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

Running head: Probiotic effects of *Lactobacillus plantarum*

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FH, TA and MS were responsible for the design of the study and contributed to the final interpretation of the data. KN and HO are employees of Nomura Dairy Products Co., and produced three types of yogurts. FH assigned subjects using a computer-generated allocation sequence. TA is a medical doctor in Hiroshima University Hospital. MN and TA were responsible for the data collection of the study.

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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 are also evaluated by measuring serum lipid contents and liver functional indicators as 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) yogurt made by plant-derived LAB (mainly *Lactobacillus* (*Lb.*) *plantarum* SN35N); (type B) yogurt made by plant-derived LAB (mainly *Lb. plantarum* SN13T); (type C) yogurt made by animal-derived LAB (mainly *Lactococcus lactis* A6 and *Streptococcus thermophilus* 510) as a control. The subjects consumed 100g of yogurt daily for 6 weeks. Data were collected from clinical visits at 2-week 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 LDL cholesterol. The serum concentrations of liver functional parameters were improved by the type B yogurt (12-25% reduction).

Conclusions: 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.

Key words:

probiotics, *Lactobacillus plantarum*, defecation, cholesterol, gamma glutamyl transpeptidase (γ-GTP)

Introduction

Studies of lactic acid bacteria (LAB) have been conducted from the viewpoint that the microorganism 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 pathological disorders, including multisystem organ failure, colon cancer, and inflammatory bowel diseases [11, 12]. A recent study showed that intestinal microflora may influence the

production of autoantibodies against appetite-regulating peptide hormones and neuropeptides [13]. Since 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 level; however, there have been other reports that suggested no effect [2, 14-21]. Serum lipid-lowering effects in human by LAB have not been conclusive, therefore, further human studies seems to be necessary to accumulate the evidences.

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 the gut microflora conditions. Now, functional foods, which are potentially beneficial and affect a variety of bodily functions, are spreading in the worldwide marketplace. LAB contribute to the manufacture of these products. The physiological 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].

LAB 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 microorganisms 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, since 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 evaluate the effects on human health of yogurts produced by the 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, fluctuated 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 a total of 68 male and female volunteers (66 Japanese and 2 Chinese) aged 21-65 years 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), H1 blockers (n=1), gastric proton pump inhibitors (n=1), bis-phosphonate (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 the 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 3 parallel groups from October through December 2007. Subjects were assigned to one of 3 types of

yogurts, using stratified randomization by the defecation frequencies of preliminary inquiries: (A type) yogurt produced by plant-derived LAB (the contents of *Lb. plantarum* SN35N and SN13T are 95% and 5%, respectively); (B type) yogurt produced by plant-derived LAB (the contents of *Lb. plantarum* SN13T and SN35N are 98% and 2%, respectively); (C type) yogurt produced by animal-derived LAB (the contents of *Lc. lactis* A6, *S. thermophilus* 510, and *Lb. bulgaricus* C6 are 86.1%, 13.8%, and 0.1%, respectively). Allocation sequence, which was generated by a computer and kept in the numbered container, was used for random allocation. All yogurts remained viable at more than 2×10^8 LAB per gram throughout their shelf-life and were manufactured by Nomura Dairy Products Co., Ltd., 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 in time-independently for 6 weeks. 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 the case of an emergency. In a case in which 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 prior to 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 type 1 to 7 according to the "Bristol Stool Form Scale" (Type 1: Separate hard lumps, like nuts; Type 2: Sausage-shaped but lumpy; Type 3: Like a sausage but with cracks on the surface; Type 4: Like a sausage or snake; smooth and soft; Type 5: Soft blobs with clear-cut edges; Type 6: Fluffy pieces with ragged edges; a mushy stool; Type 7: Watery 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. Week-averages of the scores were individually calculated for the evaluation.

Analyses of serum biochemical parameters

Biochemical parameters, such as total cholesterol, LDL-cholesterol, HDL-cholesterol, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma 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 anti-hyperlipidemic agents and four subjects who withdrew and lacked the final data point (week 6) were excluded from analyses for total, LDL-, and HDL-cholesterols. 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 the statistical software program SPSS (version 16.0: Statistical Package for the Social Sciences, SPSS Japan, Inc.). One-way ANOVA was performed on all baseline data among the types. Differences in variables between the baseline and after the treatment were assessed with paired *t*-tests. Differences among the treatment types were analyzed by a two-way repeated measured ANOVA or by independent *t*-tests for the treatment types *versus* the control type. Data are presented as the mean ± the standard deviations. All statistical analyses were two-tailed ($P < 0.05$ is 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 3 of 5 clinical visits). The other three remained until week 4 (completed 4 of 5 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 4 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 shown in **Table 1**. There were no significant differences in the treatment types with regard to age, body weight, BMI, 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, blood urea nitrogen, and creatinine clearance) were observed during the clinical trial.

Defecation

In the present study, participants chronically had defecation troubles, such as

constipation, diarrhea, abdominal pain, abdominal bloating, straining, and feeling of incomplete evacuation. Based on the frequency of defecation at the baseline, the subjects were separated into three categories: 1) less than 5 times/week; 2) between 5 and 10 times/week; 3) more than 10 times/week. When the defecation frequencies were analyzed in category 1, drastic increases were observed in the plant-derived LAB yogurts, type A and B, which, at week 6, averaged 1.50 and 1.73 times that at the baseline, respectively; on the other hand, there was only a modest increment in the type C yogurt (1.17 times the frequency at the 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 the baseline, “Bristol scale < 4” subjects and “Bristol scale >4” subjects were analyzed separately with regard to any improvements in stool consistency. The smaller number in the Bristol scale stool form indicates harder stools, and the larger numbers, softer stools and diarrhea. Remarkably, not

only did individuals who experienced 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**). 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 the subject group B reduced significantly from 214.3 mg/dL (5.55 mmol/L) to 203.2 mg/dL (5.26 mmol/L, P=0.012) for 6 weeks, but not in the subject groups A and C. Next we analyzed the subjects with moderately high level of total cholesterol, within 180-260 mg/dL (4.66-6.73 mmol/L; T-Cho 180-260 subjects) at the baseline (week 0), excluding the subjects with low or remarkably high lipid levels. There were significant decreases of total cholesterol in the T-Cho 180-260 subjects of type B and C. However, the total cholesterol in the subject group A reduced statistically neither in all subjects nor the T-Cho 180-260 subjects. Similarly, LDL cholesterol was lowered only in type B subjects when all subjects were analyzed, and significant decreases were observed in types B and C subjects when T-Cho 180-260 subjects were analyzed. As expected, HDL cholesterol did not change regardless of the 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 to the other

types, all AST, ALT, and γ-GTP were remarkably reduced to 88%, 75%, and 78% of the values recorded at the baseline, respectively (**Table 5**).

Discussion

Yogurt is generally considered to improve 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 co-culture of *Lc. lactis* with *S. thermophilus*). The three types of yogurt are available on the markets in Hiroshima, Japan. We observed that consumption of these yogurts resulted in satisfactory defecation from the first week for the subjects originally experiencing constipation. 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/week on 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 the continuous consumption of yogurt due to the restriction of the survival of the bacteria within the gastrointestinal tract. The defecation frequency/week at week 6 increased by 1.5 and 2.6 times/week from the baseline with type A and B yogurts, respectively, but by only 0.6 times/week with type C. The three types of yogurt improved both hard and soft stools. In addition, volume and odor of stool, feeling during defecation, abdominal pain, and feeling of incomplete evacuation were also 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. Total cholesterol and LDL cholesterol were significantly decreased with 6 weeks of intake of type B and C yogurts. Total cholesterol 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. It has been reported that serum lipid-lowering effects were observed by *Lb. plantarum*, *Lb. acidophilus*, and *Lb. bifidobacterium* [14-18]. There have, however, been reports with contrasting results [19-21]. This inconsistent result was also observed in the current 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/ nonalcoholic fatty liver, the values of AST, ALT, and γ-GTP in serum, as hepatic indicators, are significantly increased. Nonalcoholic fatty liver disease (NAFLD) is a common liver pathology and includes a wide histological spectrum that ranges from simple steatosis to nonalcoholic steatohepatitis (NASH) [27, 28]. No treatment has yet been established for patients with NASH. LAB have been shown effective on improving liver function exclusively in animal model experiments [29-32]. In the present study, we observed that type B yogurt contributes to diminish all of 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% reduction). Type A yogurt reduced the ALT value. This is the first report of a human clinical trial in which a certain strain of LAB is shown 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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Table 1. Characteristics of subjects at the 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

Group A: Intake of yogurt produced by mainly *Lb. plantarum* SN35N.

Group B: Intake of yogurt produced by mainly *Lb. plantarum* SN13T.

Group C: Intake yogurt produced by mainly *Lc. lactis* A6 and *S. thermophilus* 510.

Data are shown as the means ± S.D.

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

frequency of defecation (times/week)	Group A (n=8)	Group B (n=9)	Group C (control) (n=11)	*P* [a]		
				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			

[a] Two-way repeated measured ANOVA.

Data are shown as the means ± S.D.

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

Table 3. Changes in stool consistency defined by the Bristol scale stool form.

		Group A	Group B	Group C (control)
Bristol scale <4 [a]	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 [b]	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 *

The Bristol scale of each subject at the baseline was averaged and grouped into <4 and >4.

1. Nut-like; 2. Lumpy sausage; 3. Sausage with cracks; 4. Smooth snake; 5. Soft blobs; 6. Fluffy pieces; 7. Watery.

[a] n=10, 7, and 7 for groups A, B, and C, respectively.

[b] n=9, 10, and 10 for groups A, B, and C, respectively.

Data are shown as the means ± S.D.

Significant difference from the baseline (before yogurt intake), * $P < 0.05$ or ** $P < 0.01$ (paired *t*-test).

Table 4. Total, LDL, HDL cholesterol at the baseline (week 0) and week 6.

	Group A (n=24)	Group B (n=18)	Group C (control) (n=20)
Total cholesterol (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; 2.0)	-11.1 * (-18.8; -3.3)	-9.7 (-19.2; -0.1)
T-Cho 180-260 subjects [a]			
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; 4.5)	-12.3 * (-21.4; -3.1)	-11.9 * (-20.8; -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; 4.0)	-7.3 * (-13.4; -1.2)	-6.8 (-14.4; 0.8)
T-Cho 180-260 subjects [a]			
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; 7.0)	-10.2 * (-18.4; -1.9)	-10.1 * (-18.5; -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; 0.2)	-0.7 (-3.9; 2.4)	0.0 (-5.0; 5.0)
T-Cho 180-260 subjects [a]			
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; 0.6)	1.1 (-2.6; 4.7)	2.5 (-2.5; 7.4)

[a] n=13, 11, and 11 for groups A, B, and C, respectively.

Data are shown as the means ± S.D.

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

Table 5. Liver functional parameters at the 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; 0.6)	-2.2 * (-4.1; -0.3)	-0.7 (-2.2; 0.9)
	Relative change (%) (95% CI)	-1.6 (-8.3; 5.1)	-6.4 (-12.7; -0.1)	-0.9 (-7.2; 5.4)
20-80 subjects [a]				
	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; 0.5)	-4.0 * (-7.0; -1.0)	-2.8 (-7.3; 1.6)
	Relative change (%) (95% CI)	-7.4 (-20.5; 5.6)	-11.7 * (-21.0; -2.4)	-8.2 (-21.7; 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; -0.2)	-4.7 * (-9.1; -0.3)	-1.9 (-3.7; 0.0)
	Relative change (%) (95% CI)	-5.4 (-13.7; 2.8)	-8.4 (-17.4; 0.6)	-5.7 (-13.6; 2.2)
20-80 subjects [b]				
	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; -1.5)	-8.0 * (-13.0; -3.0)	-6.4 (-11.9; -0.9)
	Relative change (%) (95% CI)	-11.7 * (-19.3; -4.0)	-25.2 * (-39.1; -11.2)	-21.4 (-37.1; -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; -1.0)	-5.8 * (-10.9; -0.7)	-2.0 (-4.3; 0.4)
	Relative change (%) (95% CI)	-10.4 ** (-17.0; -3.7)	-8.4 (-16.6; -0.1)	-4.6 (-11.9; 2.7)
25-90 subjects [c]				
	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; -0.5)	-16.0 * (-27.2; -4.8)	-4.9 (-10.1; 0.3)
	Relative change (%) (95% CI)	-11.1 (-23.3; 1.1)	-22.1 * (-39.0; -5.3)	-10.9 (-23.3; 1.5)

[a] n=10, 12, and 6 for groups A, B, and C, respectively.

[b] n=9, 7, and 5 for groups A, B, and C, respectively.

[c] n=7, 6, and 8 for groups A, B, and C, respectively.

Data are shown as the means ± S.D.

Significant difference from the baseline (before yogurt intake), * $P < 0.05$ or ** $P < 0.01$ (paired *t*-test).

Figure legend

Figure 1. Flow of subjects through the trial.