efficacy of a probiotic intervention and previous studies have shown differences between active and placebo groups reach a statistical significance only after 60 days of treatment.[36] The current study was a 4-week study, and longer studies will be of value and may be effective in

discerning improvements over the placebo. Additionally, there were some improvements seen in the male subgroups, while it is plausible that sex hormones play a potential role in gender-related differences, no conclusions could be drawn due to the small number of participants in this subgroup and future research is warranted.

Previous studies in healthy persons demonstrate that *B. subtilis* acts rapidly,[37,38] promotes the regularity of bowel movements,[24] and reduces the incidence of antibiotic associated diarrhea.[29] Given this, MB40 has the potential to act as a prophylactic or as a quickly acting bacteria that requires a time sensitive administration, but further research is necessary to explore this potential avenue. Future research should investigate a population that is without, but regularly presents with, bloating to determine the capacity of *B. subtilis* to limit the occurrence of bloating.

CONCLUSIONS

Consumption of MB40 for 4-weeks was well tolerated in this double-blind randomized study. Clinical improvements indicate that MB40 may have a potential role in alleviating symptoms of abdominal discomfort, gas, and bloating in an otherwise healthy population. Study results indicate that regular consumption of MB40 at doses up to 5×10^9 CFU per day were safe and well tolerated.

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