## Original Article

Title

Beneficial effects of Fuji FF (inulin)-containing tea on gut microbiota balance and bowel habit

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# ABSTRACT

Inulin-type fructans have various functions as prebiotics. In particular, the intestine-regulating function has been reported with a focus on bifidogenic effects and bowel habits. However, because previous studies have been conducted on high inulin intake, this was a factor in the development of functional foods using inulin. The aim of this placebo-controlled, randomized, double-blind, crossover trial was to determine the effect of low inulin intake on the gut microbiota and bowel habits in 30 healthy Japanese adults. As a result of the intake of green tea containing Fuji FF (5 g/day) for 4 weeks, fecal bifidobacteria significantly increased (p < 0.05). In addition, in subjects with constipation, the changes in stool frequency and amount were significantly increased (p < 0.05). The present study revealed that relatively low inulin intake improved gut microbiota and bowel habits.

KEYWORD Inulin, Bifidobacterium, Bowel Habit, Stool Frequency, Stool Amount

#### Introduction

Inulin, a storage polysaccharide and water-soluble dietary fiber, is commonly found in foods, such as wheat, barley, onions, leeks, and garlic (1). Inulin that extracted and purified from chicory roots has traditionally been used as a food in Europe and the United States. Moreover, inulin has recently been produced via enzymatic reactions using sugar as the raw material and has been incorporated into many foods (2). The inulin structure consists of fructose molecules arranged in a linear chain linked by  $\beta$  (2-1) bonds to the fructose residues of sucrose, with approximately 2–60 degree of polymerization (1).

The physiological functions of inulin have been extensively studied in animals and humans. In particular, several studies have supported the ability of inulin to increase beneficial gut bacteria, such as *Bifidobacterium* and *Lactobacillus* (commonly known as probiotics), thereby enhancing the gut microbiome balance (3-6). Consequently, inulin has been recognized as a prebiotic. Additionally, the functional aspects of gastrointestinal effects, including bowel habit improvement by increasing bowel frequency and volume, have been demonstrated (7-10).

However, most studies examining the regulatory effects of inulin on bowel habits used relatively high doses, whereas few clinical trials have been conducted using low doses. Therefore, we aimed to investigate the effect of relatively low inulin intake on gut microbiota and bowel habits.

#### Participants and Methods

#### 1. Test food

The test food used in this study was a tea-based beverage. A tea beverage containing 5 g Fuji FF (≥90% inulin content) was used as the test food, and a tea beverage without Fuji FF was used as the placebo.

#### 2. Participants

This trial adhered to the ethical principles of the Declaration of Helsinki, the "Ethical Guidelines for Medical Research Involving Human Subjects (Notification No. 3 of the Ministry of Education, Culture, Sports, Science and Technology, and the Ministry of Health, Labour and Welfare, 2014)," and the "Act on the Protection of Personal Information (Law No. 57 of May 30, 2003)." This study was approved by the Nihonbashi Egawa Clinic Clinical Research Ethics Committee (Approval Date: July 14, 2017) and conducted after registration in the UMIN Clinical Trials Registry (UMIN-CTR) (UMIN Trial ID: UMIN000028826). Huma R & D K.K., a contract research organization, recruited participants for the trial. Prior to the trial, the participants were thoroughly informed of the content of the trial and their voluntary consent was obtained. The principal investigator explained the trial details to the participants during their initial visit and ensured that they provided written informed consent based on their voluntary decision to participate. The selection criteria for the participants were healthy Japanese men and women aged 20–60 years. Following screening tests, 30 individuals (8 men, 22 women, mean age 41.5±10.2 years, mean body mass index [BMI] 20.6±3.2) were selected based on the discretion of the principal investigator.

#### 3. Study design

This was a double-blind, randomized, placebo-controlled, crossover comparison trial. The trial schedule is shown in Fig. 1. Thirty participants were randomly assigned to Group A and Group B using a stratified randomization method based on sex and age, with each group comprising 15 participants. In Period I, Group A received placebo and Group B received inulin. During Period II, Group A received inulin and Group B received placebo. The ingestion period of the test food was set at 4 weeks, preceded by 1-week pre-observation period before each test food ingestion period in Phase I and Phase II. These were labeled as the "before Phase I" and "before Phase II". Additionally, 2-week rest period was implemented after the completion of Phase I and before the pre-observation period (before Phase II).

## 4. Stool examination (gut microbiota analysis)

The fourth week of Phases I and II was designated as the stool sampling period. During this

period, stool samples were collected from each participant using a stool collection kit. The gut microbiota was analyzed using the terminal restriction fragment-length polymorphism (T-RFLP) method to measure the relative abundance of *Bifidobacterium*, *Lactobacillus*, *Bacteroides*, *Prevotella*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster XI, *Clostridium* cluster XIVa, *Clostridium* cluster XI, *Clostridium* cluster XIVA, *Clostridium* cluster XI, *Clostridium* cluster XIVA, *Clostridium*, *Clost* 

# 5. Bowel habit questionnaire

During the trial period, participants recorded daily bowel movements, including information such as "stool color," "stool shape," "stool frequency," "stool amount," "stool odor," "straining during bowel movements," and "sense of incomplete evacuation after bowel movements." The stool amount was measured using a standard of one unit equivalent to the volume of a cylinder with a diameter of 2 cm and a length of 10 cm.

#### 6. Statistical analyses

A paired *t*-test (5% significance level, two-tailed) was conducted for gut microbiota analysis, and the results were presented as mean values and medians. For the bowel habit questionnaire, changes were calculated based on the measured values at week 4 of ingestion of the test food in Phases I and II and the respective pre-observation periods. Wilcoxon's signed-rank test (5% significance level, twotailed) was performed for the questionnaire, and the results are presented as mean  $\pm$  standard deviation. Additionally, participants with fewer than five bowel movements per week during the pre-observation period were classified as constipated, and bowel questionnaire analyses were conducted for all participants, participants without constipation, and participants with constipation.

#### Results

#### 1. Participant demographics and adverse events

Thirty participants were initially selected; however, one individual was excluded from the analysis because of a violation of the exclusion criterion related to probiotics intake. Therefore, the analysis was conducted on 29 participants (8 males, 21 females, mean age  $41.9\pm10.2$  years, BMI 20.5 $\pm3.3$ ). The test-food intake rate during the study period was 100 %. No adverse events related to the test foods were identified by the principal investigator during the study period.

#### 2. Gut microbiota analysis

T-RFLP analysis of the gut microbiota revealed a significant increase in *Bifidobacterium* during the inulin intake period (mean: 18.8±14.1%, median: 20.5% [interquartile range: 0.95–25.7%]) compared to the placebo intake period (mean: 14.1±14.2%, median: 11.6% [interquartile range: 2.5–19.1%]). No significant differences were observed in other bacterial counts between the inulin and the placebo intake periods.

#### 3. Bowel habit questionnaire

The bowel habit questionnaire analyzed all participants (with and without constipation) after classifying participants with fewer than five bowel movements per week during the pre-observation period as constipated. The characteristics of all participants, those without constipation, and those with constipation are listed in Table 1, and the stool frequency and amount are listed in Table 2. The bowel habits of all participants and those without constipation were not significantly different between the inulin intake period and the placebo period. However, although the actual stool frequency of participants with constipation were not significantly different between the inulin period (3.30±1.05 movements/week) and the placebo period  $(2.85\pm1.01 \text{ movements/week})$ , the change in stool frequency significantly increased during the inulin period (0.45±0.80 movements/week) compared to that during the placebo period (-0.25±0.99 movements/week). Similarly, the actual stool volume for participants with constipation was 10.75±5.05 units/week during the inulin period compared to 9.30±5.98 units/week during the placebo period. Although no significant difference was observed between the inulin period and the placebo period, the change in stool volume was significantly increased during the inulin period  $(2.05\pm2.48 \text{ units/week})$  compared to the placebo period  $(0.00\pm3.75 \text{ units/week})$ . The actual values or rates of change in "stool color," "stool shape," "stool odor," "straining during bowel movements," and "sense of incomplete evacuation after bowel movements" of participants with constipation were not significantly different.

#### Discussion

This study investigated the effects of a relatively low-dose inulin intake (5 g/day, Fuji FF) on the fecal microbiota and bowel habits of healthy individuals. The results revealed an increase in the abundance of *Bifidobacterium* in feces. Furthermore, stool frequency and volume increase in individuals with a tendency toward constipation.

The increase in bifidobacteria in feces following inulin intake suggests that bifidobacteria utilized and proliferated on inulin in the large intestine. Inulin contains fructose, which is polymerized through  $\beta$  (2-1) bonds, and passes through the upper digestive tract to the large intestine without substantial breakdown (11). Intestinal bacteria, including *Bifidobacterium* and lactic acid bacteria, possess specific digestive enzymes that metabolize inulin in the large intestine (12-14). Therefore, intake of relatively long-chain Fuji FF intake seems to increase the proportion of *Bifidobacterium* in the feces. As the fecal microbiota reflects the bacterial composition in the intestine, an increased proportion of beneficial bacteria such as *Bifidobacterium* and Lactobacillus (commonly known as probiotics) in the intestine is considered to improve the gut microbiota balance (15). This study showed an increased proportion of *Bifidobacterium* in feces due to inulin intake, indicating that inulin improves the gut microbiota balance by promoting *Bifidobacterium* growth in the intestines.

Inulin-induced improvement in bowel habits can be attributed to intestinal *Bifidobacterium* proliferation. *Bifidobacterium* utilize inulin for growth and produce various short-chain fatty acids (SCFAs) such as acetic, propionic, and butyric acids (14). Among these SCFAs, propionic acid enhances intestinal peristalsis through SCFA receptor activation (16). Therefore, inulin intake may stimulate *Bifidobacterium* growth in the intestine, leading to SCFA production, which, in turn, promotes intestinal motility and improves bowel habits.

Conclusion

The findings of this study revealed that ingesting relatively low inulin doses increased the proportion of *Bifidobacterium* in the intestine, thereby improving the gut microbiota. Additionally, inulin improves bowel habits, particularly in individuals with constipation. These findings indicate that inulin enhances the intestinal environment and regulates bowel movement in healthy individuals.

Conflict of interest (COI)

No declaration related to the content of this paper.

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# Figure 1

	Pre-observation period (Before Phase I)	Phase I	Washout period	Pre-observation period (Before Phase II)	Phase II	
	1 week	4 weeks	2 weeks	1 week	4 weeks	
Group a $(n = 15)$		Placebo food			Test food	
Group b ( $n = 15$ )		Test food			Placebo food	
Interview	•		•	•	•	
Bowel habit questionnaire	•				•	
Stool sampling			•		•	

Figure 1 Study schedule

# Table 1

	All subjects (n = 29)	Non-constipated subjects a) (n = 9)	Constipated subjects b) (n = 20)
Male/Female	8/21	1/8	7/13
Age	$41.9\pm10.2$	$41.4\pm9.8$	$42.1\pm10.4$
Body mass index (BMI)	$20.5\pm3.3$	$19.7\pm1.8$	$20.9\pm3.7$
Number of bowel movements	$3.7\pm1.7$	$5.4 \pm 2.1$	$3.0\pm0.6$
(times/week)			

Table 1. Subject characteristcs of all subjects, non-constipated subjects, and constipated subjects

a) Subjects with five or more bowel movements per week during the pre-observation period

b) Subjects with less than five bowel movements per week during the pre-observation period

	Subject characteristics		Placebo			Inulin		
Evaluated item		n	Pre-observation period	Fourth week of intake	Amount of change	Pre-observation period	Fourth week of intake	Amount of change
Stoo frequency (times/week)	All subjects	29	3.93 ± 2.15	3.72 ± 2.20	$-0.21 \pm 1.83$	$3.55 \pm 1.61$	3.86 ± 1.63	0.31 ± 1.44
	Non-constipated subjects a)	9	$5.78\pm2.97$	$5.67\pm2.79$	$-0.11 \pm 2.92$	$5.11 \pm 1.79$	$5.11 \pm 1.97$	$0.00\pm2.26$
	Constipated subjects b)	20	$3.10\pm0.70$	$2.85\pm1.01$	$\textbf{-0.25}\pm0.99$	$2.85\pm0.85$	$3.30\pm1.05$	$0.45\pm0.80^{\ast}$
Stool amout (units/week)	All subjects	29	$9.83 \pm 7.04$	$10.17 \pm 6.70$	$0.34 \pm 4.37$	$9.31 \pm 5.59$	$10.59\pm5.40$	$1.28 \pm 3.41$
	Non-constipated subjects a)	9	$11.00\pm10.13$	$12.11 \pm 7.71$	$1.11\pm5.40$	$10.67\pm7.82$	$10.22\pm6.09$	$\textbf{-0.44} \pm \textbf{4.42}$
	Constipated subjects b)	20	$9.30\pm4.97$	$9.30\pm5.98$	$0.00\pm3.75$	$8.70\pm4.08$	$10.75\pm5.05$	$2.05\pm 2.48^{\ast}$

Table 2. Impact of intake of inulin-containing beverage on stool frequency and stool amount

a) Subjects with more than five bowel movements per week during the pre-observation period

b) Subjects with less than five bowel movements per week during the pre-observation period

\*p < 0.05