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Prebiotic effect of two grams of lactulose in healthy Japanese women: a randomised, double-blind, placebo-controlled crossover trial

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RESEARCH ARTICLE

Abstract

Sixty healthy Japanese women with a defaecation frequency of 2-4 times/week participated in this randomised, double-blind crossover trial. Participants received 2 g/day lactulose for 2 weeks and placebo in a random order, separated by a washout period of 3 weeks. Eight participants were excluded who did not satisfy the conditions, and therefore data from 52 were analysed. The primary outcome was defaecation frequency and the secondary outcomes were the number of defaecation days, faecal consistency, faecal volume, and the number and percentage of *Bifidobacterium* in faeces. The defaecation frequency (times/week) was significantly higher during lactulose (4.28±0.23) than placebo (3.83±0.23) treatment (delta (Δ) 0.45 [95% confidence interval (CI) 0.10-0.80], *P*=0.013). The defaecation days (days/week) was significantly higher during lactulose (3.77±0.17) than placebo (3.47±0.17) treatment (Δ0.30 [95% CI 0.04-0.56], *P*=0.024). Faecal consistency using the Bristol Stool Scale (/defaecation) was significantly higher during lactulose (3.84±0.10) than placebo (3.68±0.10) treatment (Δ0.16 [95% CI 0.00-0.31], *P*=0.044). Faecal volume (/week) was significantly higher during lactulose (21.73±3.07) than placebo (17.65±3.07) treatment (Δ4.08 [95% CI 0.57-7.60], *P*=0.024). The number of *Bifidobacterium* in faeces (log colony forming units/g faeces) was significantly higher during lactulose (9.53±0.06) than placebo (9.16±0.06) treatment (Δ0.37 [95% CI 0.23-0.49], *P*<0.0001). The percentage of *Bifidobacterium* in faeces was also significantly higher during lactulose (25.3±1.4) than placebo (18.2±1.4) treatment (Δ7.1 [95% CI 2.9-11.4], *P*=0.0014). Finally, straining at defaecation (/defaecation) during lactulose (3.62±0.24) treatment was significantly lower than during placebo (3.97±0.24) treatment (Δ0.35 [95% CI -0.69 – -0.02], *P*=0.037). No significant difference was observed between lactulose and placebo with regard to flatulence. Severe adverse effects did not occur. Thus, oral ingestion of 2 g/day lactulose had a prebiotic effect, increasing the number and percentage of bifidobacteria in faeces, softening the faeces, and increasing defaecation frequency, but without increasing flatulence.

Keywords: bowel movement, indigestible oligosaccharide, gut microbiome, constipation, intestinal regulation

1. Introduction

The Rome IV Criteria for Constipation represent a widely accepted definition of constipation. In addition, in 2017, an Evidence-based Clinical Practice Guideline for Chronic Constipation was published in Japan. According to the guideline, constipation is defined as 'a situation in which faeces that should be discharged outside the body are not sufficiently and comfortably discharged'. In Japan, 3.55% of people complain of constipation, but the problem is commoner in women than men and its prevalence increases with age (Ministry of Health, Labour and Welfare of Japan, 2018). Such constipation is likely to be alleviated by the ingestion of foods that promote easier defaecation.

Lactulose is a laxative that has been shown to be effective at improving the frequency of defaecation and faecal hardness (Bharucha *et al.*, 2013), and it is widely used for this purpose worldwide (Aït-Aissa and Mohammed, 2014). In addition to this application, it is also widely used as a prebiotic (bifidus factor) (Seki and Saito, 2012). Indeed, since Petuely (1957) reported that lactulose is capable of promoting the proliferation of bifidobacteria, it has been added to infant formula in Japan as a prebiotic (Kiyosawa *et al.*, 1986). The most common bacterial genus in the intestinal microbiota is bifidobacteria at the time breast feeding is commenced after birth (Mitsuoka, 1990). Although the proportion of bifidobacteria decreases with age in Japanese people, it remains the dominant genus even after weaning, and is considered to be a beneficial to human health, due to the competitive exclusion of pathogenic and putrefactive bacteria, immune stimulation, control of intestinal function, and enhancement of mineral absorption (Yaeshima, 1996). *Blautia* is also one of the most common bacterial genus in the intestine of Japanese people, and is known to produce acetic acid from hydrogen gas (Nishijima, 2016). However, its method of acetic acid synthesis differs from that of bifidobacteria, which use the bifid shunt. Hydrogen gas produced in the intestine can be associated with health benefits, but can also be a cause of flatulence, depending on the amount generated (Nishimura, 2014).

Lactulose is thought to function as laxative because of its osmotic effect, and the recommended dose for this purpose is 10-20 g/day (Schiller, 2001), which is higher than the quantity included in the diet when it is used as a prebiotic. A prebiotic is defined as 'a substrate that is selectively utilised by host microorganisms, conferring a health benefit' (Gibson *et al.*, 2017), and the amount required for a laxative effect is generally higher than for a prebiotic effect. For example, 'Maiasa-Sokai', which is designated as a Food for Specified Health Use in Japan contains 4 g lactulose. The mechanism of the prebiotic effect of lactulose is thought to rely upon it reaching the large intestine without being absorbed or digested, where it can be metabolised by bifidobacteria, causing the population of these bacteria to expand. This population expansion is associated with greater production of acetic acid, which promotes peristalsis in the large intestine, shortens large intestinal transit time (LITT) and increases the moisture content of the faeces, softening them. This reduces straining at defaecation and increases the frequency of defaecation. Thus, the mechanisms involved in the prebiotic and laxative effects of lactulose differ.

Oku and Okazaki (1998) reported that the maximum quantity of lactulose that could be ingested by Japanese women without having a laxative effect is 0.26 g/kg. Therefore, the use of lactulose as a prebiotic may be accompanied by a risk of diarrhoea. However, if a prebiotic effect of lactulose could be demonstrated, such as an increase in defaecation frequency, at a low dose, it should be possible to minimise the risk of diarrhoea associated with its use.

We previously reported that the administration of just 1 g/day lactulose increases defaecation frequency and the number of *Bifidobacterium* in the faeces (Sakai *et al.*, 2019). However, this was a preliminary trial that was conducted as a single-arm study, aiming to determine the optimum dose. Here, we conducted a double-blind crossover study to evaluate the prebiotic effect of 2 g/day lactulose, following on from the previous study. We aimed not only to evaluate defaecation frequency, faecal consistency, and faecal volume, but also the effects on the intestinal microbiota, which are also thought to have an influence on defaecation frequency. In addition, the degree of straining at defaecation, faecal moisture content, and faecal acetic acid concentration were also assessed, to test the hypothesis described above regarding the mechanism of the prebiotic effect of lactulose.

2. Materials and methods

Trial design

This randomised, double-blind, placebo-controlled crossover trial consisted of a pre-observation period, two ingestion periods and a washout period between these. The prebiotic effect of 2 g/day lactulose ingested for 2 weeks was evaluated (Figure 1). The study was conducted at Showa Women's University (Japan) between May and December 2017 and was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Institutional Review Board of Showa Women's University. All participants provided their written informed consent. The protocol for this study is registered with the University Hospital Medical Information Network Clinical Trials Registry (No. UMIN000027305).

Participants

The participants were healthy students, staff members and affiliates of Showa Women's University. The inclusion criteria were: (1) age 18-65 years; and (2) defaecation frequency 2-4 times/week. The exclusion criteria were: (1) severe hepatic, renal, cardiac, gastrointestinal, cerebrovascular, endocrine, metabolic or infectious disease; (2) history of gastrointestinal resection; (3) gastrointestinal dysfunction, such as irritable bowel syndrome or inflammatory bowel disease; (4) use of medicines or supplements that could influence defaecation frequency (e.g. antibiotics, probiotics, laxatives, anti-diarrhoeal drugs and fibre); (5) milk allergy; (6) lactose intolerance; (7) participation in another study; and (8) individuals who were judged inappropriate for the study by the investigator or a physician.

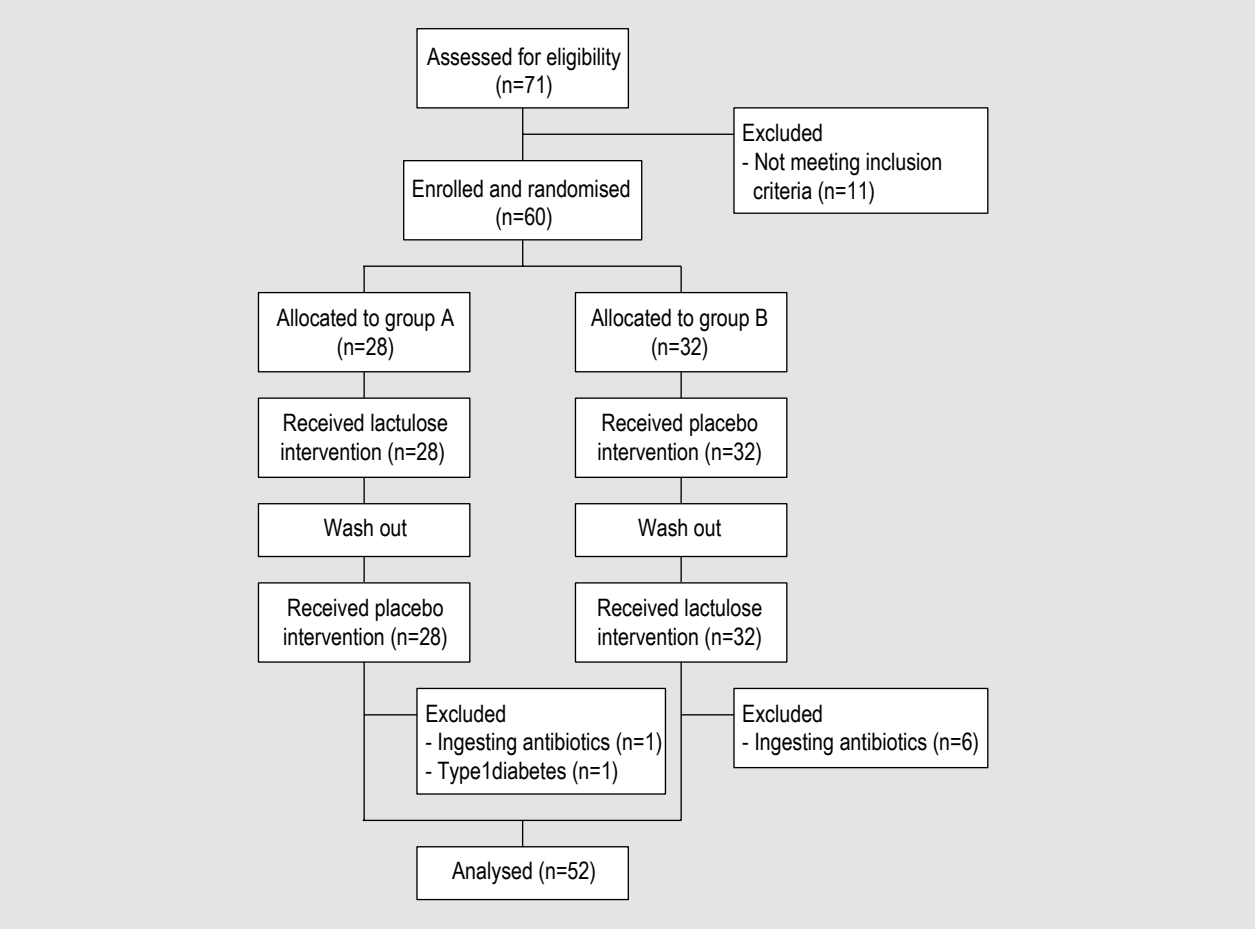

Figure 1. Flow chart of participant numbers throughout the trial.

Randomisation

Participants were randomly assigned to group A (lactulose first) or group B (placebo first). An assignment manager, who was independent of the trial staff, created the allocation order using the replacement block method (block size 4). The allocation ratio was 1:1. According to the allocation order, the test food number was displayed on the test food package for each participant. The correspondence chart between the test food number and the assignment group was hidden from the authors, participants and trial staff, including the intestinal microbiome analyst and the statistician, until the completion of the trial. The trial staff assigned the test foods in ascending order of test food number, corresponding to the order of participant registration.

Intervention

Two-gram portions of lactulose crystal anhydrate powder (MLC-97, ≥97%, Morinaga Milk Industry Co., Ltd., Tokyo, Japan) were provided in aluminium sachets. Glucose crystal anhydrate powder (Nihon Shokuhin Kako Co., Ltd., Tokyo, Japan) was used as the placebo, because this could not be visually discriminated from the lactulose. Participants in group A received lactulose during the first intervention period and placebo during the second intervention period, for 2 weeks each, whereas participants in group B received the placebo during the first intervention period and lactulose during the second intervention period. A 3-week washout period was used between the two intervention periods (Figure 2). The time of day for ingestion was not specified. The participants were instructed in advance to avoid the use of pharmaceuticals and supplements (e.g. antibiotics, laxatives, anti-diarrhoeal drugs, probiotics, prebiotics and fibre), which could affect defaecation during the study period.

Outcomes

The primary outcome was defaecation frequency and the secondary outcomes were the number of days on which defaecation occurred (defaecation days), faecal consistency and faecal volume. The data for each outcome were extracted from a diary written by the participants.

The mean weekly values for defaecation frequency were calculated using data from the entire intervention period

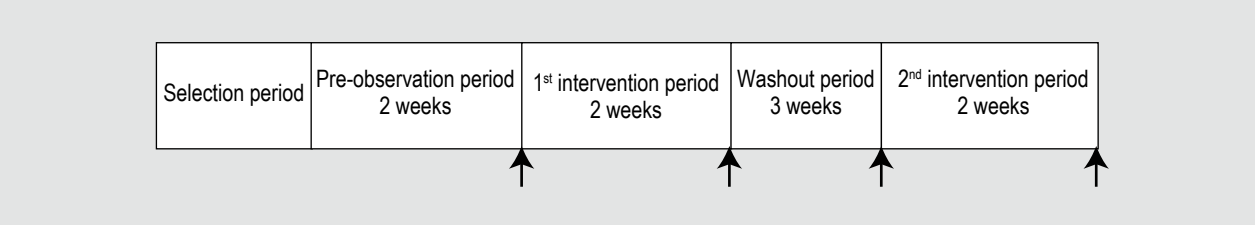

Figure 2. Trial design. Defaecation frequency, faecal consistency, faecal volume and health conditions were recorded in the participants' diaries during the study periods. Faecal sample collection is indicated with an arrow.

(2 weeks) for each participant. A 'defaecation day' was recorded when a participant defaecated one or more times, and weekly means were calculated for the entire intervention period (2 weeks) for each participant. Faecal consistency was evaluated using the Bristol Stool Scale (BSS), using scores between 1 (hard) and 7 (watery) (Lewis and Heaton, 1997). Mean weekly faecal consistency on BSS values per defaecation were calculated for the entire intervention period (2 weeks) for each participant. Faecal volume was measured using a 'faecal model', a 20-mm diameter, 50-mm long cylinder. The participants recorded their faecal volumes in increments of 0.5 × faecal model size. Mean weekly faecal volumes were calculated for the entire intervention period (2 weeks) for each participant. The mean values calculated for each outcome were statistically analysed.

Furthermore, the same diary was used to record whether the participant felt refreshed after defaecation, whether evacuation felt complete and the degree of straining associated with defaecation, using visual analogue scales (VAS). VAS scores were assigned using a horizontal 10-cm long line, on which participants indicated their score by placing a cross on the line at the point that corresponded to their experience, with the left-hand side of the scale representing zero and the right-hand side representing the highest score they could imagine. The mean values for each VAS score per defaecation were calculated for the entire intervention period (2 weeks) for each participant. The frequency of flatulence was also recorded daily by the participants in the same diary, then the mean weekly flatulence frequency was calculated for the entire intervention period (2 weeks) for each participant. The mean values calculated for each outcome were also statistically analysed.

The number and percentage of *Bifidobacterium* in faeces were also determined. Faecal samples were collected before and at the end of each intervention period (Figure 2) and were placed in storage immediately at <-18 °C until they arrived at the laboratory, and at -80 °C thereafter. Because the participants' defaecation frequencies were 2-4 times per week and it was difficult to designate a specific date for sample collection, this was scheduled to occur before administration or the last day of administration ±2 days. DNA was extracted from these samples, as described by Sugahara *et al.* (2015), and used to determine both the number and percentage of *Bifidobacterium*.

To determine the number of *Bifidobacterium* per gram of faeces, a quantitative PCR method was used, with *Bifidobacterium* genus-specific forward (5'-CTCCTGGAAACGGGTGG-3') and reverse (5'-GGTGTTCTTCCCGATATCTACA-3') primers (Matsuki *et al.*, 2004). A standard curve was prepared using dilutions of *Bifidobacterium longum* ATCC 15707 cells. The data before and at the end of intervention were logarithmically transformed, and the latter set was statistically analysed.

To determine the percentage of each bacterial taxon of interest in the faecal microbiome, including those of *Bifidobacterium* and *Blautia*, the same DNA extract was sequenced using Illumina Miseq (Illumina, Inc., San Diego, CA, USA), as described previously (Kato *et al.*, 2018). After removing sequences consistent with data from the Genome Reference Consortium human build 38 (GRCh38) and phiX reads from the raw Illumina paired-end reads, the sequences were analysed using the QIIME2 software package, version 2017.10 (https://qiime2.org/). Potential chimeric sequences were removed using DADA2 (Callahan *et al.*, 2016), then 30 and 90 bases of the 3'-region of the forward and reverse reads were trimmed, respectively. Taxonomical classification was performed using a Naive Bayes classifier trained on Greengenes 13.8, with a 99% threshold for operational taxonomic unit full-length sequences. UniFrac distances were calculated using QIIME2 software (Bolyen *et al.*, 2018). The data collected at the end of the intervention period was statistically analysed.

Faecal acetic acid concentration, moisture content and pH were quantified by the Techno Suruga Laboratory Co., Ltd. (Shizuoka, Japan). A diet survey was conducted before the first intervention period and at the end of the second intervention period, using a brief self-administered diet history questionnaire.

Statistical methods

Statistical analysis was conducted per protocol set, meaning that the participants who took medicines or supplements that could affect defaecation, or those who had a disease, were excluded from the analysis. The baseline was calculated as mean ± standard deviation or frequency.

The primary and secondary outcomes, VAS and the percentage of *Bifidobacterium* were analysed using a linear mixed model in which the test food group and timing were fixed effects, and participant identity was a variable effect. The least mean square value and standard error for each group, the difference between groups, and the associated 95% confidence interval (CI) and *P*-value were calculated. The two-tailed significance level was set at 5%.

The carry-over effect was analysed using a model in which the interaction between the test food group and time was added to the fixed effect of the main analysis model. The numbers of *Bifidobacterium* were logarithmically transformed and analysed using a linear mixed model in which the number of *Bifidobacterium* after ingestion was the objective variable; the food group, the number of *Bifidobacterium* before ingestion and timing were fixed effects; and participant identity was a variable effect. The least mean square value and standard error for each food group, the difference between groups, and the associated 95% CI and *P*-value were calculated. The two-tailed significance level was set at 5%. Differences in the frequency of adverse events between periods of lactulose and placebo ingestion were evaluated using the McNemar test.

Statistical analyses of the data were performed using JMP 13.2 (SAS Institute Inc., Cary, NC, USA). On the basis of the results of the preliminary trial (Sakai *et al.*, 2019), the number of participants required was calculated for a two-tailed significance level of 5% and a power of 90%, with the assumption that the difference in defaecation frequency between the groups would be 0.7 and the standard deviation would be 1.5. The number of participants required was calculated to be 51. Assuming that the withdrawal and dropout rate would be ~10%, the number of participants required was determined to be 60.

3. Results

Participants

Sixty healthy participants were enrolled, and 28 were assigned to group A and 32 to group B (Figure 1). None dropped out. Seven participants who used antibiotics during the trial and one who was type I diabetic were excluded, leaving 52 for analysis. There is a risk of bias due to the use of per protocol set analysis. The mean value and standard deviation of ingestion rate for each participant was calculated as the compliance. The compliance (percentage ± standard deviation) of group A was 95.4±7.4% during the first intervention period and 96.9±5.1% during the second intervention period. The compliance of group B was 95.9±8.1% during the first intervention period and 96.1±6.6% during the second intervention period.

Baseline data

Table 1 shows the baseline data for the participants. There were no significant imbalances in this.

Primary outcome

The least square mean value for defaecation frequency (times/week) was 3.83±0.23 in the placebo group and 4.28±0.23 in the lactulose group. The difference between the groups was 0.45 (95% CI 0.10-0.80, *P*=0.013) (Table 2).

Table 1. Anthropometric data at the start of the trial.[1]

Parameter	Whole cohort n=52	Group A lactulose first n=26	Group B placebo first n=26	*P*-value
Sex (women/men)	52/0	26/0	26/0	–
Age (years)	20.2±2.4	19.8±1.1	20.6±3.2	0.23
Body mass (kg)	51.4±5.2	51.9±4.1	50.8±6.2	0.47
Height (m)	1.58±0.1	1.59±0.1	1.58±0.1	0.44
Body mass index	20.5±1.8	20.6±1.7	20.4±2.0	0.76
Previous medical history (Y/N)	0:52	0:26	0:26	–
Morbidities (Y/N)	0:52	0:26	0:26	–
Smoking (>1 cigarette per week) (Y/N)	0:52	0:26	0:26	–
Drinking (>2 times per week) (Y/N)	10:42	7:19	3:23	0.29

[1] Values are expressed as the simple mean ± standard deviation (paired *t*-test).

Table 2. Effects of 2 g/day lactulose on each outcome.[1]

Outcome	Placebo n=52	Lactulose n=52	Delta (95% CI)[2]	*P*-value
Defaecation frequency over entire intervention period (times/week)	3.83±0.23	4.28±0.23	0.45 (0.10 to 0.80)	0.013
Defaecation days over entire intervention period (days/week)	3.47±0.17	3.77±0.17	0.30 (0.04 to 0.56)	0.024
Faecal consistency over entire intervention period (/defaecation)	3.68±0.10	3.84±0.10	0.16 (0.00 to 0.31)	0.044
Faecal volume over entire intervention period (/week)	17.65±3.07	21.73±3.07	4.08 (0.57 to 7.60)	0.024
Number of *Bifidobacteria* after intervention (log cfu/g faeces)	9.16±0.06[3]	9.53±0.06[3]	0.37 (0.23 to 0.49)	<0.0001
Percentage *Bifidobacteria* after intervention	18.2±1.4[3]	25.3±1.4 [3]	7.1 (2.9 to 11.4)	0.0014
Percentage *Blautia* after intervention	22.8±1.1[3]	21.7±1.1 [3]	-1.1 (-4.3 to 2.0)	0.47
Feeling refreshed after defaecation over entire intervention period (/defaecation)	4.81±0.24	4.78±0.24	-0.03 (-0.30 to 0.25)	0.86
Feeling of incomplete evacuation over entire intervention period (/defaecation)	3.84±0.23	3.68±0.23	-0.16 (-0.53 to 0.22)	0.41
Straining at defecation over entire intervention period (/defaecation)	3.97±0.24	3.62±0.24	-0.35 (-0.69 to -0.02)	0.037
Flatulence frequency over entire intervention period (days/week)	0.76±0.16	0.76±0.16	0.00 (-0.37 to 0.37)	0.99
Faecal acetic acid concentration after intervention (mg/g faeces)	3.03±0.18[3]	3.14±0.18[3]	0.11 (-0.27 to 0.49)	0.57
Faecal moisture content after intervention (%)	72.2±1.2[4]	74.1±1.1[5]	1.9 (-0.6 to 4.5)	0.13
Faecal pH after intervention	6.89±0.08[3]	6.87±0.08[3]	-0.02 (-0.22 to 0.18)	0.83

[1] Values are expressed as the least square mean ± standard error (generalised linear mixed model).
[2] CI = confidence interval.
[3] n=49, because there were three participants who were not willing to collect faeces.
[4] n=43, because there were six samples that were too small for measurement of their moisture content.
[5] n=45, because there were four samples that were too small for measurement of their moisture content.

Secondary outcomes

The secondary outcome data are shown in Table 2. The least square mean value for defaecation days (days/week) was 3.47±0.17 for the placebo group and 3.77±0.17 for the lactulose group. The difference between the groups was 0.30 (95% CI 0.04-0.56, *P*=0.024). The least square mean value for faecal consistency on BSS (/defaecation) was 3.68±0.10 for the placebo group and 3.84±0.10 for the lactulose group. The difference between the groups was 0.16 (95% CI 0.00-0.31, *P*=0.044). The least square mean value for faecal volume (/week) was 17.65±3.07 for the placebo group and 21.73±3.07 for the lactulose group. The difference between the groups was 4.08 (95% CI 0.57-7.60, *P*=0.024).

Other findings

Other outcome data are also shown in Table 2. The least square mean values for the number of *Bifidobacterium* (log cfu/g faeces) were 9.16±0.06 for the placebo group and 9.53±0.06 for the lactulose group. The difference between the groups was 0.37 (95% CI 0.23-0.49, *P*<0.0001). The least square mean values of the percentage of *Bifidobacterium* were 18.2±1.4% for the placebo group and 25.3±1.4% for the lactulose group. The difference between the groups was 7.1% (95% CI 2.9-11.4%, *P*=0.0014). The least square mean values for *Blautia* were 22.8±1.1% during placebo ingestion and 21.7±1.1% during lactulose ingestion. The difference between these periods was -1.1% (95% CI -4.3-2.0%, *P*=0.47). The correlation coefficient for the relationship between the number and percentage of *Bifidobacterium* was 0.38 (*P*=0.0065). There were no significant carry-over effects (Table 3). Three participants were not willing to collect faeces.

No significant differences were observed in the least square mean values for 'feeling refreshed after defaecation', 'feeling of incomplete evacuation', or flatulence frequency. However, the least square mean values for straining at defaecation (/defaecation) were 3.97±0.24 for the placebo group and 3.62±0.24 for the lactulose group. The difference between the groups was -0.35 (95% CI -0.69 – -0.02, *P*=0.037). The diet survey showed no significant differences in the intakes of specific nutritional components between the start and end of the trial (Table 3).

Carry-over effect

No significant carry-over effects were detected (Table 4). However, because the *P*-value of the interaction (Intervention vs Period) with respect to the percentage of *Bifidobacterium* was 0.10, there might have been a slight carry-over effect that was not revealed using the method described.

Table 3. Diet survey results.[1]

Parameter	n	Before the intervention	At the end of the intervention	*P*-value
Energy (kcal/day)	52	1,431±334	1,414±365	0.61
Mass (g/day)	52	1,563±444	1,531±414	0.41
Water (g/day)	52	1,259±396	1,231±363	0.45
Protein (g/day)	52	52.2±12.8	53.8±14.6	0.28
Fat (g/day)	52	47.6±12.3	48.1±15.0	0.71
Carbohydrate (g/day)	52	187.0±50.6	178.0±48.2	0.06
Soluble dietary fibre (g/day)	52	2.3±0.8	2.4±0.8	0.44
Insoluble dietary fibre (g/day)	52	6.1±1.9	6.3±2.0	0.36
Total dietary fibre (g/day)	52	8.6±2.8	8.9±2.9	0.39

[1] A diet survey was conducted before the first intervention period and at the end of the second intervention period using a brief self-administered diet history questionnaire. Values are expressed as the simple mean ± standard deviation (paired *t*-test).

Table 4. Carry-over effect.[1]

Outcome	Group	n	First intervention period		Second intervention period		*P*-value of interaction (intervention vs period)
			Before the intervention	At the end of the intervention	Before the intervention	At the end of the intervention	
Number of *Bifidobacterium* (log cfu/g faeces)							
	Group A lactulose first	24	8.98±0.89	9.44±0.76	9.15±0.90	9.17±0.82	0.57
	Group B placebo first	25	9.33±0.63	9.26±0.61	9.21±0.72	9.52±0.53	
Percentage *Bifidobacterium*							
	Group A lactulose first	24	13.6±11.9	24.8±14.3	19.3±17.2	19.0±16.3	0.10
	Group B placebo first	25	20.6±12.3	19.6±14.4	20.0±12.9	23.5±10.6	

[1] The carry-over effect was analysed using a model in which the interaction between the test food group and time was added to the fixed effect of the main analysis model. Values are expressed as the simple mean ± standard deviation.

Adverse events

No side effects or serious adverse events were observed in any of the participants. The main secondary symptoms were gastrointestinal symptoms, but there were no significant differences in the incidence of these between the periods of placebo and lactulose ingestion (Tables 5 and 6).

Table 5. Adverse events: principal secondary abdominal symptoms.[1]

Abdominal symptom	Lactulose	Placebo
Stomach ache	2	2
Heartburn	0	1
Abdominal pain	22	22
Diarrhoea	9	7
Constipation	0	1
Flatulence	2	0
Abdominal fullness	47	56
Flatus	50	53
Total	132	142

[1] The number of significant secondary abdominal symptoms during each intervention.

4. Discussion and conclusions

This is the first study to have quantitatively and comprehensively characterised the prebiotic effect of the ingestion of 2 g/day lactulose in a randomised, double-blind, placebo-controlled crossover trial. When lactulose or placebo were ingested by 60 healthy participants, their defaecation frequency, defaecation days, faecal consistency on BSS, faecal volume, and the number and percentage of *Bifidobacterium* significantly differed. However, there was

Table 6. Adverse effects in the form of gastrointestinal symptoms.[1]

		Lactulose			*P*-value
		Number not reporting	Number reporting	Total	
Placebo	Number not reporting	16	4	20	0.25
	Number reporting	8	32	40	
	Total	24	36	60	

[1] McNemar's test was performed for the presence or absence of adverse events between treatment groups.

no significant difference in flatulence frequency (Figure 3). Thus, we have demonstrated that a dose of only 2 g/day lactulose has a prebiotic effect.

In this study, not only defaecation frequency and the number of defaecation days, but also faecal consistency on BSS, improved significantly. However, the trend for the moisture content of the faeces to be higher during the lactulose ingestion period did not reach significance (Table 2). This is probably because the participants had a low defaecation frequency, and therefore the water would have been largely absorbed by the large intestine prior to defaecation. It has been reported that there is little difference in the moisture content of faeces, even if faecal consistency differs (Blake *et al.*, 2016), and this explanation may also apply to the data obtained in the present study. In addition, a significant increase in faecal bifidobacteria was observed, but no effect on acetic acid concentration in the faeces (Table 2). Analogous to the explanation given for the faecal moisture content data, this is probably because the acetic acid was mostly absorbed by the large intestine prior to defaecation. LITT is thought to be approximately inversely proportional to faecal consistency (Degen and Phillips, 1996), and might be shortened if the faeces are softened during lactulose ingestion. Nevertheless, the LITT in the present study was relatively long. Thus, because the defaecation frequency was significantly higher during lactulose ingestion, a prebiotic effect is implied, which was associated with a gradual improvement in the intestinal environment.

Tomoda *et al.* (1991) reported that 0.65 g/day lactulose significantly increased the number of faecal *Bifidobacterium*. However, there are a number of differences between their study and ours. In Tomoda's study yoghurt was used as a test food, the test duration varied between 3 and 6 weeks, defaecation frequency and faecal consistency were not measured quantitatively, and the number of *Bifidobacterium* were assessed using culture rather than quantitative PCR, as in this study. Thus, the present study is the first to show a quantitative effect of low-dose lactulose on defaecation frequency.

This is also the first study to analyse the effect of lactulose on the percentage of *Bifidobacterium* in the intestinal microbiome using next-generation sequencing. This percentage was significantly higher after the ingestion of lactulose than after placebo. After ingesting placebo for 2 weeks, the percentage of *Blautia*, which was the commonest faecal microbe in the trial participants, was higher than that of *Bifidobacterium* (Table 7). However, neither the percentage of *Blautia*, nor the percentages of the other dominant intestinal microbes, differed significantly after the ingestion of lactulose (data not shown). Thus, 2 g/day lactulose preferentially increases the bifidobacterial population, which is thought to be beneficial to human health, while not significantly affecting the population of other types of bacteria or disrupting the balance of the intestinal microbiome.

Flatulence, which often occurs following the ingestion of indigestible oligosaccharides, is recognised as an adverse event. Mizota *et al.* (2002) reported that a drink containing 3 g/day or 5 g/day lactulose significantly increases flatulence and that the flatulence score associated with 5 g/day is significantly higher than that associated with 3 g/day. However, ingestion of 2 g/day lactulose did not result in significant flatulence. Such flatulence is thought to be caused by hydrogen and/or methane produced as a metabolite by intestinal microbes (Kurbel *et al.*, 2006). However, methane is usually detected in very low concentrations in Japanese people in breath gas tests (Morii *et al.*, 2003), so it is likely that the flatulence of the participants in the present study was mainly due to hydrogen gas. It is likely that the hydrogen generated following 2 g/day lactulose is dispersed throughout the body and mainly discharged by exhalation, rather than in the form of substantial flatulence. The fact that the dominant bacterial genus, *Blautia*, did not decrease in number, may be one of the factors that did not increase flatulence.

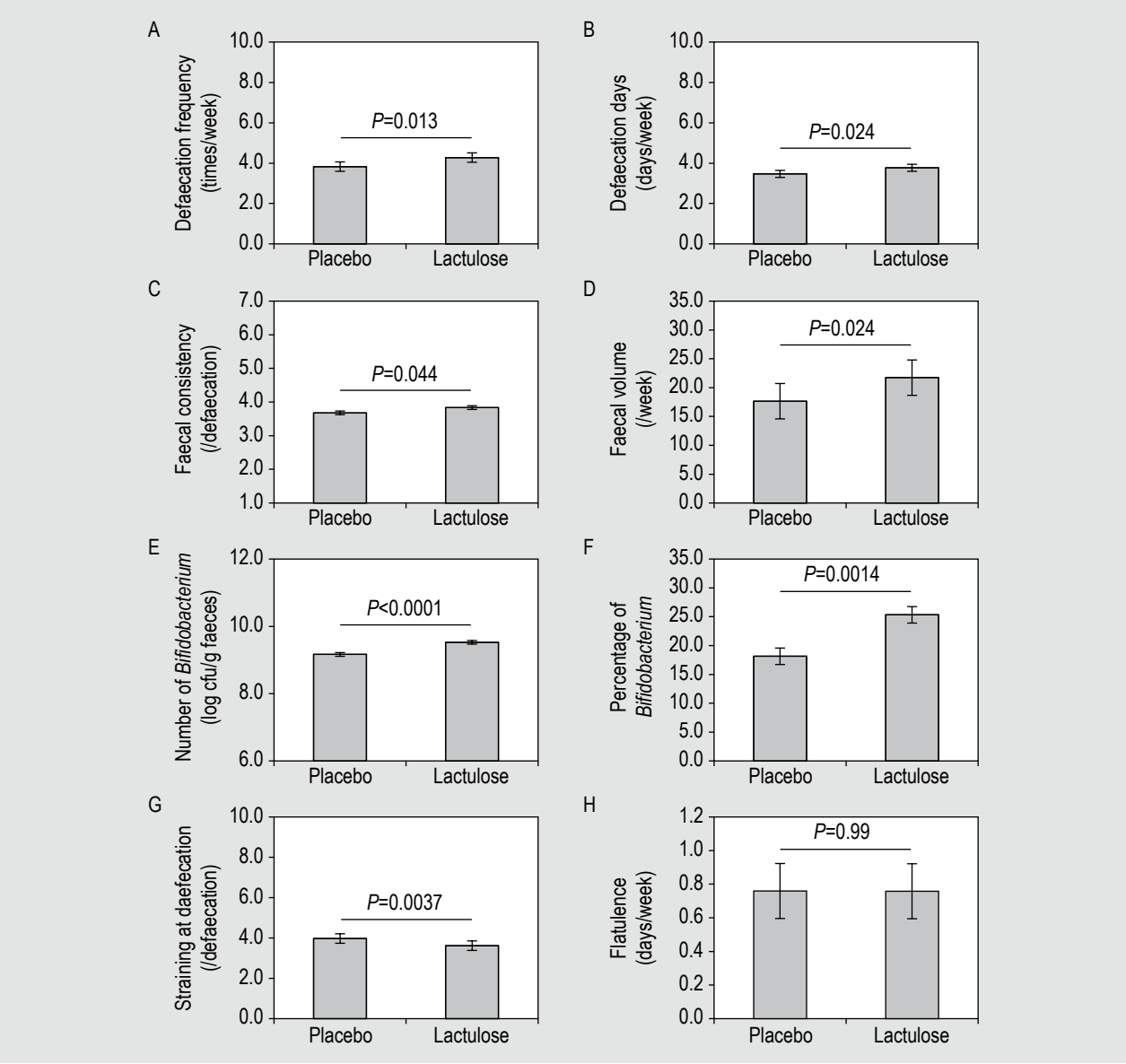

Figure 3. Prebiotic effects of 2 g/day lactulose. (A) defaecation frequency, n=52, (B) defaecation days, n=52, (C) faecal consistency, n=52, (D) faecal volume, n=52, (E) number of *Bifidobacterium*, n=49, (F) percentage of *Bifidobacterium*, n=49, (G) straining at defaecation, n=52 and (H) flatulence frequency, n=52. Values are expressed as the least square mean ± standard error (generalised linear mixed model).

Terada *et al.* (1992) reported that the number of *Bifidobacterium* returns to its original level 7 days after the ingestion of 3 g/day lactulose for 2 weeks. On the basis of our recent findings (Sakai *et al.*, 2019), we used a washout period of 3 weeks in this study. Because a carry-over effect was not observed, it seems that the number of *Bifidobacterium* returns to its original level within 3 weeks. But by the same token, this suggests that it is necessary to continue ingesting lactulose to maintain the beneficial effects of lactulose ingestion. In addition, habituation to the effects of lactulose was not identified in this study because only a 2-week ingestion period was used and the participants demonstrated a low frequency of defaecation.

In conclusion, in this study, 2 g/day lactulose significantly increased the defaecation frequency of Japanese women with a baseline defaecation frequency of 2-4 times/week. This seems to be due to a significant and selective increase in *Bifidobacterium* numbers, which is considered to be beneficial for human health. These results indicate that 2 g/day lactulose has a prebiotic effect.

Table 7. Simple mean values for each outcome.[1]

Outcome	Placebo n=52	Lactulose n=52
Defaecation frequency over entire intervention period (times/week)	3.83±1.50	4.28±1.84
Defaecation days over entire intervention period (days/week)	3.47±1.21	3.77±1.30
Faecal consistency over entire intervention period (/defaecation)	3.68±0.72	3.84±0.75
Faecal volume over entire intervention period (/week)	17.65±16.86	21.73±26.30
Number of *Bifidobacterium* before intervention (log cfu/g faeces)	9.24±0.77[2]	9.10±0.81[2]
Number of *Bifidobacterium* after intervention (log cfu/g faeces)	9.21±0.71[2]	9.48±0.65[2]
Percentage *Bifidobacterium* before intervention	20.0±14.8[2]	16.9±12.7[2]
Percentage *Bifidobacterium* after intervention	19.3±15.2[2]	24.2±12.5[2]
Percentage *Blautia* before intervention	24.1±10.4[2]	24.4±9.6[2]
Percentage *Blautia* after intervention	22.8±8.7[2]	21.7±9.4[2]
Feeling refreshed after defaecation over entire intervention period (/defaecation)	4.81±1.78	4.78±1.67
Feeling of incomplete evacuation over entire intervention period (/defaecation)	3.84±1.65	3.68±1.71
Straining at defaecation over entire intervention period (/defaecation)	3.97±1.72	3.62±1.68
Flatulence frequency over entire intervention period (days/week)	0.76±1.01	0.76±1.32
Faecal acetic acid concentration after intervention (mg/g faeces)	3.04±1.13[2]	3.14±1.35[2]
Faecal moisture content after intervention (%)	72.1±8.1[3]	74.1±7.4[4]
Faecal pH after intervention	6.89±0.50[2]	6.87±0.57[2]

[1] Values are expressed as the simple mean ± standard deviation.
[2] n=49, because there were three participants who were not willing to collect faeces.
[3] n=43, because there were six samples that were too small for measurement of their moisture content.
[4] n=45, because there were four samples that were too small for measurement of their moisture content.

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