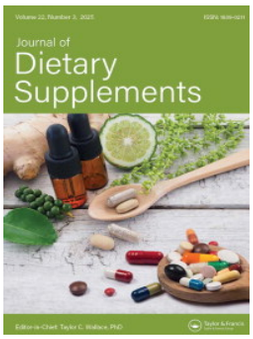

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Efficacy of Probiotic Supplementation with *Lactiplantibacillus plantarum* Strains on Gastrointestinal Tract Function – A Randomized Controlled Trial

Ruma G. Singh[a], Fumiki Aoki[b,c], Maria Rodriguez-Palmero Seuma[b], Meritxell Aguilo[b], Motohisa Washida[c], Jordi Espadaler-Mazo[b], Huda Al-Wahsh[a], David C. Crowley[a], Najla Guthrie[a], Malkanthi Evans[a,d], Marc Moulin[a] and Erin D. Lewis[a]

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ABSTRACT

Gastrointestinal (GI) dysfunction in older adults may be associated with gut microbiota activity or composition changes. *Lactiplantibacillus plantarum* strains KABP031 and KABP032 have been shown to beneficially influence the frequency of bowel movements (BMs) and nutritional status in older adults. This study investigated the efficacy of this probiotic blend on defecation/stool consistency, GI symptoms, nutrient uptake, and mental well-being in older adults with occasional constipation. Subjects 50–85 years of age with infrequent BMs, straining during defecation and hard stool consistency, were randomized to either the Probiotic or Placebo group for 84 days. Changes in bowel function, GI symptoms, and stress were assessed by the daily bowel habits diary, Gastrointestinal Symptoms Rating Scale (GSRS) and the Perceived Stress Scale (PSS), respectively. Improvements in bowel movement frequency ($p = 0.027$, 95% CI: 0.22–2.39) and stool consistency ($p = 0.002$; 95% CI: 0.32–1.30) with the Probiotic were significantly greater compared to Placebo after 42 days. There was also significant decrease in the percentage of weekly BMs with a Bristol Stool Scale score of ≤2 with Probiotic vs. Placebo (-28.6% vs. −3.2%, $p < 0.001$). A significantly lower proportion of participants in the Probiotic group reported moderate stress following 84 days of supplementation compared to the Placebo group (9.4% vs. 37.9%, $p = 0.013$). Further, within-group significant improvements in stool consistency and BM frequency from baseline at days 42 and 84 were observed with the Probiotic ($p < 0.001$), but not the Placebo. The findings suggest the probiotic blend alleviated constipation symptoms and improved the mental well-being in older adults with occasional constipation.

Clinical trial registry number and website: NCT04147923; https://clinicaltrials.gov/study/NCT04147923

KEYWORDS

Constipation; defecation; gastrointestinal tract; *Lactiplantibacillus plantarum*; probiotic

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Introduction

Digestive health disorders including altered bowel functions and nutrient malabsorption are commonly observed in older adults and may develop due to age-associated changes in metabolic and physiological processes. Altered bowel function characterized by difficult and infrequent bowel movements (BMs), straining, abdominal pain, and bloating can negatively impact quality of life (QoL), and result in major social and healthcare burden (Nag et al. 2020). Current treatment options to relieve gastrointestinal (GI) symptoms and improve BMs include over-the-counter (OTC) remedies such as laxatives and fiber supplements (Johanson and Kralstein 2007). However, most consumers are dissatisfied with traditional treatment options due to a lack of predictability, ineffective relief of constipation, bloating, and other symptoms (Johanson and Kralstein 2007). These limitations have prompted interest in other effective therapeutic strategies. Further, nutrition is essential for regulation of physiological functions. However the ability of the GI tract to absorb nutrients may be impaired in older adults, which may lead to malnutrition (Amarya et al. 2015). The alterations in nutrient absorption and GI health observed in older adults may be associated with changes in gut microbiota activity or composition (He et al. 2003; Blaut et al. 2006). Therefore, probiotics have emerged as an attractive and safe alternative that may be useful in improving nutrient absorption (Judkins et al. 2020) as well as improving GI symptoms and altered bowel habits in the older adult population (Miller and Ouwehand 2013; Martínez-Martínez et al. 2017; Takeda et al. 2023).

Probiotics are defined as 'live microorganisms which when administered in adequate amounts confer a health benefit on the host' (Hill et al. 2014). Furthermore, probiotic effects are strain-specific and there is general consensus that recommendations, especially in clinical settings, should be based on human studies showing the claimed benefits (Hill et al. 2014; McFarland et al. 2018; Guarner et al. 2024). The effect of probiotics, primarily bifidobacteria and lactobacilli, in the management of constipation has been investigated in several randomized controlled trials, systematic reviews, and meta-analyses (Dimidi et al. 2014; Martínez-Martínez et al. 2017; Ding et al. 2024). However, the majority of studies have been conducted in individuals with functional or chronic constipation with a limited number in healthy populations (Del Piano et al. 2010).

A pre-clinical investigation of two *Lactiplantibacillus plantarum* (*L. plantarum*) strains, KABP031 and KABP032, reported their functional probiotic properties including high tolerance to GI tract conditions and antimicrobial activity against pathogens (Bosch et al. 2012). Therefore, it is important to investigate the effect of this probiotic blend on human health. A previous study by Bosch et al. conducted in an elderly cohort in Spain demonstrated that consumption of low dose *L. plantarum* KABP031 and KABP032 significantly improved intestinal transit and nutritional status (Bosch Gallego et al. 2011). The ability of these *L. plantarum* strains to improve nutritional status in older adults may be attributed to the beneficial effect of probiotics on digestive health (Markowiak and Śliżewska 2017). Further, a growing body of evidence suggests that stress may lead to alterations in gut microbiota composition further contributing to bowel dysfunction characterized by decreased gut motility, inflammation, and increased permeability (Chang et al. 2014). Supplementation with specific

probiotic strains has shown to have beneficial effects on mental health (Messaoudi et al. 2011) *via* the gut-brain axis. There is evidence demonstrating the link between probiotic-induced changes in gut microbiota and reduction of anxiety in stressed adults (Ma et al. 2021). Therefore, investigating the effect of *L. plantarum* strains KABP031 and KABP032 on mental health is warranted. The objective of this study was to examine the effects of a probiotic blend containing *L. plantarum* KABP031 and KABP032 strains on bowel function and GI symptoms, nutrient absorption, as well as emotional and general well-being in older adults with occasional constipation.

Methods

Ethics and regulatory approvals

The study was reviewed by the Natural and Non-Prescription Health Products Directorate (NNHPD), Health Canada and approved on September 5, 2019. Ethics Board approval was granted on September 24, 2019, by the Institutional Review Board Services (Aurora, Ontario, Canada; Pro00038766). The study was conducted according to the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki guidelines and subsequent amendments. The trial followed CONSORT guidelines for randomized controlled trials (Moher et al. 2012) (Supplementary Table S1). Informed consent was obtained from participants prior to performing any study procedures. The study was registered with Clinicaltrials.gov (NCT04147923).

Study design

This study was a randomized, double-blind, placebo-controlled, parallel clinical trial conducted at KGK Science Inc. (London, Ontario, Canada) from November 2019 to June 2023. The study consisted of a 21-day run-in period followed by an 84-day supplementation period in which participants were randomized to receive a multi-strain Probiotic or Placebo.

Study population

Participants were males and females between the ages of 50 and 85 years, had a body mass index of 18.5–29.9 kg/m^2, and met the following bowel habits criteria at screening and baseline: ≤5 complete BMs per week AND at least 25% of BMs are Bristol Stool Scale (BSS) type 1 or 2 collectively with excessive straining (≥3 min) for most of the BMs (≥50%) OR at least 50% of BMs are BSS type 1 and 2 OR ≤3 complete spontaneous BMs per week. Participants were healthy as determined by their medical history and review of current health status by the Medical Director (MD), the Qualified Investigator for the study.

Individuals were excluded if they had; an allergy, sensitivity, or intolerance to the investigational product's active or inactive ingredients or milk, chronic constipation, history or ongoing clinically significant diseases of the GI tract, pancreatitis, short

bowel syndrome, malabsorption, kidney or liver diseases, used narcotics or concomitant prescribed (e.g. antibiotics, diuretics, anticholinergics) or OTC medications or supplements (e.g. probiotics, prebiotics, synbiotics, laxatives) that could affect bowel function or GI symptoms, any other medical condition that may have adversely affected the participant's ability to complete the study or its measures or which may have posed a significant risk to the participant, as assessed by the MD.

Investigational product and placebo

The investigational product (IP) was a capsule containing a total of ≥1 x 10^9 CFU of a probiotic blend (known as INNERIM™, manufactured by Kaneka Americas Holdings Inc.) that is a 1:1 CFU basis blend of *L. plantarum* KABP031 (CECT 7315) and *L. plantarum* KABP032 (CECT 7316). At the time of manufacturing, 5 x 10^9 CFU of each strain were input into the capsule to maintain the total CFU at ≥1 x 10^9 CFU throughout the entire product use in the study. The CFU content per capsule was monitored throughout the study by ISO17025-accredited company Eurofins Microbiology Laboratories (Wisconsin US), and all IP lots were confirmed to consistently meet the total CFU concentration of ≥1 x 10^9 CFU per capsule, with the lowest total CFU content of a capsule being 1 x 10^9 CFU. The IP contained excipients identical to Placebo. The Placebo contained maltodextrin, magnesium stearate and capsule shell (hypromellose, and titanium dioxide).

Participants were instructed to take the Probiotic or Placebo with a multivitamin and mineral supplement (Multi 100% Complete for Adults 50+ (Jamieson Laboratories Ltd, Canada)) after breakfast for 84 days. The multivitamin and mineral supplement contained 14 vitamins, 12 minerals, lutein and lycopene (detailed composition presented in Supplementary Table S2). Clinic staff instructed participants to save all unused and opened packages of study products and return them to the clinic site for determination of compliance. Compliance was further measured using the study diary, in which participants recorded daily IP use. If a dose was missed participants were instructed to consume the missed dose with their next meal as soon as they remembered. Participants were instructed not to exceed more than one dose per day.

Randomization and blinding

Investigators, study personnel, and participants were blinded to the products. A blinded investigator assigned a randomization number to each participant from the randomization list (www.randomization.com). Allocation concealment was attained with the use of opaque sealed envelopes labeled with a randomization number, which contained information regarding the treatment assigned. For blinding, the study products were identical in appearance (size, color, taste, texture) and were sealed in blister packs that appeared identical. The labels on each bottle were affixed by personnel not involved in study assessments per the requirements of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH) Guideline for Good Clinical Practice (GCP) and applicable local regulatory guidelines.

Study outcomes

The primary outcome was the difference in bowel function (BM and stool consistency), GI symptoms (as assessed by the Gastrointestinal Symptom Rating Scale (GSRS), and nutrient uptake between the Probiotic and Placebo from baseline on days 42 and 84. A subset of participants ($n = 40$) were selected for nutrient uptake analysis.

Secondary outcomes were the difference in perceived stress, assessed by the Perceived Stress Scale (PSS), and QoL assessed by the 12-Item Short Form Survey (SF-12) between the Probiotic and Placebo following 84 days of supplementation. Safety was assessed by the incidence of adverse events (AEs), and clinically relevant changes in vital signs, clinical chemistry, and hematology after 84 days of supplementation.

Study assessments

Daily bowel habits diary with Bristol Stool Scale (BSS)

The daily diary consisted of a seven-item questionnaire for evaluating each BM, and a BSS for evaluating stool shape and consistency of each BM. The number of BMs in the week leading up to each in-clinic visit was summed to obtain the number of BMs per week at baseline, day 42 and, day 84. The stool consistency in the week leading up to each visit was calculated by taking the average of the available stool consistency measurements. The percent of bowel movements with a score of 1 and 2 (i.e. hard stools) was also calculated across the week leading up to each visit.

Gastrointestinal Symptom Rating Scale (GSRS)

The GSRS is a validated 15-item scale for assessment of GI symptoms (Svedlund et al. 1988). Five subscales classified as Reflux, Abdominal Pain, Indigestion, Diarrhea, and Constipation Syndrome were identified from participant responses to the 15 items on the questionnaire. All items were scored on a 4-point Likert scale, ranging from 0 (no discomfort) to 3 (severe discomfort).

Perceived Stress Scale (PSS)

The PSS is a widely used psychological instrument for measuring the perception of stress in clinical studies. The scale includes generic questions about current levels of experienced stress, designed to assess how unpredictable, uncontrollable, and overloaded individuals find their lives. For each item, participants were asked how often they felt a certain way over the past month. A total score was calculated by summing participant responses to all 10 questions. A total score ≤13 was classified as 'Low', >13 and ≤26 was 'Moderate', and >26 was 'High' perceived stress (Cohen et al. 1983; State of New Hampshire Employee Assistance Program, n.d.).

12-Item Short Form Survey (SF-12)

The SF-12 is a brief version of the SF-36 Health Survey (Lacson et al. 2010), comprising of six items each for assessment of physical and mental health-related QoL. Responses were combined, scored, and weighted to calculate physical and mental

component summary scores (Hays 2004). A higher score indicated better physical or mental health-related QoL (Ware et al. 1995).

Nutrient uptake

Participants selected for nutrient uptake testing were instructed to fast for at least 12 h prior to collection of blood samples at baseline, day 42, and day 84. Nutrient uptake of study participants was determined by analyzing serum levels of albumin, total protein, vitamins and minerals (vitamin A, vitamin B_{12}, folate, vitamin C, vitamin D, vitamin E, calcium, iron, and magnesium) as well as plasma levels of vitamin B_6, zinc, and coenzyme Q10 (CoQ10). Albumin, total protein, calcium, iron, magnesium, vitamin C were analyzed using colorimetric assays, vitamins B_{12} and D were analyzed using chemiluminescence immunoassay, vitamins A and E were analyzed using high-performance liquid chromatography, folate was analyzed using enzyme-linked immunosorbent assay, vitamin B_6 and CoQ10 were analyzed using liquid chromatography/tandem mass spectrometry, and zinc was analyzed using inductively coupled plasma mass spectrometry. All these blood parameters were analyzed by Dynacare (London, Ontario, Canada) except for folate which was analyzed at CannaLabs (London, Ontario, Canada).

Safety

The severity of an AE was classified as 'mild', 'moderate', or 'severe', and the degree of relationship between the study product and an AE was categorized as 'not related', 'unlikely', 'possibly', 'probably', and 'most probably', by the MD. Clinical chemistry parameters included liver function (alanine aminotransferase, aspartate aminotransferase, total bilirubin), kidney function (creatinine, electrolytes (sodium, potassium, and chloride), fasting glucose, glycated hemoglobin, and estimated glomerular filtration rate). Hematology parameters included white blood cell (WBC) count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), red blood cell (RBC) count, hemoglobin, hematocrit, platelet count, and RBC indices (mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, mean platelet volume, and red cell distribution width). All blood parameters were analyzed by Dynacare using standardized procedures. The clinical significance of abnormal clinical chemistry and hematology laboratory values was assessed by the MD.

Study procedures

All participants were advised not to change their diet or physical activity throughout the study period. Participants completed the bowel habits diary daily starting on day −21 (first day of the run-in period) through to the end of the supplementation period. The GSRS was completed at each in-clinic visit (baseline, day 42, day 84), with PSS and SF-12 questionnaires completed at baseline and day 84. At baseline, day 42, and day 84, fasting blood samples for nutrient uptake testing were collected, processed and stored at 2–8 °C or −80 °C per guidelines provided by the laboratory. All samples were shipped under their specific storage conditions for analysis the same day. Safety

assessments, including clinical chemistry and hematology, were completed at screening and day 84, and vital signs and AEs were assessed at each clinic visit.

Statistical analysis

A sample size of 60 participants per group was estimated to detect a difference in mean change in the frequency of BMs of 0.80 (Ojetti et al. 2014) between the Probiotic and Placebo groups. However, the trial ended early with enrollment of a total of 70 participants as the COVID-19 pandemic period and region-wide lockdowns impacted timely recruitment of study participants. Further, the first 60 participants of the 120 enrolled participants were to be randomized to nutrient uptake analysis and stratified into 50–64 and 65–85 years old groups. However, the required number of participants to be stratified into the older age group were not enrolled in the nutrient uptake analysis at the time when the trial ended. Therefore, a sample size of 35 participants per group was analyzed in this study, with 20 participants from each group selected for nutrient uptake analysis.

Summary statistics including mean and standard deviation are presented for continuous outcomes for each group with frequencies and proportions presented for categorical outcomes. Given that the coronavirus outbreak happened during the conduct of this study the statistical analyses of continuous outcomes were adjusted for the intervention period of study participants (from Day −21 to day 84), and classified into three time periods according to the timeline of the Ontario government's SARS-CoV-2-related lockdown restrictions: 'Before March 1, 2020', 'Overlapping with lockdown restrictions between March 1, 2020, and March 21, 2022', and 'After March 21, 2022'. The differences between and within groups in BMs, stool consistency, GSRS scores (continuous), nutrients, PSS (continuous), and QoL subscale scores were evaluated using linear mixed models, with fixed effects including group, visit, intervention period, and their interactions. This statistical method accounts for the difference at baseline between groups by including the value of the outcome as a dependent variable at each visit, including baseline. Therefore, the interaction term between groups at each visit represents the difference in change from baseline between groups at that visit. For GSRS indigestion score, SF-12 mental health subscale score, and folate concentrations, values were rank transformed to meet the linear mixed model assumptions. For assessment of the number of BMs, if participants had missing diary days or data, the average of the days completed was computed and multiplied by seven to address missing data. Between-group and within-group differences in categorical outcomes (GSRS, PSS) were analyzed using Fisher's exact test and McNemar or Bhapkar's test, respectively. A post hoc analysis was conducted to assess percentages of BMs with BSS score 2 or lower using a mixed logistic model with fixed effects including group, visit, intervention period, and their interactions. The model used in this post hoc analysis also considered the value of the outcome as a dependent variable at each visit, including baseline.

Analyses are reported for the Per Protocol (PP) population consisting of all participants who consumed at least 80% of the Probiotic or Placebo doses, did not have any protocol violations related to the primary outcome, and completed all study visits and procedures connected with measurement of the primary variable. All statistical

analyses were performed using the R Statistical Software Package Version 4.2.1. p values ≤0.05 were considered statistically significant.

Results

Study population

A total of 187 participants were screened, with 70 eligible participants consented and enrolled in the study. There were nine participants excluded from the PP population (Figure 1). The PP population consisted of 50 females and 11 males, with an median age of 58 (range of 50 – 80) years. There were no significant differences in demographics and anthropometric variables between the Probiotic and Placebo groups (Table 1). Body weight was significantly increased from baseline to Day 84, in both the Probiotic group (0.84 ± 1.62 kg, within-group $p = 0.008$) and Placebo group (0.76 ± 2.41 kg, within-group $p = 0.022$), but there was no significant difference between groups.

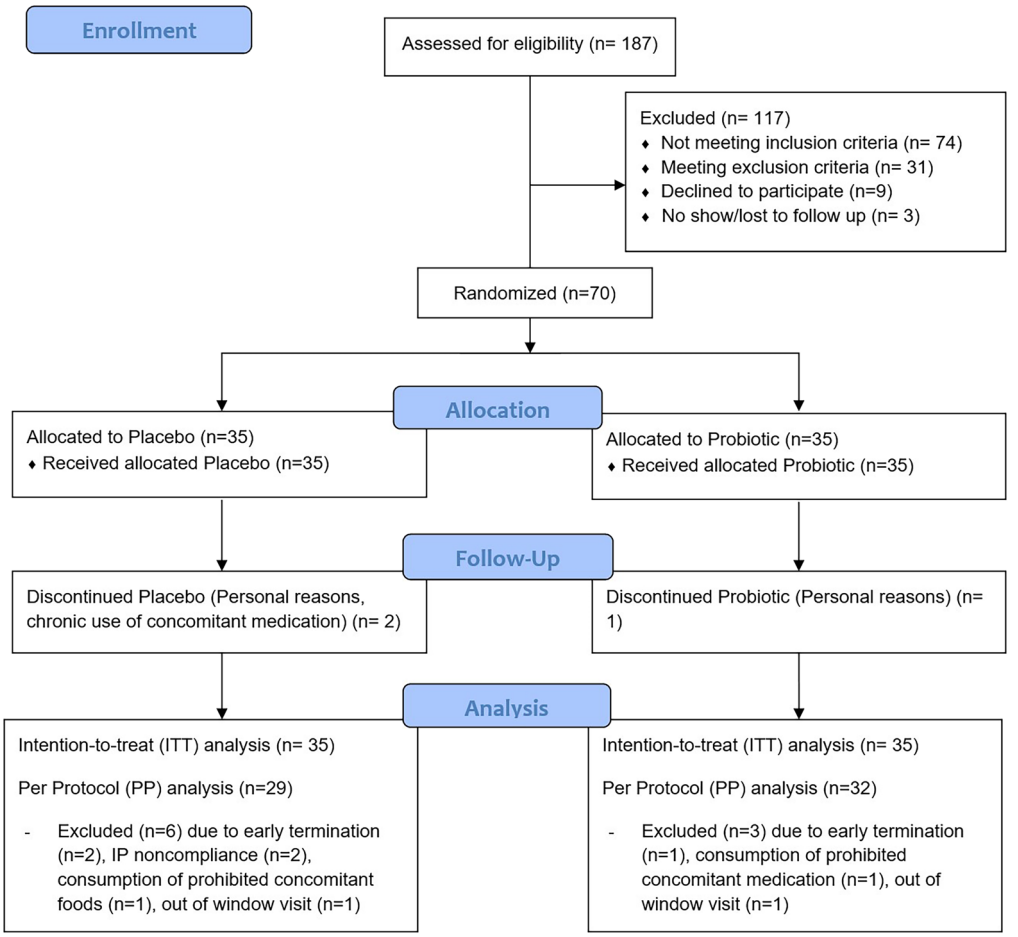

Figure 1. Disposition of study participants.

Table 1. Characteristics of study participants ($n = 61$).

Variable	Placebo ($n = 29$)	Probiotic ($n = 32$)	p Value between groups
Age (years)	58.41 ± 7.05	58.22 ± 7.26	0.916
Weight (kg) (median, range)	58.00 (50.00 to 80.00)	57.50 (50.00 to 81.00)	0.593
BMI (kg/m^2)	24.72 ± 2.86	25.55 ± 2.59	0.242
Sex			
Female	21 (72.41%)	29 (90.62%)	0.096
Male	8 (27.59%)	3 (9.38%)	
Race			
Western or Eastern European White	26 (89.66%)	26 (81.25%)	0.804
Other	1 (3.45%)	2 (6.25%)	
Native American	0 (0.00%)	2 (6.25%)	
South or Southeast Asian	1 (3.45%)	1 (3.12%)	
Middle Eastern	1 (3.45%)	0 (0.00%)	
South American	0 (0.00%)	1 (3.12%)	
Ethnicity			
Hispanic or Latino	0 (0.00%)	1 (3.12%)	1.00
Not Hispanic or Latino	29 (100.00%)	31 (96.88%)	
Baseline stool consistency (% of BMs with BSS ≤2)	50.4 ± 34.7	66.6 ± 30.0	0.137

Data presented as mean ± SD, or frequency (percentage) unless otherwise stated; BBS, Bristol stool scale; BM, bowel movement; n, number; SD, standard deviation; p values for categorical variables were calculated using fisher's exact test; p values for continuous variables were calculated using t-test.

Bowel movements (BMs)

At baseline, the number of BMs per week in the Probiotic and Placebo groups were 5.59 ± 3.89 and 6.26 ± 4.06, respectively. A significant increase in the frequency of BMs between Probiotic and Placebo groups was found at day 42 ($p = 0.027$), but this difference faded away at day 84 because of the increasing response in the Placebo group. Participants supplemented with the Probiotic had significant increases in BM frequency by 2.08 ± 2.52 and 2.07 ± 2.93 BMs/week after 42 days and 84 days, respectively (within-group $p < 0.001$ for both time points), while changes in those taking placebo were of 0.49 ± 2.48 BM/week ($p = 0.342$) and 0.93 ± 2.65 BM/week ($p = 0.073$) (Figure 2).

Stool consistency

At baseline, average BSS score were 2.31 ± 0.85 and 2.79 ± 1.10 for probiotic and placebo group, respectively ($p = 0.079$). Using linear mixed models, which account for baseline values as dependent variable, supplementation with the Probiotic resulted in a greater improvement in stool consistency compared to Placebo after 42 days (between-group $p = 0.002$) and 84 days (between-group $p = 0.058$) (Figure 3A). The Probiotic group had significant improvements in average BSS scores from 2.31 ± 0.85 at baseline to 3.14 ± 1.10 and 3.05 ± 0.93 at days 42 and 84, respectively (within-group $p < 0.001$), while average BSS scores in the Placebo group changed from 2.79 ± 1.10 at baseline to 2.81 ± 1.20 and 3.06 ± 1.07 ($p \geq 0.10$). In a post hoc analysis, there was no significant difference in the percentage of bowel movements at baseline with a BSS score of ≤2 (constipation) in the Probiotic and Placebo groups, respectively ($p = 0.137$) (Table 1). From baseline at day 42, there was a significant decrease in the percentage of bowel movements per week with a BSS score of ≤2 (constipation) for participants supplemented with the Probiotic compared to those on Placebo ($p < 0.001$) (Figure 3B).

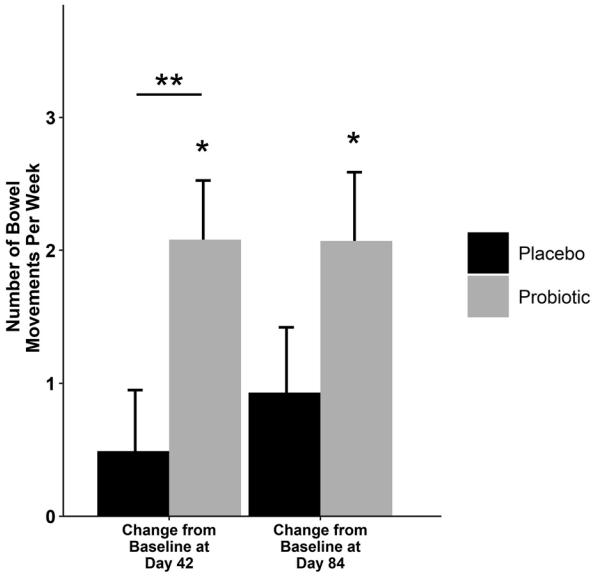

Figure 2. Change in bowel movements. Change in number of bowel movements per week from baseline at days 42 and 84. The graphs are presented as mean and SEM; * indicates significant within-group difference; ** indicates significant between-group difference; – indicates between-group comparison.

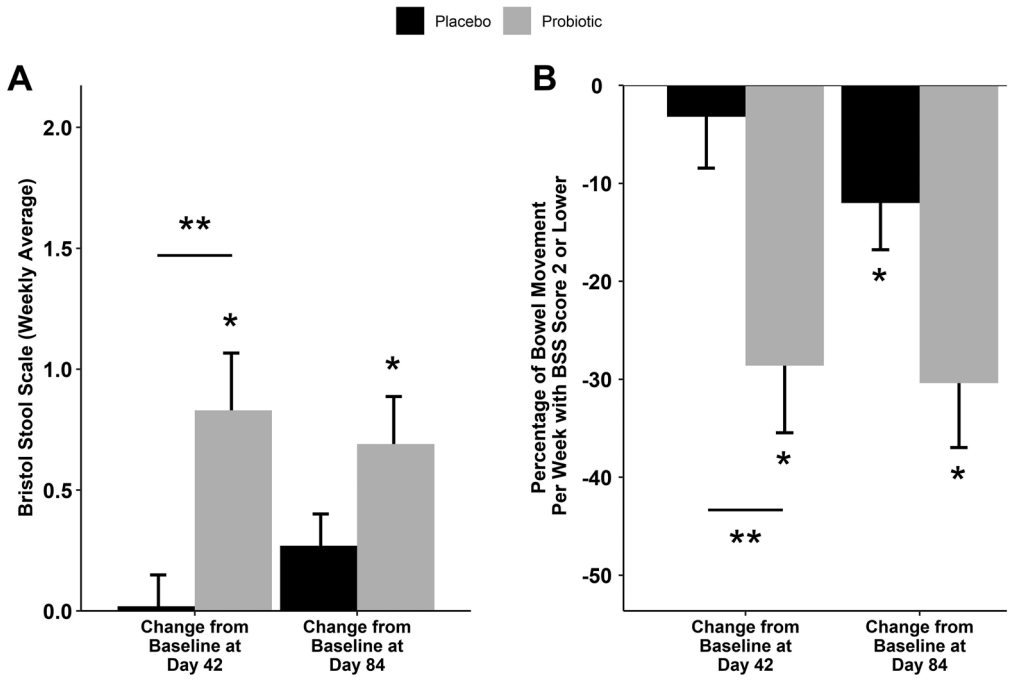

Figure 3. Change in stool consistency. (A) Absolute bristol stool scale (BSS) from baseline at days 42 and 84. (B) Percentage of bowel movements per week with a BSS of ≤ 2 from baseline at days 42 and 84. The graphs are presented as mean and SEM; * indicates significant within-group difference; ** indicates significant between-group difference; – indicates between-group comparison; BSS, bristol stool scale.

Gastrointestinal symptoms

There were no significant differences between the Probiotic and Placebo groups in gastrointestinal symptoms at baseline and following supplementation. Participants supplemented with the Probiotic showed significant improvements in total GSRS scores on days 42 and 84 (within-group $p < 0.001$ for both timepoints), respectively, while those taking the Placebo also had significant improvements at both timepoints (within-group $p < 0.001$ for both timepoints) (Table 2). Further, both groups showed significant improvements in indigestion and constipation symptoms after 84 days of supplementation ($p < 0.05$).

Perceived stress and quality of life

At baseline, the mean PSS scores were not significantly different between the Probiotic and Placebo groups (9.91 ± 5.48 vs. 10.62 ± 5.85, respectively, $p = 0.61$). All participants reported low or moderate stress, and none of the participants had PSS score >26 classified as 'high' stress. Participants supplemented with the Probiotic had a significant reduction in PSS scores after 84 days of supplementation (change in score: −1.62 ± 3.97, within-group $p = 0.048$), while the Placebo group was unchanged (change in score: −0.21 ± 5.12; $p = 0.807$) (Figure 4). Similarly, there was no significant difference between the Probiotic and Placebo groups in the proportion of participants with moderate stress (21.9% vs. 37.9%, respectively) at baseline (between-group $p = 0.261$). At day 84, the proportion of participants in the Probiotic group compared to the Placebo group with moderate PSS score was significantly lower (9.4% vs. 37.9% respectively, between-group $p = 0.013$). There were no significant changes in physical and mental health subscale scores of the SF-12 questionnaire in the Probiotic and Placebo groups.

Table 2. Gastrointestinal Symptoms Rating Scale (GSRS) total score during the study period ($n = 61$).

Variable	Placebo ($n = 29$)	Probiotic ($n = 32$)	p Value between groups [95% CI]
Baseline (Day 0)	1.96 ± 1.12	2.16 ± 1.43	0.564 0.18 [-0.41 to 0.77]
Day 42	1.35 ± 0.74	1.56 ± 1.33	0.553 0.18 [-0.41 to 0.78]
Day 84	1.35 ± 1.01	1.37 ± 1.40	0.998 −0.00 [-0.59 to 0.59]
Change from baseline at Day 42	−0.61 ± 0.83	−0.60 ± 0.93	0.984 0.00 [-0.45 to 0.46]
p Value within group from baseline at Day 42	<0.001	<0.001	
95% CI	−0.61 [-0.93 to −0.28]	−0.60 [-0.91 to −0.29]	
Change from baseline at Day 84	−0.61 ± 0.90	−0.79 ± 1.21	0.443 −0.18 [-0.63 to 0.27]
p Value within group from baseline at Day 84	<0.001	<0.001	
95% CI	−0.61 [-0.94 to −0.28]	−0.79 [-1.10 to −0.48]	

Data presented as mean ± SD; n, number; SD, standard deviation;
p values were calculated using linear mixed models adjusted for intervention period

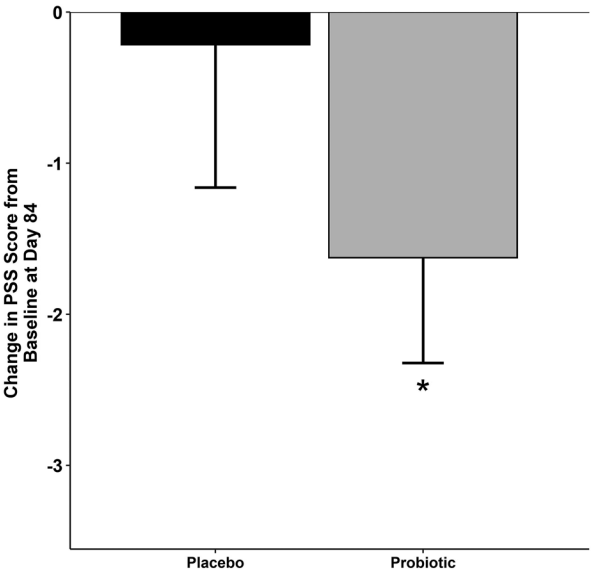

Figure 4. Change in perceived stress scale score from baseline at day 84. The graph is presented as mean and SEM; * indicates significant within-group difference; PSS, perceived stress scale.

Nutrient uptake

A total of 35 participants were included in nutrient uptake analysis in the PP population. The concentrations of nutrients in the plasma following supplementation were not significantly different between the Probiotic and Placebo groups. Regarding the changes in concentrations from baseline, both groups showed increases in plasma vitamins B_{12} and B_6 at days 42 and 84. Similarly, significant changes from baseline were also observed in plasma vitamins A, C, E magnesium, folate, and protein levels at one or both timepoints in the Probiotic and/or Placebo groups (Supplementary Table S3). Of note, these changes in nutrient concentrations remained within the normal range (Pai 2022; Sood et al. 2024; Mayo Clinic: Mayo Medical Laboratories, n.d.; Medical Council of Canada: List of Normal Lab Values., 2020; Merck Manual Professional Version, 2024). Additionally, the findings suggest that the changes were not time dependent as it relates to the multivitamin and mineral supplement intake over the course of the study.

Safety

Probiotic supplementation for 84 days was found to be safe and well tolerated in the population investigated. All hematology and clinical chemistry values outside the normal laboratory range were deemed not clinically relevant by the MD. A total of 47 post-emergent AEs were reported by 23 unique participants, with a total of 27 in the Probiotic group and 20 in the Placebo group. None of the AEs were classified as 'possibly' or 'probably related' to study products. All AEs resolved by the end of the study period or upon subsequent follow-up.

Discussion

Occasional constipation is defined as intermittent or symptomatic alterations in bowel habits, including a distressing reduction in the frequency of BMs and/or difficulty with the passage of stools without alarming features (Rao et al. 2022). Findings from previous studies investigating probiotic supplements in managing gastrointestinal disorders are promising (Lorenzo-Zúñiga et al. 2014; Parker et al. 2018; Zhang et al. 2020; Sato et al. 2022; Cano-Contreras et al. 2022), providing support to the use of safe and efficacious options to improve an individual's quality of life, possibly without the need for pharmaceutical intervention. This study provides important evidence demonstrating the efficacy and safety of long-term supplementation of a probiotic blend of KABP031 and KABP032 in healthy older adult population with occasional constipation. Enrolled participants reported at least one of the following at baseline: (1) ≤5 BMs per week and 25% of BMs with hard stool consistency (BSS type 1 and 2) collectively with excessive straining for most of the BMs, (2) at least 50% of BMs with hard stool consistency (BSS type 1 and 2), or (3) ≤3 complete spontaneous BMs per week. There were significant improvements in BM frequency and average consistency observed after 42 days of supplementation, consistent with improvements in constipation. This was further supported by a significant decrease in the percentage of BMs having a stool consistency (BSS ≤2) with Probiotic supplementation, compared to Placebo. This equated to a minimal clinically important difference (MCID) in weekly BM frequency with mean increases of 2.08 and 2.07 BM following 42 and 84 days of Probiotic supplementation, respectively, whereas the Placebo group had increases of 0.49 and 0.93 BM. It is important to note that the statistical model used in this study was adjusted for baseline. Although established in a diseased population, a previous study of 813 individuals suffering from chronic functional constipation and treated with a non-pharmacological intervention to reduce the severity of their constipation symptoms, reported an MCID of ≥1.6 BM per week (Ai et al. 2022). Overall, probiotic supplementation resulted in improvements in frequency of BM previously established to potentially provide meaningful and valuable change for participants (McGlothlin and Lewis 2014). Given the observed significant improvements in the frequency of BMs from baseline in individuals supplemented with the Probiotic, significant differences compared to the Placebo may have been achieved with a larger sample size. Therefore, future studies investigating bowel function should consider a larger sample size while exploring MCID for frequency of BMs in healthy populations.

As reflected in the defining criteria for occasional constipation, improvements in stool consistency may also be an important component for providing relief and establishing meaningful change for these individuals. Participants supplemented with the probiotic blend had significantly greater improvements in stool consistency compared to Placebo after only 42 days of supplementation, further demonstrating the beneficial effect of the probiotic blend in improving overall bowel function. There was an improvement in stool consistency at day 84 as well, but the difference between the Probiotic and Placebo groups only approached statistical significance ($p = 0.058$). It is possible the improvement in BM frequency and consistency not reaching statistical significance is the result of the Placebo group improving over time due to the transient nature and natural course of occasional constipation. Participants may have enrolled

in the study at a time where their occasional constipation was worse, for which changes over the course of the study may have naturally occurred.

A growing body of evidence has demonstrated that changes in the composition of gut microbiota impact both stool frequency and consistency (Vandeputte et al. 2016; Kwon et al. 2019). Notably, disturbances in the composition of gut microbiota have been observed in individuals with constipation (Mancabelli et al. 2017). Beneficial lactic acid bacteria play an important role in digestion by facilitating breakdown of dietary fibers and producing several biologically active metabolites such as short-chain fatty acids (SCFAs) in the gut. Short-chain fatty acids affect gut motility by stimulation of central and enteric nervous system, increasing intestinal concentration of excitatory neurotransmitters or directly acting on gut smooth muscle. On the other hand, a reduction in fecal water content is associated with longer intestinal transit times, which negatively impacts microbial growth and metabolic activity (Procházková et al. 2023). Thus, improvement in stool consistency in our study is indicative of improved intestinal transit time and water content (Lewis and Heaton 1997). Furthermore, as individuals age there is a general decrease in bacterial diversity in the gut, with a decline in beneficial microorganisms such as *Bacteroides* spp, bifidobacteria, and lactobacilli species, and an increase in facultative anaerobes such as staphylococci and Enterobacteriaceae (Claesson et al. 2011). Overall, a shift in microbial diversity and concurrent changes in concentrations of metabolites may have a direct impact on digestion, secretory functions, and intestinal motility. Additionally, aging negatively affects physiological coping mechanisms against stressors causing increased inflammatory responses and increased intestinal permeability that weakens the immune system (Salminen et al. 2008; Shalim et al. 2019). Previous studies investigating the probiotic features of *L. plantarum* strains KABP031 and KABP032 demonstrated survivability in the GI tract allowing antimicrobial activity and improvement in systemic immunity in elderly (Mañé et al. 2011; Bosch et al. 2012).

The findings of this study showed significant improvements in total GSRS scores in participants in both the Probiotic and Placebo groups. This is to be expected as subjective measures such as self-reported questionnaires are reported to be more prone to a Placebo effect (Estevinho et al. 2018) as well as recall bias. This may provide a rationale for the lack of significant improvements in objective measures such as BM and stool consistency but significant improvements in subjective total GSRS scores in the Placebo group. Considering the association between subjective measures and the Placebo effect, improvements in BM and stool consistency may be a better indicator of improved bowel function. Although gut microbiota composition nor microbial metabolites were measured in this study, which is a limitation, our hypothesis is that improvements in stool frequency and consistency in older adults following 42 days and 84 days of supplementation, with the two *L. plantarum* strains, may have been the result of favorable changes in the composition of the microbiota and/or gut environment. A recent systematic review has demonstrated improvements in functional constipation were accompanied by changes in the relative abundances of specific strains (Ding et al. 2024). However, this hypothesis must be confirmed in future studies in a similar population and probiotic strains used in the current study.

The findings of the current study suggest that improvements in frequency of BM and stool consistency may have had corresponding benefits to participants' mental health and general well-being. Probiotic supplementation for 84 days significantly reduced

perceived stress levels, moving more than 50% of participants with a PSS score between >13 and ≤26 to score of ≤13 after 84 days of supplementation. Previous studies in individuals with functional constipation reported a higher prevalence of anxiety, depression, and psychosocial distress compared to those with normal bowel function (Mason et al. 2000; Nehra et al. 2000). Although the majority of participants reported what is considered to be low baseline levels of stress, the findings of the current study suggest that the probiotic blend may have improved perceived stress due to improvements to overall digestive health. It has been reported that stress can negatively affect gut microbiota (Knowles et al. 2008), increase intestinal permeability, and indirectly inhibit intestinal motility by activating the enteric nervous system. A randomized controlled trial investigating the effect of an 84-day probiotic supplementation on stress and GI symptoms in community-dwelling older adults demonstrated significant improvements in both stress scores and GI symptoms (Kim et al. 2021). Further, individuals with depressive symptoms have been found to have changes in gut microbiota with an increase in pro-inflammatory species (Jiang et al. 2015). Therefore, digestive disorders and mood disorders may reflect dysfunctional composition of gut microbiota and associated chronic inflammation. Emerging evidence suggests that the gut microbiome and the brain communicate in a bidirectional manner (Osadchiy et al. 2019). Therefore, probiotics have gained increasing attention for their potential to regulate brain health *via* the gut-brain axis. A recent meta-analysis based on randomized controlled trials revealed that probiotic supplementation can reduce stress levels in healthy volunteers and may alleviate stress-induced anxiety and depression (Zhang et al. 2020). The findings of this study suggest that the probiotic blend may not only alleviate irregular BM and GI symptoms but may also influence the gut-brain axis and improve the mental well-being of an older adult population. However, the direct effect of probiotic supplementation on stress was not determined in the current study and warrants further investigation.

A growing body of evidence suggests a bidirectional relationship between dietary nutrients and gut microbiota (Barone et al. 2022). Nutrients are utilized by microorganisms for growth and metabolic activities that are essential for maintaining gut microbial functions and their composition. On the other hand, the gut microbiome regulates nutrient bioavailability by influencing nutrient absorption and synthesis of essential nutrients such as B vitamins (Barone et al. 2022). Therefore, age-associated changes in microbial diversity may act as a risk factor for nutritional deficiencies in older adults. Dietary supplementation with multivitamins, minerals and/or probiotics may influence the composition of the gut microbiome, facilitating colonization of beneficial bacteria in the colon and adequate nutrient absorption. Albumin and total protein levels in the participants in this study were within normal range with no clinically relevant changes following probiotic supplementation for 84 days. Although an increase in albumin and protein levels within normal ranges may indicate an improvement, maintenance of albumin and total protein levels during the study period suggests adequate nutritional status and normal hepatic and renal function in our study population (Moman et al. 2022). Further, weight maintenance is important for healthy aging as involuntary weight loss in older adults is indicative of nutritional deficiency, which is associated with higher mortality risk (Park et al. 2018). Notably, participants in both groups had significant increases in their weight from baseline, which further supports their adequate nutritional status during the study period, but this was not different between groups.

This study did not include a follow-up period and therefore, the effect of the probiotic blend on bowel function and GI symptoms in the post-supplementation period was not determined. Further, this study did not examine changes in inflammatory markers, or any microbiome analysis including metabolites, microbial diversity or analysis of the individual probiotic strains following supplementation. Therefore, although compliance was assessed through return of study products and confirmation through study diaries, the evaluation of compliance through analysis of fecal samples was a limitation of this study. Future studies investigating mechanisms by which the probiotic blend exerts its beneficial effect on the GI system are warranted. In this study, nutrient uptake following study product and multivitamin supplementation was examined in a subset of study participants ($n = 35$). The changes in concentrations of vitamins B_{12}, B_6, C, and E over the study period in the Probiotic group were not significantly different compared to the Placebo group, which may have been impacted by the small subgroup of participants included in the analysis and large inter-individual variations. Future studies with larger sample size in populations with high risk of nutritional deficiencies are warranted to investigate the effect of the probiotic blend on nutrient uptake. Further, the findings of this study suggest that the probiotic blend may positively influence the mental health and well-being of individuals who experience stress due to GI problems. However, future studies may consider exploring the efficacy of the probiotic blend in moderately stressed individuals independent of GI disturbances, as literature suggests that the gut microbiome may play a role in stress regulation.

Conclusion

This study demonstrated that 84-day supplementation with a probiotic blend of KABP031 and KABP032 *L.plantarum* improved bowel function and mental well-being in an otherwise healthy population of older adults with occasional constipation. Specifically, the probiotic blend demonstrated early efficacy in the improvement of frequency and consistency of bowel movements after 42 days of supplementation, highlighted by significantly greater improvement compared to Placebo. Notably, this was associated with a reduction in the percentage of bowel movements with ≤ type 2 stool consistency (constipation) for participants supplemented with the Probiotic vs. Placebo, further supporting the early efficacy of the probiotic blend on bowel function in this population. Improvements in bowel habits and GI symptoms corresponded with a significant reduction in perceived stress from baseline in the Probiotic group and a significantly lower proportion of participants reporting moderate stress compared to Placebo at day 84. The current study demonstrated that supplementation with the probiotic blend alleviated GI disturbances in the studied population, providing improvements in bowel functions, GI symptoms, and stress levels.

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Author contributions

CRediT: **Jordi Espadaler-Mazo**: Conceptualization, Writing – review & editing.

Author's contributions

FA, MRP, MA, MW, JEM, EDL, ME, NG designed research; EDL, ME, RGS, MM, DCC conducted research; RGS, MM, HA, DCC analyzed data; RGS, MM, ME, EDL wrote the paper; FA, MRP, MA, JEM, HA, MM and EDL revised the paper. All authors read and approved the final manuscript.

Disclosure statement

FA, MRP, MA and JEM are employees of AB-Biotics SA, FA and MW are employees of Kaneka Americas Holding Inc., RGS, MM, EDL, HA, DCC., and NG are employees of KGK Science Inc., ME is an adjunct professor at Western University.

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Data availability statement

Data will be made available from the corresponding author upon reasonable request.

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