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Improvement of constipation and liver function by plant-derived lactic acid bacteria: A double-blind, randomized trial

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Abstract

Objective: Lactic acid bacteria (LAB) contribute to human health; however, the probiotic properties vary among strains classified into the same species. The primary objective of this study was to evaluate the effects of yogurts made by different types of LAB on the gastrointestinal system. The yogurts were also evaluated by measuring serum lipid contents and liver functional indicators as a secondary objective.

Methods: Healthy human adults ($n = 68$) with some complaints with regard to intestinal health, including constipation and diarrhea, were randomly assigned to receive one of three types of yogurt in a double-blind manner: type A, a yogurt made by plant-derived LAB (mainly *Lactobacillus [Lb.] plantarum* SN35N); type B, a yogurt made by plant-derived LAB (mainly *Lb. plantarum* SN13T); and type C, a yogurt made by animal-derived LAB (mainly *Lactococcus lactis* A6 and *Streptococcus thermophilus* 510) as a control. The subjects consumed 100 g of yogurt daily for 6 wk. Data were collected from clinical visits at 2-wk intervals and by diaries used to record defecation and health conditions.

Results: Drastic and constant increments of defecation frequency in subjects with constipation were observed with type A and B yogurts but not with type C yogurt. Type B and C yogurts resulted in decreases in total and low-density lipoprotein cholesterol. The serum concentrations of liver functional parameters were improved by the type B yogurt (12–25% reduction).

Conclusion: The present study suggests that *Lb. plantarum* SN13T exhibits a superior probiotic effect on constipation in addition to improving the serum lipid contents and liver function.

Keywords: Probiotics; *Lactobacillus plantarum*; Defecation; Cholesterol; γ-Glutamyl transpeptidase

Introduction

Studies of lactic acid bacteria (LAB) have been conducted from the viewpoint that the micro-organism contributes to the prevention and improvement of constipation, diarrhea, inflammatory bowel disease, *Helicobacter pylori* infection, lactose intolerance, colon cancer, serum cholesterol level, and allergies [1–7]. Constipation accompanied by infrequent defecations, hard or lumpy stools, straining, bloating, feeling of incomplete evacuation after a defecation, and abdominal discomfort is common among the general population. Health-related quality of life is impaired by chronic constipation [8–10]. Gut flora has important, metabolic, and protective functions and could be essential for certain pathologic disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases [11,12]. A recent study has shown that intestinal microflora may influence the production of autoantibodies against appetite-regulating peptide

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hormones and neuropeptides [13]. Because colonic microflora also influences the peristalsis of the colon, imbalance in the colonic microflora has been suggested as a cause of constipation. Some clinical studies have shown that LAB can reduce serum cholesterol levels; however, there have been other reports that suggested no effect [2,14–21]. Serum lipid-lowering effects in humans by LAB have not been conclusive; therefore, further human studies seem to be necessary to accumulate the evidence.

Probiotics are defined as a live microbial food supplement that beneficially affects the host by improving the intestinal microbial balance [22]. LAB have been suggested to improve gut microflora conditions. Functional foods, which are potentially beneficial and affect a variety of bodily functions, are currently spreading in the worldwide marketplace. LAB contribute to the manufacture of these products. The physiologic and functional properties of LAB, however, differ even in strains classified into the same species [11,23]. The bacteria are classified on the basis of their phenotypic properties, e.g., morphology, mode of glucose fermentation, growth at different temperatures, lactic acid configuration, and fermentation of various carbohydrates. Molecular typing with 16S ribosomal RNA is a valuable method to identify the species [24].

Lactic acid bacteria can be roughly classified into two groups. The first group is derived from animal sources, such as raw milk and intestines, and has been used to make yogurt or cheese. The second group is isolated from plant sources, including grasses, vegetables, and fruits, and has been used in traditional Asian foods, such as *miso*, soy sauce, pickled vegetables, and *kimuchi*. Although almost all ingested micro-organisms are killed by gastric acid, bile, and pancreatic secretion before they reach the large intestine, some LAB strains are, interestingly, resistant to these digestive fluids [23]. In general, plant-derived LAB are more resistant to severe environment than animal-derived LAB. Indeed, the plant-derived LAB strains that we isolated, including the strains SN13T and SN35N, which belong to *Lactobacillus* (*Lb.*) *plantarum*, are much more resistant to artificial gastric juices and bile than animal-derived *Lactococcus* (*Lc.*) *lactis* and *Lb. acidophilus*, which are generally used to produce yogurts (unpublished). Thus, because plant-derived LAB can reach the intestine in the living state, they may be more functional as probiotics than animal-derived LAB. The combination of prominent health care function with high resistance properties against gastrointestinal digestive juices of a certain LAB strain promises to be significantly beneficial for human health.

In this study, we evaluated the effects on human health of yogurts produced by plant-derived LAB. In addition to evaluating the effects of yogurt consumption on the function of defecation, we tried to determine whether the biochemical parameters, such as serum cholesterol and liver functional indicators, fluctuate with consumption of these yogurts.

Materials and methods

Subjects

Healthy adults who had some complaints with regard to intestinal health, such as constipation, diarrhea, abdominal pain, and bloating, were recruited by advertisement in Hiroshima, Japan, and 68 male and female volunteers (66 Japanese and 2 Chinese) aged 21–65 y participated in the study. Of the 68 subjects who began, four dropped out during the study for reasons unrelated to the study. Pregnant or breast-feeding women were excluded from the study population. Four men and five women were taking the following medicines: minor tranquilizers ($n = 2$), calcium channel blockers ($n = 1$), angiotensin II receptor blockers ($n = 2$), H_1 blockers ($n = 1$), gastric proton pump inhibitors ($n = 1$), bisphosphonate ($n = 1$), or hypolipidemic agents ($n = 2$). Medication dosages were kept constant throughout the trial. The study was approved by the ethics committee of Hiroshima University and performed according to guidelines of the Helsinki Declaration. Before starting the clinical evaluation study for functional foods, we obtained informed consent from all study participants.

Study design

This study was carried out using a double-blind, randomized design with three parallel groups from October through December 2007. Subjects were assigned to one of three types of yogurts using stratified randomization by the defecation frequencies of preliminary inquiries: type A yogurt was produced by plant-derived LAB (with contents of *Lb. plantarum* SN35N and SN13T at 95% and 5%, respectively); type B yogurt was produced by plant-derived LAB (with contents of *Lb. plantarum* SN13T and SN35N at 98% and 2%, respectively); and type C yogurt was produced by animal-derived LAB (with contents of *Lc. lactis* A6, *Streptococcus* (*S.*) *thermophilus* 510, and *Lb. bulgaricus* C6 at 86.1%, 13.8%, and 0.1%, respectively). An allocation sequence, which was generated by a computer and kept in a numbered container, was used for random allocation. All yogurts remained viable at more than 2×10^8 LAB/g throughout their shelf-life and were manufactured by Nomura Dairy Products Co., Ltd. (Hiroshima, Japan), with plain packages to prevent the study subjects from learning the type of yogurt they were receiving. All subjects in all treatment types consumed 100 g of each yogurt every day independent of time for 6 wk. Subjects were instructed to maintain their ordinary dietary habits during the study, and they were asked to avoid other fermented foods and medicines for intestinal disorders except in case of an emergency. If subjects consumed these foods or medicines, they were asked to make a record in their daily diaries, and the data in the medication category were excluded from analyses. Clinical assessment, body weight, blood samples, and blood pressure were obtained at weeks −2, 0, 2, 4, and

6. Urine samples were also collected at weeks −2, 0, and 6. Furthermore, the subjects were asked to fill out questionnaires with regard to defecation and to describe matters concerning their health, medication, and yogurt intake in their daily diaries from a week before the start of the trial through the end. Diaries were collected at every clinical visit to encourage compliance.

Analyses of defecation

Subjects were instructed to keep diaries about defecation, including frequency, form, volume, odor, feeling during evacuation, abdominal pain, and feeling of incomplete evacuation. The stool forms were scored from 1 to 7 according to the Bristol Stool Form Scale (1, separate hard lumps, like nuts; 2, sausage-shaped but lumpy; 3, like a sausage but with cracks on the surface; 4, like a sausage or snake, smooth and soft; 5, soft blobs with clear-cut edges; 6, fluffy pieces with ragged edges, a mushy stool; 7, watery with no solid pieces) [25,26], and the subjects received instructions with a stool illustration and explanation in advance for the purpose of objectively selecting the stool form. Weekly averages of the scores were individually calculated for the evaluation.

Analyses of serum biochemical parameters

Biochemical parameters, such as total cholesterol (TC), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and γ-glutamyl transpeptidase (γ-GTP) in serum, were measured for a preliminary human clinical evaluation. Urine was also examined to assess any undesirable changes because of adverse events. Two subjects who had taken antihyperlipidemic agents and four subjects who withdrew and lacked the final data point (week 6) were excluded from analyses for TC, LDL cholesterol, and HDL cholesterol. For liver functional parameters (AST, ALT, and γ-GTP), two were excluded because they could not visit Hiroshima University Hospital at week 4.

Statistical analysis

The data were analyzed using SPSS 16.0 (SPSS Japan, Inc.). One-way analysis of variance was performed on all baseline data among the types. Differences in variables between baseline and after treatment were assessed with paired *t* tests. Differences among treatment types were analyzed by a two-way repeated measured analysis of variance or by independent *t* tests for the treatment types versus the control type. Data are presented as mean ± standard deviation. All statistical analyses were two-tailed ($P < 0.05$ was significant for all statistical tests).

Results

Subjects and characteristics

Four of 68 subjects dropped out during the study for reasons unrelated to the study. One individual participated until week 2 (completed three of five clinical visits). The other three remained until week 4 (completed four of five clinical visits). One of four submitted all diaries even though the final visit to the hospital had not been completed; therefore, 65 subjects completed the defecation study. Data collected from these four subjects until dropping out were used for analyses. The flow of study subjects is illustrated in Figure 1. The compliance of yogurt intakes was 97.8 ± 3.0% (88.1–100%) according to the daily diaries of the study subjects. The characteristics of the subjects at the baseline are listed in Table 1. There were no significant differences in the treatment types with regard to age, body weight, body mass index, systolic blood pressure, and diastolic blood pressure. No subject reported any significant adverse events resulting from yogurt intake throughout the trial. No abnormal changes in urine analysis or serum biochemical parameters (e.g., lactate dehydrogenase, choline esterase, alkaline phosphatase, amylase, Na^+, K^+, Cl^-, total protein, serum urea nitrogen, and creatinine clearance) were observed during the clinical trial.

Defecation

In the present study, participants had persistent defecation difficulties, such as constipation, diarrhea, abdominal pain, abdominal bloating, straining, and feeling of incomplete evacuation. Based on the frequency of defecation at baseline,

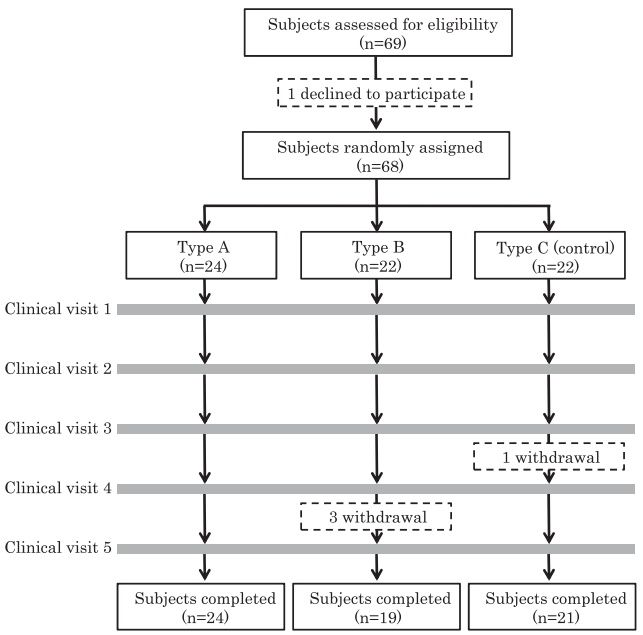

Fig. 1. Flow of subjects through the trial.

Table 1
Characteristics of subjects at baseline*

	Group A ($n = 24$)	Group B ($n = 22$)	Group C (control) ($n = 22$)	P at baseline
Male/female	6/18	7/15	6/16	—
Age (y)	37.3 ± 12.5	35.1 ± 11.6	33.0 ± 13.0	0.505
Body weight (kg)	58.1 ± 11.8	57.0 ± 16.2	55.6 ± 10.3	0.807
BMI (kg/m^2)	22.5 ± 3.5	21.4 ± 3.8	21.2 ± 3.1	0.362
Systolic blood pressure (mmHg)	116.6 ± 17.0	118.9 ± 15.3	114.9 ± 14.3	0.697
Diastolic blood pressure (mmHg)	70.5 ± 9.2	72.6 ± 9.7	70.5 ± 8.6	0.670

BMI, body mass index; group A, intake of yogurt produced by mainly *Lactobacillus plantarum* SN35N; group B, intake of yogurt produced by mainly *Lactobacillus plantarum* SN13T; group C, intake of yogurt produced by mainly *Lactococcus lactis* A6 (*Streptococcus lactis* A6) and *Streptococcus thermophilus* 510 (*Streptococcus salivarius subsp. thermophilus* 510).

* Data are presented as mean ± SD.

the subjects were separated into three categories: 1) fewer than 5 times/wk; 2) 5 to 10 times/wk; or 3) more than 10 times/wk. When the defecation frequencies were analyzed in category 1, drastic increases were observed in the plant-derived LAB yogurts, types A and B, which, at week 6, averaged 1.50 and 1.73 times that at the baseline, respectively; conversely, there was only a modest increment with type C yogurt (1.17 times the frequency at baseline), which contained the animal-derived LAB (Table 2). Significant increases in defecation frequency versus each baseline were determined in all treatment types at some points; however, type B was especially outstanding, showing significant enhancements from the second through the final week. Despite the large degree of improvement, there was no statistical difference between the study types (types A and B) and the control (type C). In categories 2 and 3, the intake of all yogurts resulted in no significant increase or decrease in defecation frequencies, although we had anticipated that the individuals in category 3 would have a normal defecation frequency.

According to the individual average of the Bristol scale at baseline, subjects with a Bristol scale <4 and those with a Bristol scale >4 were analyzed separately with regard to any improvements in stool consistency. The smaller number in the Bristol Stool Form Scale indicates harder stools, and the larger numbers, softer stools and diarrhea. Remarkably, not only did individuals who produced hard stools (Bristol scale <4) achieve a more normal stool form, but also individuals with a soft stool (Bristol scale >4) tended to move toward the middle range in all types (Table 3). A similar efficacy in all treatment types was observed in the stool consistencies by yogurt intake, and the responses started in the first week.

Serum biochemical parameters

Total cholesterol in group B decreased significantly from 214.3 mg/dL (5.55 mmol/L) to 203.2 mg/dL (5.26 mmol/L, $P = 0.012$) for 6 wk, but not in groups A and C. Next we analyzed the subjects with moderately high levels of TC, 180–260 mg/dL (4.66–6.73 mmol/L; TC 180–260 subjects) at baseline (week 0), excluding the subjects with low or remarkably high lipid levels. There were significant decreases of TC in the TC 180–260 subjects with type B and C yogurts. However, TC in group A did not decrease statistically in all subjects or the TC 180–260 subjects. Similarly, LDL cholesterol was lowered only in group B subjects when all subjects were analyzed, and significant decreases were observed in group B and C subjects when TC 180–260 subjects were analyzed. As expected, HDL cholesterol did not change regardless of treatment (Table 4).

It is noteworthy that an improvement of liver function according to serum AST, ALT, and γ-GTP was observed by yogurt intake. In type B, especially, when compared with the other types, all AST, ALT, and γ-GTP levels were

Table 2
Effect of yogurt on frequency of defecation in subjects with constipation*

Frequency of defecation (times/wk)	Group A ($n = 8$)	Group B ($n = 9$)	Group C (control) ($n = 11$)	P†		
				Time	Treatment	Time × treatment interaction
Before yogurt intake	3.0 ± 1.7	3.4 ± 1.6	3.4 ± 1.6			
Week 1	3.4 ± 1.1	4.0 ± 2.1	4.3 ± 2.3			
Week 2	4.1 ± 2.1	4.6 ± 2.5‡	3.8 ± 2.6			
Week 3	3.5 ± 1.9	5.0 ± 3.0‡	4.4 ± 2.4	<0.005	0.557	0.414
Week 4	4.1 ± 1.1	5.1 ± 2.5‡	4.3 ± 2.3			
Week 5	3.8 ± 1.3	5.5 ± 3.0‡	4.0 ± 2.0‡			
Week 6	4.5 ± 1.5‡	6.0 ± 3.7‡	4.0 ± 2.6			

* Data are presented as mean ± SD.

† Two-way repeated measures analysis of variance.

‡ Significant difference from baseline (before yogurt intake), $P < 0.05$ (paired t test).

Table 3
Changes in stool consistency defined by the Bristol Stool Form Scale*

		Group A	Group B	Group C (control)
Bristol scale <4	Subjects	10	7	7
	Before yogurt intake	2.82 ± 0.82	3.38 ± 0.31	3.07 ± 0.68
	Week 1	3.44 ± 1.04‡	3.97 ± 0.44†	3.92 ± 0.97†
	Week 6	4.09 ± 0.47‡	3.77 ± 0.46	4.15 ± 0.70†
Bristol scale >4	Subjects	9	10	10
	Before yogurt intake	4.82 ± 0.53	4.77 ± 0.33	4.65 ± 0.43
	Week 1	4.21 ± 0.70‡	4.46 ± 0.50	4.04 ± 0.66†
	Week 6	4.22 ± 0.58†	4.34 ± 0.58	4.23 ± 0.60†

* Data are presented as mean ± SD. The Bristol scale of each subject at baseline was averaged and grouped as <4 and >4: 1, nut-like; 2, lumpy sausage; 3, sausage with cracks; 4, smooth snake; 5, soft blobs; 6, fluffy pieces; 7, watery.
† $P < 0.05$
‡ $P < 0.01$ (paired t test), significant difference from baseline (before yogurt intake).

remarkably decreased to 88%, 75%, and 78% of the values recorded at baseline, respectively (Table 5).

Discussion

Yogurt is generally considered to alleviate gastrointestinal conditions such as constipation and diarrhea [1–4]. In the present study, we compared the probiotic effects of three types of yogurt produced by different LAB strains: type A and B yogurts were produced using plant-derived LAB (*Lb. plantarum* SN13T or *Lb. plantarum* SN35N), and type C was produced by animal-derived LAB (a coculture of *Lc. lactis* with *S. thermophilus*). The three types of yogurt are available in the markets in Hiroshima, Japan. We observed that consumption of these yogurts resulted in satisfactory defecation from the first week for the subjects originally reporting constipation.

Table 4
TC, LDL cholesterol, ad HDL cholesterol at baseline (week 0) and week 6*

	Group A ($n = 24$)	Group B ($n = 18$)	Group C (control) ($n = 20$)
TC (mg/dL)			
All subjects			
Week 0	193.6 ± 34.4	214.3 ± 42.1	206.9 ± 40.6
Week 6	188.0 ± 39.2	203.2 ± 32.2†	197.3 ± 32.2
Difference (95% CI)	−5.6 (−13.2 to 2.0)	−11.1† (−18.8 to −3.3)	−9.7 (−19.2 to −0.1)
TC 180–260 subjects‡			
Week 0	212.4 ± 20.1	219.5 ± 24.0	216.6 ± 30.3
Week 6	203.2 ± 37.5	207.3 ± 17.0†	204.7 ± 23.9†
Difference (95% CI)	−9.2 (−22.8 to 4.5)	−12.3† (−21.4 to −3.1)	−11.9† (−20.8 to −3.0)
LDL cholesterol (mg/dL)			
All subjects			
Week 0	104.3 ± 28.4	120.1 ± 33.8	115.7 ± 35.8
Week 6	102.1 ± 28.3	112.7 ± 27.6†	108.9 ± 29.9
Difference (95% CI)	−2.3 (−8.5 to 4.0)	−7.3† (−13.4 to −1.2)	−6.8 (−14.4 to 0.8)
TC 180–260 subjects‡			
Week 0	117.6 ± 18.0	125.2 ± 26.5	123.4 ± 26.5
Week 6	113.5 ± 26.1	115.0 ± 22.3†	113.3 ± 20.8†
Difference (95% CI)	−4.2 (−15.3 to 7.0)	−10.2† (−18.4 to −1.9)	−10.1† (−18.5 to −1.7)
HDL cholesterol (mg/dL)			
All subjects			
Week 0	74.8 ± 17.6	76.3 ± 22.3	76.3 ± 15.0
Week 6	72.0 ± 18.7	75.6 ± 22.0	76.3 ± 13.4
Difference (95% CI)	−2.7 (−5.6 to 0.2)	−0.7 (−3.9 to 2.4)	0.0 (−5.0 to 5.0)
TC 180–260 subjects‡			
Week 0	78.2 ± 20.6	73.5 ± 24.6	78.2 ± 17.6
Week 6	74.2 ± 21.6	74.5 ± 25.1	80.6 ± 12.7
Difference (95% CI)	−4.0 (−8.6 to 0.6)	1.1 (−2.6 to 4.7)	2.5 (−2.5 to 7.4)

CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; TC 180–260 subjects, subjects with total cholesterol levels 180–260 mg/dL (4.66–6.73 mmol/L)
* Data are presented as mean ± SD.
† Significant difference from the baseline (before yogurt intake), $P < 0.05$ (paired t test).
‡ $n = 13$, 11, and 11 for groups A, B, and C, respectively.

Table 5
Liver functional parameters at baseline (week 0) and week 4*

	Group A ($n = 23$)	Group B ($n = 22$)	Group C (control) ($n = 21$)
AST (IU/L)			
All subjects			
Week 0	20.4 ± 7.1	23.8 ± 12.6	18.9 ± 5.9
Week 4	19.3 ± 4.0	21.6 ± 9.8†	18.2 ± 3.9
Difference (95% CI)	−1.1 (−2.9 to 0.6)	−2.2† (−4.1 to −0.3)	−0.7 (−2.2 to 0.9)
Relative change (%) (95% CI)	−1.6 (−8.3 to 5.1)	−6.4 (−12.7 to −0.1)	−0.9 (−7.2 to 5.4)
Subjects with 20–80 IU/L	10	12	6
Week 0	25.9 ± 7.8	29.3 ± 15.0	25.7 ± 6.8
Week 4	22.8 ± 3.3	25.3 ± 12.1†	22.8 ± 3.2
Difference (95% CI)	−3.1 (−6.7 to 0.5)	−4.0† (−7.0 to −1.0)	−2.8 (−7.3 to 1.6)
Relative change (%) (95% CI)	−7.4 (−20.5 to 5.6)	−11.7† (−21.0 to −2.4)	−8.2 (−21.7 to 5.3)
ALT (IU/L)			
All subjects			
Week 0	20.7 ± 12.7	31.1 ± 41.4	16.2 ± 6.7
Week 4	19.0 ± 11.1†	26.4 ± 34.3†	14.4 ± 3.4
Difference (95% CI)	−1.7† (−3.2 to −0.2)	−4.7† (−9.1 to −0.3)	−1.9 (−3.7 to 0.0)
Relative change (%) (95% CI)	−5.4 (−13.7 to 2.8)	−8.4 (−17.4 to 0.6)	−5.7 (−13.6 to 2.2)
Subjects with 20–80 IUL	9	7	5
Week 0	32.1 ± 14.0	29.4 ± 6.3	25.2 ± 7.1
Week 4	28.3 ± 12.6†	21.4 ± 5.0†	18.8 ± 1.3
Difference (95% CI)	−3.8† (−6.1 to −1.5)	−8.0† (−13.0 to −3.0)	−6.4 (−11.9 to −0.9)
Relative change (%) (95% CI)	−11.7† (−19.3 to −4.0)	−25.2† (−39.1 to −11.2)	−21.4 (−37.1 to −5.6)
γ-GTP (IU/L)			
All subjects			
Week 0	23.0 ± 16.1	37.0 ± 39.5	23.9 ± 15.2
Week 4	20.9 ± 15.6‡	31.2 ± 31.2†	22.0 ± 12.0
Difference (95% CI)	−2.2‡ (−3.4 to −1.0)	−5.8† (−10.9 to −0.7)	−2.0 (−4.3 to 0.4)
Relative change (%) (95% CI)	−10.4‡ (−17.0 to −3.7)	−8.4 (−16.6 to −0.1)	−4.6 (−11.9 to 2.7)
Subjects with 25–90 IU/L	7	6	8
Week 0	41.7 ± 18.3	55.5 ± 25.2	37.4 ± 17.6
Week 4	38.1 ± 19.0	39.5 ± 12.0†	32.5 ± 13.5
Difference (95% CI)	−3.6 (−6.6 to −0.5)	−16.0† (−27.2 to −4.8)	−4.9 (−10.1 to 0.3)
Relative change (%) (95% CI)	−11.1 (−23.3 to 1.1)	−22.1† (−39.0 to −5.3)	−10.9 (−23.3 to 1.5)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; γ-GTP; γ-glutamyl transpeptidase
* Data are presented as mean ± SD.
† $P < 0.05$.
‡ $P < 0.01$ (paired t test), significant difference from the baseline (before yogurt intake).

However, the probiotic effect was not the same among the three types. With consumption of type C yogurt, the averages of defecation frequency increased by 0.9 times/wk in week 1, but no further increments were observed. Type A and B yogurts resulted in constantly increasing defecation frequencies with each intake, suggesting that the plant-derived *Lb. plantarum* SN13T and SN35N reach the human intestine alive, even after the bacteria are exposed to gastric juice and bile. With the intake of type C yogurt, the defecation might be maximal within the first week, and the effect could be transient without continuous consumption of the yogurt due to the restriction of the survival of the bacteria within the gastrointestinal tract. The weekly defecation frequency at week 6 increased by 1.5 and 2.6 times/wk from baseline with type A and B yogurts, respectively, but by only 0.6 times/wk with type C. The three types of yogurt improved hard and soft stools. In addition, volume and odor of the stool, feeling during defecation, abdominal pain, and feeling of incomplete evacuation were improved by all types of yogurt (data not shown). These observations are consistent with the probiotic effects commonly attributed to yogurt consumption.

The main objective of this clinical trial was to evaluate the potential of yogurt produced by plant- or animal-derived LAB to improve intestinal conditions. Therefore, it was necessary to recruit volunteers with complaints of intestinal function, such as constipation or diarrhea. The trials were determined to be adequately long to permit gastrointestinal responses. TC and LDL cholesterol were significantly decreased with 6 wk of intake of type B and C yogurts. TC and LDL cholesterol continued to decrease as types B and C were consumed (data not shown), suggesting that the long-term intake might be more effective. Serum lipid-lowering effects have been reported with *Lb. plantarum*, *Lb. acidophilus*, and *Bifidobacterium longum* [14–18]. There have, however, been reports with contrasting results [19–21]. This inconsistent result was also observed in the

present study as follows: although strains SN35N and SN13T were classified into the same *Lb. plantarum*, the effects on serum lipid content and liver functional parameters differed in the two strains. Thus, the probiotic effects of LAB may be strain-specific.

When individuals experience chronic insults, such as viral infection, toxic damage, and alcoholic/non-alcoholic fatty liver, the values of AST, ALT, and γ-GTP in serum, as hepatic indicators, are significantly increased. Non-alcoholic fatty liver disease is a common liver pathology and includes a wide histologic spectrum that ranges from simple steatosis to non-alcoholic steatohepatitis [27,28]. No treatment has yet been established for patients with non-alcoholic steatohepatitis. LAB have been shown to be effective in improving liver function exclusively in animal model experiments [29–32]. In the present study, we observed that type B yogurt contributes to a decrease in these hepatic indicators, especially when the subjects within the moderate ranges (AST 20–80 IU/L, ALT 20–80 IU/L, and γ-GTP 25–90 IU/L) were analyzed (12–25% decrease). Type A yogurt decreased the ALT value. This is the first report of a human clinical trial in which a certain strain of LAB was found to improve liver function.

Conclusions

We confirmed that LAB have several probiotic potencies to maintain human health and that those effects are strain-specific. Plant-derived LAB, especially, *Lb. plantarum* SN13T, contributed to improvements in constipation, serum lipid, and liver function, suggesting that this LAB strain is greatly useful as a functional food for promoting human health.

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