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Full Paper

Effects of *Bifidobacterium longum* CLA8013 on bowel movement improvement: a placebo-controlled, randomized, double-blind study

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A placebo-controlled, randomized, double-blind study was conducted to evaluate the effect of taking 25 billion colony-forming units of heat-killed *Bifidobacterium longum* CLA8013 over 2 weeks on bowel movements in constipation-prone healthy individuals. The primary endpoint was the change in defecation frequency between the baseline and 2 weeks after the intake of *B. longum* CLA8013. The secondary endpoints were the number of days of defecation, stool volume, stool consistency, straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, abdominal bloating, fecal water content, and the Japanese version of the Patient Assessment of Constipation Quality of Life. A total of 120 individuals were assigned to two groups, 104 (control group, n=51; treatment group, n=53) of whom were included in the analysis. After 2 weeks of consuming the heat-killed *B. longum* CLA8013, defecation frequency increased significantly in the treatment group compared with that in the control group. Furthermore, compared with the control group, the treatment group showed a significant increase in stool volume and significant improvement in stool consistency, straining during defecation, and pain during defecation. No adverse events attributable to the heat-killed *B. longum* CLA8013 were observed during the study period. This study revealed that heat-killed *B. longum* CLA8013 improved the bowel movements of constipation-prone healthy individuals and confirmed that there were no relevant safety issues.

Key words: *Bifidobacterium longum*, bowel movements, stool consistency, straining during defecation, pain during defecation, postbiotics

INTRODUCTION

The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [1], and lactic acid bacteria and bifidobacteria are the most common probiotics [2, 3]. Inactivated probiotic, or postbiotic, are defined as "preparation of inanimate microorganisms and/or their components that confers a health benefit on the host" [4], whereas paraprobiotic are defined as "inactivated microbial cells (non-viable) that confer a health benefit on the consumer" [5]. The actions of probiotics and their ingredients, postbiotics and paraprobiotics, vary by strain and include immunostimulation [6], skin improvement [7], and anti-obesity effects [8]; one of their major effects is improved bowel movements [9]. Of the probiotics used as medicines, the *Bifidobacterium bifidum* G9-1 strain has been reported to improve constipation symptoms in patients with chronic constipation [10]. In foods, various probiotics, postbiotics, and paraprobiotics are used in supplements, drinks, yogurt, and other products. Some probiotics, postbiotics, and paraprobiotics that have been confirmed as effective and safe for human consumption in Japan have been designated as functionally active ingredients and included in foods with function claims [11].

Functional constipation, characterized by abnormal bowel movements, is a health problem that affects quality of life (QOL) [12], with a reported global prevalence of 14%–20% in adults [13, 14]. The Rome IV criteria are the international diagnostic criteria for functional constipation. These criteria are used to evaluate and diagnose defecation difficulty in terms of low defecation frequency, straining during defecation, hard stools, and feeling of incomplete evacuation. Functional constipation is diagnosed when two or more of the following criteria are met: frequency of defecation must occur less than three times per week and other criteria must occur more often than once in every four

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defecations [15]. However, constipation-prone individuals with defecation problems do not always meet these diagnostic criteria. In Japan, foods with function claims that contain functionally active ingredients for improving bowel movements, such as those that contain dietary fiber, oligosaccharides, and probiotics, are targeted at individuals prone to constipation. Defecation frequency is commonly used to evaluate the efficacy of these functionally active ingredients in improving constipation [16–18]. Meanwhile, the constipation scoring system questionnaire suggests that hard stools, straining, and pain during defecation have a significant impact on QOL, in addition to frequency of defecation [19]. Therefore, to improve the QOL of individuals prone to constipation, the improvement of straining, pain, frequency of defecation, and other symptoms affected by hard stools is also important. However, to the best of our knowledge, no food containing functionally active ingredients in Japan has demonstrated efficacy against these symptoms in addition to frequency of defecation.

As the firmness of stool depends on its water content [20], we hypothesized that by promoting water secretion into the intestinal tract and hydrating the stool, it can be softened, thereby alleviating painful bowel movements. Chloride channels in the intestinal epithelium are thought to be involved in water secretion into the intestinal tract [21]. Therefore, we screened beneficial microbes that activate chloride channels and promote water secretion into the intestinal tract, and we found *Bifidobacterium longum* CLA8013, a human-derived *Bifidobacterium* that shows high activity against chloride channels on intestinal epithelial cells [22]. This bacterium was confirmed to improve bowel movements in animal models of constipation (unpublished). Surprisingly, the function of *B. longum* CLA8013 in improving bowel movements was maintained even when using the heated-killed form of the bacteria.

Hence, to develop a food with a function claim using heat-killed *B. longum* CLA8013 as a postbiotic, we confirmed its effect in individuals who are prone to constipation in an exploratory study and found that it tended to improve bowel movements. Therefore, we conducted a randomized, placebo-controlled, double-blind study of *B. longum* CLA8013 in healthy individuals prone to constipation to evaluate its effects on symptoms related to QOL, such as straining and pain during defecation, in addition to improving bowel movements.

MATERIALS AND METHODS

Preparation

Capsules containing 25 billion colony-forming units (CFUs) of heat-killed *B. longum* CLA8013 were used as the test food. Placebo capsules, identical in appearance to the test food but not containing *B. longum* CLA8013, were used as the control food. The manufacture of both capsules was outsourced to API Co., Ltd (Gifu, Japan; Table 1).

Participants

In accordance with the principles of the Declaration of Helsinki and the Ethical Guidelines for Medical Research Involving Human Subjects, this study was approved by the Ethics Review Committee of Kobuna Orthopedic Clinic (approval date, August 5, 2021; approval number, MK-2108-01) and was registered and published in the UMIN clinical trial registration system (clinical trial registration number: UMIN000045353). Individuals were fully informed of the study details by the investigator, and written consent to participate of their own free will was obtained prior to registration. This study was conducted at a clinic in Tokyo. Medical interviews and examinations were conducted by physicians and nurses at the Shinagawa Season Terrace Health Care Clinic. KSO Corporation cooperated with this study as contract research organization and recruited the participants. Of the 651 participants who agreed to participate, 200 were considered eligible based on the inclusion and exclusion criteria.

The inclusion criteria were as follows: (1) healthy men and women aged 20–65 years at the time of providing consent to participate in the study; (2) regular dietary intake of at least two meals per day; (3) tendency for constipation with 3–5 spontaneous bowel movements per week; (4) tendency for constipation with an average Bristol Stool Form Scale (BSFS) type of less than 4; and (5) fully informed of the purpose and content of the study, had the ability to provide consent, and provided written consent to participate in the study based on a thorough understanding of its purpose and content.

The exclusion criteria were as follows: (1) BSFS types 1–2 for more than one out of every four bowel movements; (2) regular use of oral medications (e.g., laxatives and bowel regulators); (3) consumption of health foods (including food for specified health uses and functional foods) that were considered beneficial for improving constipation at the time of the screening test; (4) use of antimicrobial agents within 2 weeks prior to the screening test; (5) inability to discontinue the intake of foods containing viable

Table 1. Composition and nutrition of capsules

		Test capsule	Placebo capsule
Composition	Capsule contents	Heat-killed *B.longum* CLA8013 (25 Billion CFUs), Microcrystalline cellulose, Calcium stearate, Silicon dioxide	Microcrystalline cellulose, Calcium stearate, Silicon dioxide
	Capsule shell	Pork gelatin, Titanium dioxide, Lecithin from soybean	Pork gelatin, Titanium dioxide, Lecithin from soybean
Nutrients	Calories (kcal)	0.3766	0.3121
	Carbohydrates (g)	0.0115	0.0009
	Proteins (g)	0.0698	0.0649
	Lipids (g)	0.0060	0.0057
	Salt equivalents (g)	0.0003	0.0002

CFUs: colony-forming units.

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bacteria (e.g., lactobacilli, bifidobacteria, and *Bacillus natto*), foods fortified with oligosaccharides and dietary fiber, health foods (including foods for specified health uses and foods with function claims) that are believed to improve constipation, and foods containing large amounts of sugar alcohols, antibacterial agents, laxatives, or bowel preparations during the screening period; (6) consumption of large amounts of alcohol (more than approximately 40 g of pure alcohol equivalent) on a daily basis; (7) a history of digestive diseases or surgery affecting digestion, absorption, or defecation; (8) presence of chronic diseases (including irritable bowel syndrome and inflammatory bowel disease) and regular drug use; (9) allergies to foods or medicines; (10) presence of other serious diseases; (11) deemed unsuitable for study participation based on answers provided in the background questionnaire; (12) pregnancy, the intention to become pregnant during the study period, or breastfeeding; (13) participation in or the intention to participate in other studies, or participation in other clinical studies within 1 month of providing consent; and (14) considered unsuitable by the principal investigator for other reasons.

A control food intake screening was conducted over a period of 2 weeks to exclude participants with an excessive response to the placebo. During the control food intake screening period, those showing excessive changes in defecation frequency before and after the screening test were excluded. Finally, 120 healthy participants (26 men and 94 women, aged 46.1 ± 9.1 years) who met the inclusion criteria but not violate the exclusion criteria were selected.

The criteria for discontinuation were as follows: (1) the occurrence of serious adverse events and determination by the investigator or sub-investigator to terminate the individual's participation; (2) subjective or objective symptoms leading to the investigator or sub-investigator determining that the individual is unable to continue in the study; (3) discovering that the participant consumed foods containing live bacteria (e.g., lactobacilli, bifidobacteria, or *Bacillus natto*), foods fortified with oligosaccharides or dietary fiber, health foods (including foods for specified health uses and foods with function claims) that are proposedly effective for improving constipation, or foods containing large amounts of sugar alcohols or that the participant used antibacterial agents, laxatives, or intestinal regulators on multiple occasions during the study period; (4) becoming pregnant during the study period; (5) discovering that a participant participated in another study for which other foods were ingested, drugs or quasi-drugs were used, or cosmetics or drugs were applied during the study period or if 1 month had not passed since participation in that study before participating in this study; (6) discovering, after commencement of the study, that an individual was not eligible based on the inclusion or exclusion criteria; (7) non-compliance with the instructions provided by the principal investigator or sub-investigator; and (8) determination by the investigator or sub-investigator that participation in the study should be terminated.

In addition, participants were instructed as follows: (1) The test food should be consumed as instructed by the investigator. (2) Only the participant themselves should consume the test food. (3) No food or drink other than water should be consumed after 21:00 on the day prior to the test day. (4) The same lifestyle as before the study should be maintained (e.g., no binge drinking or eating, dieting, diet changes due to travel, abruptly stopping doing exercise, or starting new exercises). (5) During the study period, participants should refrain from consuming more than an appropriate amount of alcohol (approximately 40 g of pure alcohol equivalent per day on average) at any time. (6) The use of medicines and quasi-drugs that may improve constipation, such as intestinal regulators and laxatives, should be avoided as much as possible. Finally, (7) participants should refrain from consuming foods containing live bacteria (e.g., lactobacilli, bifidobacteria, and *B. natto*), foods fortified with oligosaccharides and dietary fiber, health foods (including foods for specified health uses and foods with function claims) that are proposed to improve constipation, and foods containing large amounts of sugar alcohols.

Intervention schedule

This was a placebo-controlled, randomized, double-blind study (Fig. 1), and the study period was from August 2021 to November 2021. The 120 participants were randomized by a statistical analyst into two groups to avoid bias in age, sex, and defecation frequency during the control food intake screening period. The JMP data analysis software (JMP Statistical Discovery LLC, Cary, NC, USA) was used for randomization, and multiple random number tables were used to divide the groups. An assignment manager who was not involved in the conduct of the study allocated the participants to two groups, one receiving the test food and the other receiving the control food. The allocation tables were kept confidential by the assignment manager until data analysis. The study consisted of a 2-week pre-observation period followed by a 2-week intervention period (test or control food intake). Participants were instructed to take one capsule per day with water after dinner. If they missed consuming the test food, they were asked to take it on the same day and not to take double the dose (two capsules) on the following day.

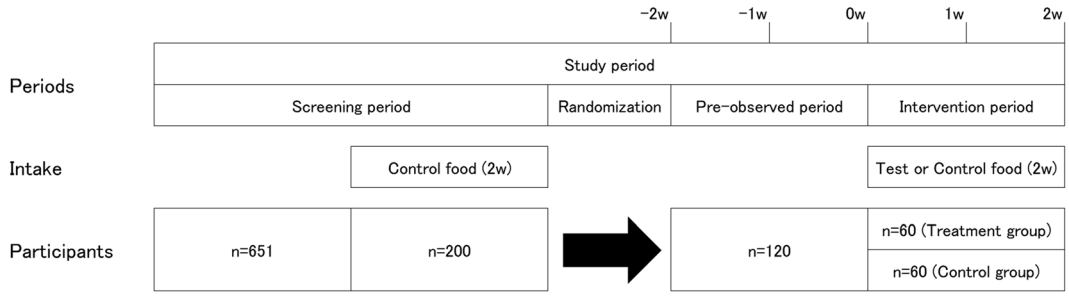

Fig. 1. Intervention schedule during the study period of August 2021 to November 2021.

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Outcome measures

The primary endpoint was the change in defecation frequency between the pre-observation period and the 2 weeks after the intake of *B. longum* CLA8013 (intervention period).

The secondary endpoints were the change in defecation frequency between the pre-observation period and the week after the intake of *B. longum* CLA8013 and the change in the number of days of defecation, stool volume, stool consistency, straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, abdominal bloating, fecal water content, and the Japanese version of the Patient Assessment of Constipation-QOL questionnaire (JPAC-QOL) score [23] between the pre-observation period and at 1 and 2 weeks after the intake of *B. longum* CLA8013.

A safety evaluation was also performed that included a medical interview, evaluation of subjective and objective symptoms (adverse events), and measurement of body mass index (BMI).

Medical interviews and examinations were conducted by a physician on the first day of the intervention period and on the day after the intervention period concluded. For a total of 4 weeks (the pre-observation period and the intervention period), participants recorded whether or not they had consumed the test food and the time of intake (during the intake period only). They also recorded whether they had used medicines; the number of bowel movements, stool volume, and stool consistency; whether they had menstruated; any other changes in their physical condition (mainly gastrointestinal symptoms); and any changes in their living situation.

The stool investigation was conducted according to the following criteria: (1) For defecation frequency, the number of defecations per day was assessed (defecation frequency was set to 0 on days with no defecation), and the number of defecations per week was totaled. (2) For number of days of defecation, the number of days per week on which defecation occurred was counted. (3) For stool volume, the stool volume per week was scored per visual measurement by the participants using one medium-sized chicken egg as a reference (if the value could not be divided by one chicken egg, 0.5 increments were used, such as 0.5 or 1.5 eggs). (4) For stool consistency (BSFS), the applicable form was selected from type 1 to type 7 (type 1, hard lumps; type 2, sausage shaped but lumpy; type 3, like a sausage but with cracks on the surface; type 4, like a sausage or snake, smooth and soft; type 5, soft blobs with clear-cut edges; type 6, fluffy pieces with ragged edges, a mushy stool; type 7, watery, no solid pieces, entirely liquid) [24]. In addition, the following symptoms were evaluated: straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, and abdominal bloating. Each symptom was measured using a visual analogue scale (VAS; integer value from 0 to 100 mm). To evaluate fecal water content, one stool sample was collected by the participants themselves during each period of the last 5 days of the pre-observed period (before intake) and the last 5 days of the intervention period (after intake); each sample was then transported in a refrigerated condition to the testing center for assessment. The assessment method comprised drying 1.0 g of feces at 80°C for 2 days, and the moisture content was then calculated by comparing the weight of the fresh feces with that of the dried feces. The JPAC-QOL was used to obtain total scores and subscores (physical discomfort, psychosocial discomfort, worries/concerns, and satisfaction) at the start of the intervention (week 0) and 2 weeks after the intervention period.

Sample size calculation

The number of participants required for this study was calculated based on the difference of 0.96 and standard deviation of 1.5 between the control and treatment groups obtained in our exploratory study. Assuming a two-tailed significance level of 5% and 90% power, the required number of participants was 52 participants per group. Considering dropout cases, the number of participants per group was set at 60.

Statistical analyses

Participants who consumed the test food at least once, the full analysis set (FAS), were included in the analysis of the safety endpoints, and those who were excluded from the FAS according to the rejection criteria, the per-protocol set (PPS), were used for the analysis of the efficacy endpoints. The rejection criteria were as follows: (1) The test food was consumed on fewer than 80% of the total number of intervention days. (2) The participant was found to be ineligible after completion of the study based on the inclusion and exclusion criteria. (3) The participant was found to have violated the instructions after completion of the study. (4) The investigator deemed the participant unsuitable to be included in the study for another justifiable reason.

Measurement values including those that were considered outliers were used in the evaluation; however, if the cause was clearly found to be a measurement error, the value was excluded from the analysis. Missing values were not compensated and were not included in the analysis.

The baseline values (0 weeks) for each endpoint were as follows. For defecation frequency, days of defecation, and stool volume, the average of the number per week for weeks 1 and 2 of the pre-observational periods was used as the baseline value. For stool consistency, straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, and abdominal bloating, the mean value of the number per defecation in the pre-observational period was used as the baseline value. For fecal water content and the JPAC-QOL score, the starting values were used as the baseline values.

For statistical analysis of the primary and secondary endpoints, the mean and standard deviation of the values at baseline (0 weeks) and weeks 1 and 2 of the intervention period and of the change from baseline (0 weeks) to weeks 1 and 2 of the intervention period were determined for each group. For both primary and secondary endpoints, including days of defecation, stool volume, straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, and abdominal bloating, the change from baseline (0 weeks) to weeks 1 and 2 after the start of the intervention was calculated, and an unpaired t-test without correspondence between the control and treatment groups was performed. For stool consistency, the change from baseline (0 weeks) to weeks 1 and 2 after intake was calculated, and Wilcoxon's rank sum test was performed for the control and treatment groups. The mean and change in fecal water content from baseline to 2 weeks of intervention were determined using unpaired t-tests for the control and treatment groups. The change in the JPAC-QOL score from baseline (0 weeks) to 2 weeks of intervention was determined using Wilcoxon's rank sum test for the control and

treatment groups. The two-tailed significance level was set at 5%. The secondary endpoints were analyzed without considering multiplicity because they were considered exploratory and aimed at supplementing the interpretation of the analysis results for the primary endpoints. IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analysis.

RESULTS

Participants

A breakdown and flowchart of the participants are shown in Fig. 2. A total of 120 participants consumed test foods for at least 1 day (FAS). Two participants discontinued their involvement in the study—one participant owing to treatment being deemed necessary for infectious gastroenteritis (criterion for discontinuation 2) and the other owing to non-compliance (criterion for discontinuation 7). Furthermore, 14 participants were excluded based on the rejection criteria—one due to consuming less than 80% of the prescribed test food (rejection criterion 1), four because they were found to be ineligible after completion of the study based on the inclusion or exclusion criteria (rejection criterion 2), and nine due to having violated the instructions (rejection criterion 3). Finally, 104 participants were included in the efficacy evaluation analysis as the PPS. The 104 participants included 51 in the control group and 53 in the treatment group. The control group contained 12 men and 39 women who were 48.0 ± 8.4 years old, while the treatment group included 10 men and 43 women who were 45.6 ± 9.3 years old (Table 2). No differences were found in age, height, weight, or defecation frequency per week between the two groups.

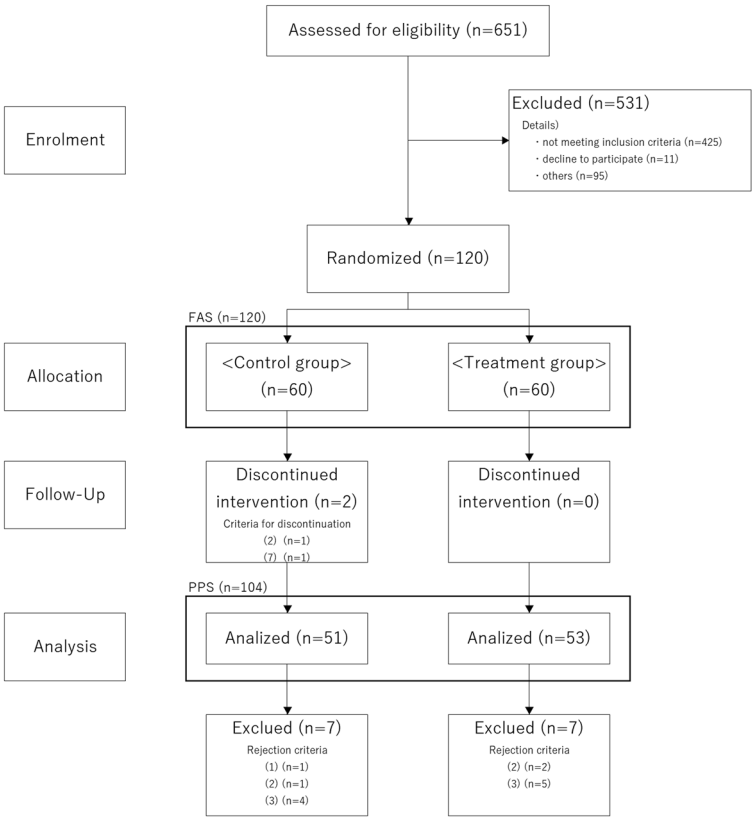

Fig. 2. Flow diagram for this study. A total of 651 individuals were registered and assessed for eligibility; 120 participants participated in the study. Of the 120 enrolled participants, data from 104 participants (control group, n=51; treatment group, n=53) were analyzed in the study.
FAS: full analysis set; PPS: per-protocol set.

Table 2. Baseline characteristics (per-protocol set: PPS)

	Control (n=51)	Treatment (n=53)	p-value
Gender (Male/Female)	12/39	10/43	-
Age (years)	48.0 ± 8.4	45.6 ± 9.3	0.183
Height (cm)	162.5 ± 8.4	160.5 ± 8.3	0.233
Weight (kg)	57.3 ± 11.3	55.3 ± 9.3	0.310
BMI (kg/m^2)	21.5 ± 2.7	21.4 ± 2.8	0.773
Defecation frequency (times/week)	4.02 ± 0.91	3.97 ± 0.93	0.791

Mean ± Standard deviation.
The analysis was performed using the unpaired t-test.

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Efficacy evaluation

The results for each endpoint are shown in Tables 3–5.

The primary endpoint, change in defecation frequency, was significantly increased in the treatment group compared with that in the control group after 2 weeks of *B. longum* CLA8013 consumption (p=0.048), with an increase of 1.16 ± 1.93 times/week.

Regarding the secondary endpoints, the change in the number of days of defecation per week was not significantly different between the groups. The stool volume per week was significantly

Table 3. Comparison of defecation frequency, number of days defecation, and stool volume scores

		0w	1w	2w	1w-0w		2w-0w	
					Δ	p-value	Δ	p-value
Defecation frequency (times/week)	Control	4.02 ± 0.91	4.51 ± 1.39	4.53 ± 1.47	0.49 ± 1.23	0.699	0.51 ± 1.35	0.048
	Treatment	3.97 ± 0.93	4.57 ± 1.58	5.13 ± 1.93	0.59 ± 1.50		1.16 ± 1.93	
Number of days defecation (days/week)	Control	3.82 ± 0.76	4.18 ± 1.09	4.18 ± 1.18	0.35 ± 0.94	0.384	0.35 ± 1.09	0.084
	Treatment	3.65 ± 0.76	4.17 ± 1.17	4.40 ± 1.36	0.52 ± 0.99		0.75 ± 1.20	
Stool volume* (score/week)	Control	9.03 ± 4.66	9.51 ± 6.02	9.61 ± 6.57	0.48 ± 2.95	0.145	0.57 ± 3.61	0.044
	Treatment	8.36 ± 4.51	9.84 ± 6.25	10.78 ± 7.47	1.48 ± 3.95		2.42 ± 5.46	

Mean ± Standard deviation.
The analysis was performed using the unpaired t-test.
*One medium-size chicken egg as a reference.

Table 4. Comparison of stool consistency, straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, and abdominal bloating

		0w	1w	2w	1w-0w		2w-0w	
					Δ	p-value	Δ	p-value
Stool consistency (BSFS)	Control	3.44 ± 0.58	3.45 ± 0.81	3.54 ± 0.79	0.02 ± 0.56	0.848	0.10 ± 0.61	0.024
	Treatment	3.34 ± 0.70	3.40 ± 0.73	3.76 ± 0.69	0.06 ± 0.73		0.43 ± 0.73	
Straining during defecation	Control	31.55 ± 21.44	29.85 ± 24.42	28.96 ± 22.53	−1.70 ± 13.38	0.309	−2.59 ± 11.81	0.004
	Treatment	38.48 ± 22.81	33.94 ± 22.65	27.90 ± 21.71	−4.53 ± 14.82		−10.58 ± 15.79	
Pain during defecation	Control	20.58 ± 19.58	21.45 ± 22.14	20.37 ± 20.93	0.87 ± 10.91	0.459	−0.20 ± 9.42	0.019
	Treatment	25.79 ± 22.44	24.98 ± 22.33	19.85 ± 20.72	−0.81 ± 12.08		−5.93 ± 14.42	
Feeling of incomplete evacuation after defecation	Control	25.49 ± 21.81	23.57 ± 21.04	22.76 ± 20.73	−1.92 ± 12.35	0.973	−2.73 ± 11.66	0.128
	Treatment	30.54 ± 22.49	28.70 ± 21.61	23.83 ± 20.35	−1.84 ± 13.44		−6.71 ± 14.67	
Abdominal bloating	Control	29.28 ± 23.28	28.38 ± 24.40	26.96 ± 23.73	−0.91 ± 11.63	0.444	−2.32 ± 10.94	0.056
	Treatment	34.05 ± 23.64	31.12 ± 23.97	26.95 ± 22.40	−2.93 ± 15.01		−7.10 ± 14.15	

Mean ± Standard deviation.
The analysis of stool consistency was performed using the Wilcoxon rank sum test. The analysis of straining during defecation, pain during defecation, feeling of incomplete evacuation after defecation, and feeling of abdominal distention was performed using the unpaired t-test.
BSFS: Bristol Stool Form Scale.

Table 5. Comparison of fecal water content and JPAC-QOL

			0w	2w	2w-0w	
					Δ	p-value
Fecal water content (%)		Control	72.8 ± 7.4	72.8 ± 8.5	0.0 ± 7.4	0.982
		Treatment	72.3 ± 7.4	72.2 ± 7.8	0.0 ± 5.2	
JPAC-QOL	Physical discomfort score	Control	7.24 ± 2.77	6.22 ± 2.17	−1.02 ± 1.69	0.481
		Treatment	7.85 ± 2.96	6.92 ± 2.88	−0.92 ± 2.43	
	Psychosocial discomfort score	Control	11.16 ± 3.56	10.24 ± 3.18	−0.92 ± 3.10	0.888
		Treatment	11.79 ± 3.88	11.09 ± 3.84	−0.70 ± 3.36	
	Worries/concerns score	Control	18.25 ± 7.46	16.18 ± 5.72	−2.08 ± 4.32	0.662
		Treatment	20.32 ± 7.93	17.87 ± 7.95	−2.45 ± 4.85	
	Satisfaction score	Control	8.73 ± 2.71	7.25 ± 3.10	−1.47 ± 3.18	0.113
		Treatment	10.08 ± 2.23	7.53 ± 3.39	−2.55 ± 3.22	
	Total score	Control	45.37 ± 14.54	39.88 ± 11.30	−5.49 ± 8.59	0.470
		Treatment	50.04 ± 15.03	43.42 ± 15.91	−6.62 ± 11.06	

Mean ± Standard deviation.
The analysis was performed using the unpaired t-test.
JPAC-QOL: Japanese version of the Patient Assessment of Constipation QOL questionnaire.

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increased in the treatment group compared with that in the control group after 2 weeks (2.42 ± 5.46 stools per week, p=0.044). The stool consistency score (BSFS) was significantly increased in the treatment group compared with that in the control group after 2 weeks (0.43 ± 0.73, p=0.024). Straining during defecation, which was evaluated with the VAS, was significantly decreased in the treatment group compared with that in the control group after 2 weeks (p=0.004), with a decrease of −10.58 ± 15.79 mm. Pain during defecation, which was also evaluated with the VAS, was significantly decreased in the treatment group compared with that in the control group after 2 weeks (p=0.019), with a decrease of −5.93 ± 14.42 mm. The feelings of incomplete evacuation and abdominal bloating after defecation did not significantly change in the treatment group compared with those in the control group after the intervention period. There was no significant difference in fecal water content or Patient Assessment of Constipation-QOL (PAC-QOL) scores (total and subscores) between the two groups.

Safety evaluation

No adverse events attributable to the test foods, such as subjective symptoms or abnormal changes in BMI, were observed during this study.

DISCUSSION

This placebo-controlled, randomized, double-blind study evaluated the effect of 25 billion CFUs of heat-killed *B. longum* CLA8013 on improving bowel movements and constipation-related QOL in healthy participants prone to constipation. To evaluate the effect of *B. longum* CLA8013, participants with a defecation frequency of 3–5 times per week were included in the study. Previous studies suggest that *B. longum* CLA8013 stimulates water secretion into the intestinal tract in animal models (unpublished). Thus, *B. longum* CLA8013 was expected to improve hard stools and pain during defecation and to affect the QOL of individuals prone to constipation.

After 2 weeks of *B. longum* CLA8013 intake, the frequency of defecation was significantly increased in the treatment group compared with that in the control group. In addition, the stool volume and QOL-related outcomes, such as stool consistency, straining during defecation, and pain during defecation, were significantly improved.

B. longum CLA8013 is known to highly activate chloride channels in the intestinal epithelial cells that secrete water into the intestinal lumen [22]. In animal models, *B. longum* CLA8013 has been shown to increase fecal water content and improve bowel movements (unpublished). In this study, *B. longum* CLA8013 was found to improve bowel movements in humans. Moreover, significant improvements in stool consistency and straining and pain during defecation were also observed in the treatment group. These results indicate that the effects of *B. longum* CLA8013 in promoting water secretion into the intestinal tract shown in *in vitro* and *in vivo* models were also shown in humans. To the best of our knowledge, this is the first study to demonstrate the effectiveness of postbiotics in promoting water secretion into the intestinal tract in a randomized controlled trial. Lubiprostone, which stimulates chloride channels and promotes water secretion into the intestinal tract, has been presumed to increase the liquidity of the intestinal contents and promote increased intestinal transit by stimulating local stretch receptors, resulting in the stimulation of smooth muscle [25]. In a clinical trial, lubiprostone was reported to improve spontaneous bowel movement, stool consistency, and straining [26]. In addition, it has been reported to alter the intestinal microbiota [27]. In postbiotics, it has been reported to alter the intestinal microbiota and improve abdominal symptoms [4]. Therefore, we considered that the effect of *B. longum* CLA8013 stimulating water secretion into the intestinal tract caused the increase in defecation frequency and stool volume and softened the stool and that the softened stool improved straining and pain during defecation. In contrast, the improvement in abdominal symptoms was possibly related not only to the promotion of water secretion into the intestinal tract but also to the alteration and activation of the intestinal microbiota. However, the intestinal microbiota changed by *B. longum* CLA8013 were not evaluated in the present study and remain a subject for future analysis.

B. longum CLA8013 modestly improved defecation in healthy individuals prone to constipation; it improved bowel movements per week from 3.97 ± 0.93 to 5.13 ± 1.93 times/week. Lubiprostone improved the bowel movements per week of constipated patients from 1.65 ± 0.78 to 5.37 ± 2.78 times/week [28]. Thus, the effects of *B. longum* CLA8013 on bowel movement are milder than the level of improvement owing to lubiprostone. *B. longum* CLA8013 increased the stool volume by an amount equivalent to 1.85 medium-sized chicken eggs, or approximately 100 g. This indicated that the increased defecation frequency did not indicate that stools were broken up into smaller stools but rather indicated that the total stool volume per week increased. These results suggest that *B. longum* CLA8013 improves bowel movements compared with the placebo. In addition, the BSFS score improved from near type 3 to type 4 in the treatment group. As stated above, we considered that improvements in straining and pain during defecation may have been caused by the improvement of the BSFS score. Furthermore, the VAS scores for straining and pain during defecation improved by more than 20% compared with those at baseline. These two difficulties during defecation lead to a rise in blood pressure, which can trigger cardiovascular events such as congestive heart failure [29]. The extent to which improvement in these two outcomes affect these diseases is unclear. Nevertheless, we consider that *B. longum* CLA8013 may contribute to a reduction in the blood pressure-related risks.

The BSFS is generally used to evaluate stool consistency, and it has been reported that stool firmness correlates with stool water content [18]. Therefore, we consider these improvements induced by *B. longum* CLA8013 to have been caused by the softening of stools via the stimulation of intestinal water secretion after the activation of chloride channels. It remains unclear why no increase in fecal water content was observed, and there was no correlation between stool volume, stool consistency, and fecal water content despite the participants not changing their quantities or qualities of food intake. However, in this study, while stool samples were transported under refrigerated conditions and assessed immediately upon arrival, the time between collection and assessment was not recorded; therefore, the actual elapsed time is unknown. Thus, it is possible that the fecal water content changed between the collection and evaluation of the stool samples and that the fecal water content could not be evaluated properly. However, given that *B. longum* CLA8013 has been shown to promote smooth defecation and improve bowel movements, this strain may soften feces by stimulating water secretion into

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the intestinal tract in humans, as was found in animal models (unpublished).

Despite the fact that *B. longum* CLA8013 improved the defecation frequency, stool volume, stool consistency, and straining and pain during defecation compared with the control food, there was no significant difference in the JPAC-QOL score between the groups. The reason for this can be attributed to the background of the participants. While the development of the PAC-QOL was based on patients with chronic constipation with a mean of 1.2 ± 0.7 bowel movements per week [30], the participants in this study were prone to constipation and had 3–5 bowel movements per week. A report examining the reliability and validity of the JPAC-QOL suggested that differences in the defecation frequency of a sample may influence the results [31]. Therefore, the JPAC-QOL was considered difficult to evaluate, but it was established with the expectation that QOL would improve along with the improvement of QOL-related endpoints. In addition, the efficacy of the PAC-QOL with other probiotics has been evaluated in longer-term studies and studies with more participants [32, 33]. Thus, it is possible that significant differences in PAC-QOL could have been obtained depending on the subject background, number of participants, and duration of administration.

B. longum CLA8013 was found to be effective in improving bowel movements and QOL-related symptoms such as stool consistency, straining during defecation, and pain during defecation in this study. An increased defecation frequency and stool volume indicate improvement of bowel movements and intestinal regulation. These results suggest that *B. longum* CLA8013 may be a versatile, functional, active ingredient for defecation and bowel regulation. Going forward, we plan to investigate the mechanism of chloride channel stimulation of *B. longum* CLA8013. Lubiprostone has been reported to alter the intestinal microbiota, improve the intestinal barrier, and reduce urinary toxins in the blood [27, 34]. As stated above, the improvement in bowel movements following the intake of *B. longum* CLA8013 may be because it promotes water secretion; however, *B. longum* CLA8013 may also alter the intestinal microbiota. In addition to improving constipation, these functions may have beneficial effects not only on the gut but also on other parts of the body. Constipation is reported to negatively affect the skin by increasing phenol and p-cresol, which can be improved by probiotic intervention [35, 36]. Other studies have reported that constipation is correlated with obesity [37], insomnia [38], and fatigue [39]. Therefore, the improvements in bowel movements and intestinal regulation induced by *B. longum* CLA8013 may have effects on a variety of conditions and functions, including constipation, intestinal barrier function, skin roughness, obesity, insomnia, and fatigue.

This study has some limitations. First, it was designed to examine the effects of *B. longum* CLA8013 in participants prone to constipation without disease; therefore, the effects in patients with disease-related constipation are unknown. Second, the effects of *B. longum* CLA8013 in healthy individuals with normal bowel movements are unknown, and soft stools or diarrhea may occur. Third, the long-term effects of *B. longum* CLA8013 remain unknown because the intake period in this study was only 2 weeks.

In conclusion, *B. longum* CLA8013 was shown to be effective in a heat-killed form. It has a wide range of potential applications; can be used in capsules, tablets, drinks, and yogurt; and may be a potential ingredient in new food products claiming the ability to improve constipation.

CONFLICTS OF INTEREST

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