

A study of the prebiotic effect of lactulose at low dosages in healthy Japanese women

Yohei SAKAI^{1*}, Nobuo SEKI¹, Hirokazu HAMANO², Hiroshi OCHI¹, Fumiaki ABE¹, Fumiko SHIMIZU³, Kazuya MASUDA³ and Hisakazu IINO⁴

¹Food Ingredients & Technology Institute, R&D Division, Morinaga Milk Industry Co., Ltd., 5-1-83 Higashihara, Zama, Kanagawa 252-8583, Japan

²R&D Management Department, Morinaga Milk Industry Co., Ltd., 5-1-83 Higashihara, Zama, Kanagawa 252-8583, Japan

³Department of Food Science and Nutrition, Faculty of Life and Environmental Sciences, Showa Women's University, 1-7 Taishidou, Setagaya-ku, Tokyo 154-8533, Japan

⁴Life Science for Living System, Graduate School, Showa Women's University, 1-7 Taishidou, Setagaya-ku, Tokyo 154-8533, Japan

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To investigate the prebiotic effect of lactulose at low dosages, we assessed changes in defaecation frequency following ingestion of 1, 2, or 3 g/day of lactulose for 2 weeks. Each test was carried out after a 2-week washout period. This was an open-label, before-after trial that enrolled 26 healthy Japanese women. The defaecation frequency, number of defaecation days, and number of faecal bifidobacteria increased significantly compared with before ingestion of 1, 2, and 3 g/day of lactulose. These results suggest that even 1 g/day of lactulose could have a prebiotic effect.

Key words: indigestible oligosaccharide, gut microbiome, constipation, intestinal regulation

A prebiotic is now defined as a substrate that is selectively utilised by host microorganisms, conferring a health benefit [1]. Lactulose, a disaccharide of galactose and fructose, has been used as a bifidobacterial growth factor (bifidus factor) in infant formula and various foods since Petuely first reported that it has the ability to increase the numbers of bifidobacteria in infant faeces [2]. Ingestion of lactulose improves the intestinal flora, and as the number of bifidobacteria increases, defaecation frequency correspondingly increases. This therefore represents a prebiotic effect. However, although several studies have investigated the prebiotic effects of lactulose [3–11], many have reported only the resulting increase in the number of bifidobacteria rather than quantitative assessments of changes in other indicators of prebiotic effects, such as defaecation frequency. In this study, we investigated the prebiotic effect of lactulose at low dosages by assessing the changes in defaecation frequency associated with an increased number of bifidobacteria following ingestion of 1, 2, or 3 g/day of lactulose.

We designed an open-label, before-after trial consisting of three 2-week ingestion periods, with a pre-observation period, and a washout period between each ingestion period (Fig. 1A). The study was conducted at Showa Women's University (Japan) between May and July 2016 and was conducted in

accordance with the principles of the Declaration of Helsinki. The trial protocol was approved by the Institutional Review Board of Showa Women's University and registered with the University Hospital Medical Information Network Clinical Trials Registry (No. UMIN000021219). All subjects, who were students recruited from Showa Women's University, provided written informed consent. The study included subjects with a defaecation frequency of two to four times per week. Exclusion criteria were as follows: (1) subjects with severe hepatic, renal, cardiac, gastrointestinal, cerebrovascular, endocrine, metabolic, or infectious diseases; (2) those with a history of gastrointestinal resection; (3) those with gastrointestinal dysfunction, such as irritable bowel syndrome or inflammatory bowel disease; (4) those using medicines or supplements that could influence defaecation frequency (e.g., antibiotics, probiotics, laxatives, antidiarrhoeals, and fibre); (5) those allergic to milk; (6) those participating in another study; and (7) those judged inappropriate for the study by the investigator or physician.

One-gram portions of lactulose crystal-anhydrate powder (MLC-97, $\geq 97\%$, Morinaga Milk Industry Co., Ltd., Tokyo, Japan) were provided in aluminium sachets. Subjects ingested the test food for 2 weeks during the ingestion period, at a dose of 1 g/day during the first ingestion period (1 g/day period), 2 g/day during the second ingestion period (2 g/day period), and 3 g/day during the third ingestion period (3 g/day period). The time of ingestion was not specified. During the research period, subjects were instructed in advance to avoid the use of pharmaceuticals and supplements (e.g., antibiotics, laxatives, antidiarrhoeals, probiotics, prebiotics, and fibre) that affect defaecation.

*Corresponding author. Yohei Sakai (E-mail: yo-sakai@morinaga-milk.co.jp)

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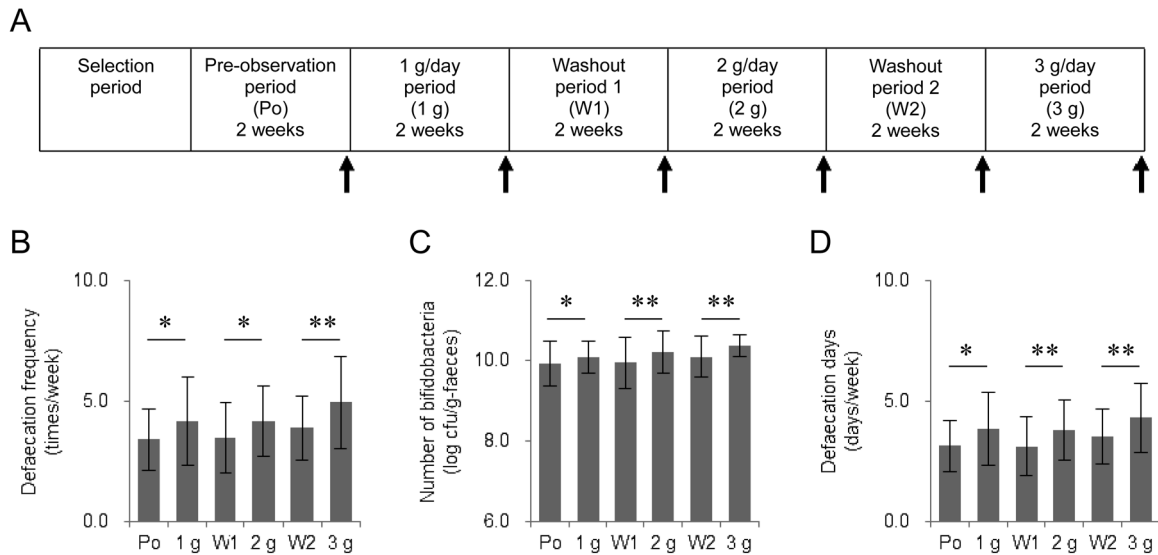


Fig. 1. Study design and the prebiotic effects of the various doses of lactulose.

(A) Study design. Defaecation, stool forms, and health conditions were recorded in a diary by each subject during the study periods. Faecal sample collection is indicated with an arrow. (B) Defaecation frequency, $n=26$. (C) Number of bifidobacteria, $n=26$. (D) Defaecation days, $n=26$. * $p<0.05$ vs. prior to the ingestion period (paired t-test). ** $p<0.01$ vs. prior to the ingestion period (paired t-test). Data are presented as the mean \pm standard deviation. Po, pre-observation period; 1 g, 1 g/day ingestion period; W1, washout period 1; 2 g, 2 g/day ingestion period; W2, washout period 2; 3 g, 3 g/day ingestion period.

Primary outcomes were defaecation frequency per week and the number of bifidobacteria in faeces. Subjects recorded defaecation between the pre-observation period and the third ingestion period in a diary. Faecal samples were collected immediately before and at the end of each ingestion period (Fig. 1A) and stored below -18°C until arrival at the laboratory and at -80°C thereafter. As described by Sugahara *et al.*, DNA was extracted from faecal samples and amplified using quantitative PCR [12]. The number of bifidobacteria per gram of faeces was quantified using *Bifidobacterium* genus-specific forward (5' CTCCTGGAAACGGGTGG 3') and reverse (5' GGTGTTCTTCCCGATATCTACA 3') primers [13]. A standard curve was prepared using dilutions of *Bifidobacterium longum* ATCC 15707 cells [14].

The secondary outcomes were the number of days the subject defaecated per week and the form of the stool on each occasion. The days when the subjects defaecated one or more times, as recorded in the diary, were counted as defaecation days. Subjects self-assessed and recorded stool form in the same diary. Evaluation of stool form was based on the Bristol Stool Scale (BSS), with scores ranging from 1 (hard) to 7 (watery) [15]. Subjects also recorded the food they consumed in a diet diary during the latter half of each period, using the non-weighed method. Nutrient calculation was performed using Excel (Microsoft, Redmond, WA, USA) add-in software (Excel-eiyokun, Ver. 8, Kenpakusha, Bunkyo-ku, Tokyo, Japan). All subjects were included in the analysis. For each pre-observation, ingestion, and washout period, the means and standard deviations of defaecation frequency and

the number of defaecation days per week were calculated using data for each 2-week period. Stool form, based on the BSS, was calculated using the mean and standard deviation per defaecation. The number of bifidobacteria was log transformed. Means and standard deviations of nutrient intake for each period were calculated from the dietary survey. The means and standard deviations of the nutrient intake before and after the ingestion period were calculated and analysed using a paired t-test. Differences in the frequency of adverse events before and during the ingestion period were tested using the McNemar test. Statistical analyses of the data were performed using SAS 9.4 (SAS Institute Inc., Cary, NC, USA), with the significance level being set at 5%.

Twenty-six healthy subjects were enrolled, and all completed the trial. The mean age of the subjects was 19.2 ± 1.0 years (range, 18–21 years); all were female, with a mean height of 158.1 ± 6.0 cm, mean body mass of 52.0 ± 5.8 kg, and mean body mass index of 20.8 ± 1.9 . The mean test food ingestion rate was $93.0 \pm 8.4\%$ over the three ingestion periods. One subject used antibiotics, and two subjects used probiotics. For the subject that used antibiotics, the outcome data for the 3 days of antibiotic use (from the last day of the pre-observation period to the second day of the 1 g/day period) and the 7 days after antibiotic use (from the third to the ninth day of the 1 g/day period) were excluded. For the first subject using probiotics, including lactic acid bacteria and bifidobacteria, the outcome data from the 1 day of probiotic use (the fourth day of the 2 g/day period) and the 2 days after probiotic use (the fifth and sixth days of the

Table 1. The prebiotic effects of lactulose

	n	Po	1 g	W1	2 g	W2	3 g
Defaecation frequency (times/week)	26	3.4 ± 1.3	4.2 ± 1.8 *	3.5 ± 1.5	4.2 ± 1.5 *	3.9 ± 1.3	4.9 ± 1.9 **
Number of bifidobacteria (log cfu/g-faeces)	26	9.93 ± 0.57	10.10 ± 0.40*	9.95 ± 0.63	10.23 ± 0.53**	10.09 ± 0.51	10.38 ± 0.28**
Defaecation days (days/week)	26	3.1 ± 1.1	3.8 ± 1.5*	3.1 ± 1.2	3.8 ± 1.2**	3.5 ± 1.1	4.3 ± 1.4**
Bristol Stool Scale score (per defaecation)	26	3.7 ± 1.0	3.8 ± 0.8	3.6 ± 0.6	3.9 ± 0.7	3.8 ± 0.6	3.9 ± 0.7

*p<0.05 vs. prior to the ingestion period (paired t-test). **p<0.01 vs. prior to the ingestion period (paired t-test). Data are presented as the mean ± standard deviation.

Po: pre-observation period; 1 g: 1 g/day ingestion period; W1: washout period 1; 2 g: 2 g/day ingestion period; W2: washout period 2; 3 g: 3 g/day ingestion period.

Table 2. Stool form, rated using the mean Bristol Stool Scale (BSS) score, during only the latter half of each period

	n	Po	1 g	W1	2 g	W2	3 g
BSS score ¹ (per defaecation)	26	3.6 ± 1.0	3.9 ± 0.9	3.4 ± 0.8	3.9 ± 1.1	3.7 ± 0.7	4.2 ± 0.8**
BSS score of <4.0 ² (per defaecation)	13	3.0 ± 0.8	3.6 ± 0.8*	3.4 ± 0.7	4.2 ± 1.1*	3.7 ± 0.8	4.2 ± 1.1*
BSS score of ≥4.0 ³ (per defaecation)	12	4.3 ± 0.6	4.2 ± 0.9	3.4 ± 1.0	3.5 ± 1.1	3.6 ± 0.6	4.2 ± 0.4**

*p<0.05 vs. prior to the ingestion period (paired t-test). **p<0.01 vs. prior to the ingestion period (paired t-test). Data are presented as the mean ± standard deviation.

¹ BSS scores during only the latter half of each period were used.

² This subgroup included subjects with a mean BSS score of <4/defaecation during the latter half of the pre-observation period, and the mean BSS score during only the latter half of each period was used.

³ This subgroup included subjects with a mean BSS score of ≥4/defaecation during the latter half of the pre-observation period, and the mean BSS score during only the latter half of each period was used.

Po: pre-observation period; 1 g: 1 g/day ingestion period; W1: washout period 1; 2 g: 2 g/day ingestion period; W2: washout period 2; 3 g: 3 g/day ingestion period.

2 g/day period) were excluded. For the other subject that used probiotics, which included *Bacillus subtilis* var. *natto* and lactic acid bacteria, the outcome data from the 2 days of probiotic use (the ninth and tenth days of the 3 g/day period) and the 2 days after probiotic use (the eleventh and twelfth days of the 3 g/day period) were also excluded. The outcome data for subjects using probiotics during the pre-observation and washout periods were not excluded, because probiotic use during these periods attenuated the prebiotic effects of lactulose.

The dietary survey found that the total quantity of energy ingested significantly decreased from 2,002 kcal/day before the second ingestion period to 1,673 kcal/day (p<0.01) after it. There were no significant changes in energy consumption during the other periods of the study. Side effects and serious adverse events were not observed in any subjects. The main secondary abdominal symptoms were gastrointestinal symptoms, but there were no significant differences in the incidence of these between the periods before and during lactulose ingestion.

The results are shown in Table 1. Defaecation frequency (times/week) significantly increased from 3.4 ± 1.3 to 4.2 ± 1.8 at 1 g/day, from 3.5 ± 1.5 to 4.2 ± 1.5 at 2 g/day, and from 3.9 ± 1.3 to 4.9 ± 1.9 at 3 g/day. The number of bifidobacteria (log colony forming units [cfu]/g-faeces) significantly increased from 9.93 ± 0.57 to 10.10 ± 0.40 at 1 g/day, from 9.95 ± 0.63 to 10.23 ± 0.53 at 2 g/day, and from 10.09 ± 0.51 to 10.38 ± 0.28 at 3 g/day. The number of defaecation days

(days/week) significantly increased from 3.1 ± 1.1 to 3.8 ± 1.5 at 1 g/day, from 3.1 ± 1.2 to 3.8 ± 1.2 at 2 g/day, and from 3.5 ± 1.1 to 4.3 ± 1.4 at 3 g/day.

There were no significant differences in stool form, assessed using the BSS, among the doses of lactulose ingested; therefore, reported stool forms were considered in more detail. Given the possibility of a time lag in the prebiotic effects of lactulose, the BSS score during the latter half of each period was examined. The BSS score only showed a significant increase during the 3 g/day period, increasing from 3.7 ± 0.7/defaecation to 4.2 ± 0.8/defaecation (Table 2). Another analysis was performed on data from subjects that had a mean BSS score of either <4/defaecation or ≥4/defaecation during only the latter half of the pre-observation period. The score in the subgroup with a BSS score of <4/defaecation significantly increased from 3.0 ± 0.8/defaecation to 3.6 ± 0.8/defaecation following ingestion of 1 g/day lactulose, from 3.4 ± 0.7/defaecation to 4.2 ± 1.1/defaecation following ingestion of 2 g/day, and from 3.7 ± 0.8/defaecation to 4.2 ± 1.1/defaecation following ingestion of 3 g/day (Table 2). The score in the subgroup with a BSS score ≥4/defaecation did not change significantly with ingestion of 1 or 2 g/day lactulose but significantly increased from 3.6 ± 0.6/defaecation to 4.2 ± 0.4/defaecation following ingestion of 3 g/day (Table 2).

We incrementally administered 1, 2, or 3 g of lactulose per day to 26 healthy Japanese women with mean defaecation frequencies of two to four times per week. The results of the trial confirmed a prebiotic effect, indicated by a significant

increase in defaecation frequency (Fig. 1B), associated with a larger number of bifidobacteria in faeces (Fig. 1C). In addition, the number of defaecation days increased (Fig. 1D). These results suggest that even 1 g/day of lactulose could have a prebiotic effect, indicated by an increase in defaecation frequency and in the numbers of faecal bifidobacteria, in healthy subjects with a defaecation frequency of two to four times per week.

In the placebo-controlled trial conducted by Tomoda *et al.* [9], which showed an increase in bifidobacteria following ingestion of 0.65 g/day of lactulose, the number of bifidobacteria in faeces was measured using the culture method, which is the method used in most studies that measure the number of bifidobacteria in faeces following lactulose ingestion. In the present study, the number of bifidobacteria was measured using quantitative PCR in an open-label trial. Our data show that ingestion of ≥ 1 g/day of lactulose for 2 weeks causes a significant increase in the number of bifidobacteria in faeces, which is consistent with the findings of Tomoda *et al.*

The dietary survey showed that the total quantity of energy consumed significantly decreased from 2,002 kcal/day to 1,673 kcal/day ($p < 0.01$) during the 2 g/day period. During that period, defaecation frequency, number of bifidobacteria in faeces, and number of defaecation days all increased significantly, as they did when 1 or 3 g/day of lactulose were ingested. Shoji *et al.* found a positive correlation between the total quantity of energy ingested and defaecation frequency [16]. Therefore, it is likely that the reduction in energy intake during the 2 g/day ingestion period would attenuate the prebiotic effects of lactulose, rather than further increasing the defaecation frequency associated with ingestion of 2 g/day of lactulose.

Our data imply that even 1 g/day of lactulose could have a prebiotic effect. However, owing the fact that there was a possibility of bias in the present study because it was an open-label trial, we cannot draw this as a conclusion. In order to have absolute confirmation of the prebiotic effect of lactulose at a low dosage, a randomised double-blind placebo-controlled trial would be necessary.

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REFERENCES

- Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, Salminen SJ, Scott K, Stanton C, Swanson KS, Cani PD, Verbeke K, Reid G. 2017. Expert consensus document: The International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat Rev Gastroenterol Hepatol* 14: 491–502. [Medline]
- Petuely F. 1957. *Lactobacillus bifidus* flora produced in artificially-fed infants by bifidogenic substances (bifidus factor). *Z Kinderheilkd* 79: 174–179. [Medline] [CrossRef]
- Ballongue J, Schumann C, Quignon P. 1997. Effects of lactulose and lactitol on colonic microflora and enzymatic activity. *Scand J Gastroenterol Suppl* 222: 41–44. [Medline] [CrossRef]
- Bouhnik Y, Attar A, Joly FA, Riottot M, Dyard F, Flourié B. 2004. Lactulose ingestion increases faecal bifidobacterial counts: a randomised double-blind study in healthy humans. *Eur J Clin Nutr* 58: 462–466. [Medline] [CrossRef]
- Förster-Fromme K, Schuster-Wolff-Bühning R, Hartwig A, Holder A, Schwiertz A, Bischoff SC, Hinrichs J. 2011. A new enzymatically produced 1-lactulose: a pilot study to test the bifidogenic effects. *Int Dairy J* 21: 940–948. [CrossRef]
- Mizota T, Mori T, Yaeshima T, Yanagida T, Iwatsuki K, Ichibashi N, Tamura Y, Fukuwatari Y. 2002. Effects of low dosages of lactulose on the intestinal function of healthy adults. *Milchwissenschaft* 57: 312–315.
- Schuster-Wolff-Bühning R, Fischer L, Hinrichs J. 2010. Production and physiological action of the disaccharide lactulose. *Int Dairy J* 20: 731–741. [CrossRef]
- Terada A, Hara H, Kataoka M, Mitsuoka T. 1992. Effect of lactulose on the composition and metabolic activity of the human faecal flora. *Microb Ecol Health Dis* 5: 43–50. [CrossRef]
- Tomoda T, Yasuo N, Takashi K. 1991. Effect of yogurt and yogurt supplemented with *Bifidobacterium* and/or lactulose in healthy persons: a comparative study. *Bifidobact Microflora* 10: 123–130. [CrossRef]
- Tuohy KM, Ziemer CJ, Klinder A, Knöbel Y, Pool-Zobel BL, Gibson GR. 2002. A human volunteer study to determine the prebiotic effects of lactulose powder on human colonic microbiota. *Microb Ecol Health Dis* 14: 165–173. [CrossRef]
- Vanhoutte T, De Preter V, De Brandt E, Verbeke K, Swings J, Huys G. 2006. Molecular monitoring of the fecal microbiota of healthy human subjects during administration of lactulose and *Saccharomyces boulardii*. *Appl Environ Microbiol* 72: 5990–5997. [Medline] [CrossRef]
- Sugahara H, Odamaki T, Hashikura N, Abe F, Xiao JZ. 2015. Differences in folate production by bifidobacteria of different origins. *Biosci Microbiota Food Health* 34: 87–93. [Medline] [CrossRef]
- Matsuki T, Watanabe K, Fujimoto J, Kado Y, Takada T, Matsumoto K, Tanaka R. 2004. Quantitative PCR with 16S rRNA-gene-targeted species-specific primers for analysis of human intestinal bifidobacteria. *Appl Environ Microbiol* 70: 167–173. [Medline] [CrossRef]
- Satoh T, Odamaki T, Namura M, Shimizu T, Iwatsuki K, Nishimoto M, Kitaoka M, Xiao JZ. 2013. *In vitro* comparative evaluation of the impact of lacto-N-biose I, a major building block of human milk oligosaccharides, on the fecal microbiota of infants. *Anaerobe* 19: 50–57. [Medline] [CrossRef]
- Lewis SJ, Heaton KW. 1997. Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 32: 920–924. [Medline] [CrossRef]
- Shoji R, Mitsukuchi C, Hayase S, Kumagai Y, Tokudome Y, Yamanaka K, Hayakawa H, Awaji H, Nonaka C, Kawai M, Fujiki K. 2017. The influence of dietary habits and the intake of fermented milk containing *Bifidobacterium* on bowel habits in young Japanese women. *Journal for the Integrated Study of Dietary Habits* 27: 249–257 (in Japanese). [CrossRef]