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Safety Evaluation of Excessive Intake of *Lactococcus lactis* Subsp. *lactis* JCM 5805: A Randomized, Double-Blind, Placebo-Controlled, Parallel-Group Trial

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Abstract

Background/Aims: Administration of a lactic acid bacterial strain, *Lactococcus lactis* subsp. *lactis* JCM 5805 (LC-Plasma), is reported to prevent viral infection via activation of plasmacytoid dendritic cells in mouse and human studies. As it is assumed that LC-Plasma is taken in excess when it is commercially provided as a supplement, we conducted a trial using capsules to give 250 mg LC-Plasma (5 times the effective anti-viral dose) every day for four weeks to healthy volunteers to investigate the safety of excessive intake of LC-Plasma. **Trial Design:** A randomized, double-blind, placebo-controlled, parallel-group trial was conducted. **Methods:** Forty healthy subjects were randomly assigned to the LC-Plasma group (daily intake of five capsules containing 50 mg heat-killed LC-Plasma cells per capsule) or the placebo group (daily intake of five placebo capsules with no LC-Plasma). Physical, hematological, biochemical and urinary examinations and medical interviews were used to evaluate safety. **Results:** No abnormal differences were observed after excessive intake of LC-Plasma capsules when compared to the intake of placebo capsules. **Conclusions:** There are no safety concerns associated with the excessive intake of heat-killed LC-Plasma capsules.

Keywords

Lactic Acid Bacteria, *Lactococcus lactis*, Excessive Intake, Safety

1. Introduction

Lactic acid bacteria (LAB) have been traditionally used in the production of fermented foods, such as yogurt and cheese, and are regarded as highly safe food materials. It is reported that LAB have many health functions, such as the regulation of intestinal cells, immune cells and microbiota, and they have been used in the production of many functional health foods [1] [2]. We previously found that *Lactococcus lactis* strain Plasma (LC-Plasma), which is a synonym of *Lactococcus lactis* subsp. *lactis* JCM 5805, stimulated and activated plasmacytoid dendritic cells (pDC), regardless of whether they were live or heat-killed [3]. pDC are immune cells, responsible for early reaction to viral infection and play a key role in preventing the proliferation and spread of viruses by production of type I interferons, a key factor in various immune reactions against viruses [4] [5]. We previously reported that LC-Plasma induced the production of interferon-α (IFN-α) in an *in vitro* study [3] and prevented viral infection in mouse and human studies [6]-[11]. In addition, oral administration of LC-Plasma significantly suppressed lung inflammation and death caused by parainfluenza virus infection in mice via enhancement of lung anti-viral immunity [6]. LC-Plasma also alleviated the following symptoms of rotaviral infection: weight loss, aggravation of fecal scores and rotavirus outgrowth in mice via increasing mature pDC in the small intestine [7]. In a clinical trial, the expression levels of anti-viral genes in human peripheral blood mononuclear cells (PBMC) stimulated by inactivated human influenza virus A/H1N1 (A/PR/8/34) were higher in the subjects who took LC-Plasma compared with subjects who did not take LC-Plasma [8]. In other clinical studies, the intake of yogurt or supplement containing 50 mg (1.0×10^{11} cells or more) of LC-Plasma activated human pDC and alleviated the symptoms of flu and common cold [9] [10] [11]. Although it was demonstrated that the intake of 50 mg (1.0×10^{11} cells or more) of LC-Plasma did not produce any safety concerns, it is assumed that LC-Plasma is taken in excess when it is provided as a supplement. To investigate the safety of excessive intake of LC-Plasma, we conducted a trial where healthy volunteers ingested capsules containing 250 mg LC-Plasma (5 times the effective anti-viral dose) every day for four weeks.

2. Subjects and Method

This trial was approved by the Institutional Review Board in Shinagawa Season Terrace Health Care Clinic (Minato-ku, Tokyo) in accordance with the Declaration of Helsinki and the Ethical Guidelines for Medical and Health Research Involving Human Subjects (approved on April 28, 2017, UMIN test ID: UMIN000027309).

2.1. Subjects

This trial was conducted at Shinagawa Season Terrace Health Care Clinic. Healthy male and female adult volunteers (20 to 64 years old) were recruited in

May 2017 by KSO Corporation. All subjects were provided a sufficient explanation regarding the trial including the trial purpose, contents, methods, and expected adverse events. Written informed consent was obtained from all volunteers before the start of the trial. The exclusion criteria were as follows: excessive alcohol-drinking behavior; unable to abstain from drinking alcohol from two days before each examination; chronic illness and regular use of medication; under treatment or with a history of serious disease (e.g., diabetes, liver disease, kidney disease or heart disease), thyroid gland disease, adrenal gland disease, or other metabolic disorder; any food allergy; under treatment for or with a history of hay fever; under treatment for or with a history of inflammatory bowel disease (e.g., ulcerative colitis or Crohn's disease); history of digestive disease affecting digestion or absorption; frequent diarrhea after ingesting dairy products; under treatment for or with a history of drug addiction or alcoholism; possible pregnancy, pregnancy or lactating; unfavorable blood test result at the baseline period; participation or possible participation in another clinical trial using any other food, medicine or cosmetics during this trial; any other reason for ineligibility as judged by the principal investigator.

Sample size was determined to be twenty subjects for each group: guidelines from the Japan Society of Health and Nutrition Food Association recommend at least twenty subjects when conducting a single safety trial [12].

2.2. Trial Design

The trial design was a randomized, double-blind, placebo-controlled, parallel group comparison experiment, conducted from May to July 2017. The baseline period before capsule intake was two weeks, capsule intake period was four weeks, and the follow-up observation period after the completion of intake was two weeks.

Subjects were randomly divided into two groups (the LC-Plasma group or placebo group) containing 20 subjects each by the person responsible for statistical analysis so that no bias was generated in the baseline data (sex, age or BMI). Subjects consumed either five LC-Plasma capsules containing 50 mg (1.0×10^{11} or more) of heat-killed LC-Plasma per capsule or five placebo capsules that did not contain LC-Plasma, daily for four weeks. Capsules were consumed at any time of the day during the intake period. Capsules were manufactured under the responsibility of Kirin Co., Ltd. The placebo capsules were made from the same materials and according to the same prescription as the LC-Plasma capsules except that LC-Plasma was replaced with cornstarch. Neither the investigators nor the subjects could distinguish between the capsules. The allocation table was sealed and stored securely by the controller; in addition, the capsule codes were blinded and the data set was locked until all analyses were complete. The examinations included: physical examination (height, weight, body mass index (BMI), systolic blood pressure, diastolic blood pressure, and pulse rate), hematological examination (white blood cell count, red blood cell count, hemoglobin

(Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, differential leucocyte count [neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOSINO), and basophils (BASO)]), blood biochemical examination (total protein, albumin, total bilirubin, aspartate aminotransferase (AST (GOT)), alanine aminotransferase (ALT (GPT)), lactic dehydrogenase (LDH), alkaline phosphatase (ALP), γ-glutamyl transpeptidase (γ-GTP), creatine phosphokinase (CPK), urea nitrogen, creatinine, uric acid, sodium (Na), chlorine (Cl), potassium (K), calcium (Ca), inorganic phosphorus, magnesium, serum iron, total cholesterol, low density lipoprotein (LDL)-cholesterol, high density lipoprotein (HDL)-cholesterol, triglyceride (TG), fasting blood glucose, and HbA1c (NGSP value)), urinalysis (urine protein, urine glucose, urinary bilirubin, urinary ketone body, urinary occult blood reaction, urinary urobilinogen, urine pH, and urine specific gravity), and a medical interview conducted by a physician. Hematological examination, blood biochemical examination and urinalysis were performed by LSI Medience Corporation under consignment.

Capsule intake, physical health, living conditions and the intake of health food products and medicines were listed in the subject's diary from fourteen days before the start of capsule intake (in week 0) and until the day before the medical examination during the follow up observation period (in week 6). All meals (including alcohol) ingested during the period from week 0 until week 6 were recorded in a dietary diary. In addition, the contents of meals taken during the period from 7 days before the day of examinations in week 0, week 2, week 4 and week 6 (28 days in total) were recorded in detail in the dietary diary. The records in the diary were checked on the day of each visit date, and subjects were strictly guided if any violations such as surfeit were found.

2.3. Statistical Analysis

The mean and standard deviation were calculated for all parameters measured at the baseline period before intake. Statistical analysis was performed by one-way analysis of variance (ANOVA) and the measured values were tabulated (**Table 1(a)**). Cross-tabulation tables were prepared for data on gender and qualitative urinalysis, and the results of qualitative urinalysis were scored and analyzed by Wilcoxon's rank sum test (**Table 1** and **Table 2**).

The mean and standard deviation were calculated for all parameters measured in each group. Statistical analysis was performed by paired t-test (two-tailed test) for comparison of the data obtained at the start of ingestion (in week 0), and those obtained at the examination in weeks 2, 4 (intake period) and 6 (at the end of the follow up). In addition, statistical analysis was conducted using unpaired t-tests (two-tailed test) for comparison of the data at each examination between the LC-Plasma group and placebo group (**Tables 2-8**). Stratified analysis by sex was conducted for the measurement parameters in which the reference values

Table 1. (a) Characteristics of the study subjects at baseline period; (b) Characteristics of the study subjects at baseline period.

(a)

Parameter	Unit	LC-Plasma (n = 20)	Placebo (n = 20)	*p* value	Whole (n = 40)
		mean ± SD	mean ± SD		mean ± SD
Age	years	44.3 ± 12.0	43.8 ± 9.9	0.875	44.0 ± 10.9
Height	cm	164.8 ± 9.3	165.2 ± 8.7	0.881	165.0 ± 8.9
Body weight	kg	59.6 ± 9.2	60.4 ± 7.9	0.770	60.0 ± 8.5
BMI		21.9 ± 1.7	22.1 ± 1.9	0.660	22.0 ± 1.8
Systolic blood pressure	mmHg	119.4 ± 14.1	121.4 ± 13.1	0.644	120.4 ± 13.5
Diastolic blood pressure	mmHg	72.6 ± 11.3	71.7 ± 10.8	0.786	72.1 ± 10.9
Pulse rate	bpm	72.5 ± 10.5	71.2 ± 7.8	0.646	71.8 ± 9.1
White blood cell count	/mL	5640 ± 1188	6300 ± 1505	0.132	5970 ± 1380
Red blood cell count	$\times 10^4$/mL	476.6 ± 44.8	466.1 ± 36.7	0.423	471.3 ± 40.8
Hemoglobin	g/dL	14.5 ± 1.5	14.3 ± 1.5	0.634	14.4 ± 1.5
Hematocrit	%	44.9 ± 4.0	43.9 ± 4.2	0.460	44.4 ± 4.1
Platelet count	$\times 10^4$/mL	27.3 ± 5.8	25.7 ± 3.5	0.289	26.5 ± 4.8
MCV	fl	94.4 ± 4.5	94.4 ± 3.2	0.968	94.4 ± 3.9
MCH	pg	30.4 ± 1.9	30.6 ± 1.2	0.788	30.5 ± 1.6
MCHC	%	32.2 ± 0.9	32.4 ± 0.7	0.371	32.3 ± 0.8
NEUT/leukocyte fractionation	%	56.3 ± 9.3	57.6 ± 7.3	0.615	56.9 ± 8.2
LYMPH/leukocyte fractionation	%	34.7 ± 7.8	33.6 ± 6.3	0.608	34.2 ± 7.0
MONO/leukocyte fractionation	%	5.5 ± 1.4	5.0 ± 1.0	0.208	5.3 ± 1.2
EOSINO/leukocyte fractionation	%	3.0 ± 2.3	3.2 ± 2.3	0.805	3.1 ± 2.3
BASO/leukocyte fractionation	%	0.5 ± 0.3	0.6 ± 0.4	0.195	0.6 ± 0.3
Total protein	g/dL	7.1 ± 0.4	7.2 ± 0.4	0.517	7.1 ± 0.4
Albumin	g/dL	4.4 ± 0.3	4.4 ± 0.3	0.604	4.4 ± 0.3
AST (GOT)	U/L	19.1 ± 4.0	18.1 ± 3.3	0.392	18.6 ± 3.6
ALT (GPT)	U/L	16.1 ± 7.9	16.1 ± 5.9	0.982	16.1 ± 6.9
LD (LDH)	U/L	181.9 ± 23.0	168.8 ± 17.2	**0.049**	175.3 ± 21.1
Total bilirubin	mg/dL	0.80 ± 0.26	0.74 ± 0.21	0.459	0.77 ± 0.23
ALP	U/L	181.4 ± 48.4	199.9 ± 52.8	0.255	190.6 ± 50.8
γ-GT (γ-GTP)	U/L	20.0 ± 10.0	20.0 ± 8.9	1.000	20.0 ± 9.4
CK (CPK)	U/L	116.3 ± 56.2	96.1 ± 46.9	0.226	106.2 ± 52.1
Urea nitrogen	mg/dL	12.7 ± 3.7	13.0 ± 3.2	0.779	12.9 ± 3.4
Creatinine	mg/dL	0.76 ± 0.18	0.75 ± 0.11	0.782	0.75 ± 0.15
Uric acid	mg/dL	5.3 ± 1.4	5.2 ± 1.0	0.689	5.2 ± 1.2
Sodium (Na)	mEq/L	141.3 ± 1.9	141.4 ± 1.4	0.848	141.3 ± 1.6
Chloride (Cl)	mEq/L	104.5 ± 1.9	104.9 ± 2.2	0.545	104.7 ± 2.1

Continued

Potassium (K)	mEq/L	4.4 ± 0.3	4.4 ± 0.3	0.364	4.4 ± 0.3
Calcium (Ca)	mg/dL	9.7 ± 0.3	9.6 ± 0.3	0.151	9.7 ± 0.3
Inorganic phosphorus	mg/dL	3.3 ± 0.5	3.5 ± 0.4	0.230	3.4 ± 0.5
Magnesium	mg/dL	2.3 ± 0.1	2.3 ± 0.1	0.347	2.3 ± 0.1
Serum iron	mg/dL	109.6 ± 43.2	101.0 ± 26.3	0.455	105.3 ± 35.6
Total cholesterol	mg/dL	200.1 ± 24.5	200.1 ± 26.6	0.995	200.1 ± 25.2
LDL-cholesterol	mg/dL	115.4 ± 23.0	112.1 ± 20.7	0.641	113.7 ± 21.7
HDL-cholesterol	mg/dL	62.3 ± 13.0	69.3 ± 16.0	0.137	65.8 ± 14.8
Triglyceride (TG)	mg/dL	93.7 ± 49.3	83.7 ± 45.6	0.509	88.7 ± 47.1
Fasting blood glucose	mg/dL	84.5 ± 7.8	82.8 ± 5.6	0.433	83.7 ± 6.7
HbA1c	%	5.5 ± 0.3	5.4 ± 0.3	0.616	5.5 ± 0.3
Urine pH		6.3 ± 0.6	6.5 ± 0.5	0.203	6.4 ± 0.6
Urine specific gravity		1.020 ± 0.008	1.020 ± 0.007	0.984	1.020 ± 0.008

p value: ANOVA.

(b)

Parameter	LC-Plasma (n = 20)	Placebo (n = 20)	*p* value	Whole (n = 40)
	mean ± SD	mean ± SD		mean ± SD
Urine protein	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00
Urine glucose	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00
Urobilinogen	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00
Urine bilirubin	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00
Urine ketone body	0.00 ± 0.00	0.00 ± 0.00	1.000	0.00 ± 0.00
Occult blood reaction	0.05 ± 0.22	0.00 ± 0.00	0.317	0.03 ± 0.16

p value: Wilcoxon's rank sum test (asymptotic significance probability).

Table 2. Physical parameters at the start of intake, during the intake period and during follow up observations of all subjects.

Parameter	Reference range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
Height (cm)		LC-Plasma	20	164.6 ± 9.3	0.849	164.6 ± 9.3	0.869	0.410	164.5 ± 9.2	0.808	0.248	164.6 ± 9.3	0.860	0.957
		Placebo	20	165.1 ± 8.7		165.1 ± 8.7		0.841	165.2 ± 8.7		0.529	165.1 ± 8.7		0.487
Body weight (kg)		LC-Plasma	20	59.0 ± 9.2	0.726	58.8 ± 9.0	0.735	0.527	59.0 ± 9.1	0.774	0.876	58.9 ± 9.2	0.793	0.676
		Placebo	20	59.9 ± 7.7		59.7 ± 7.5		0.054	59.8 ± 7.7		0.516	59.6 ± 7.5		0.079
BMI		LC-Plasma	20	21.6 ± 1.8	0.622	21.6 ± 1.6	0.599	0.463	21.7 ± 1.7	0.756	0.662	21.6 ± 1.8	0.728	0.747
		Placebo	20	21.9 ± 1.8		21.9 ± 1.8		0.109	21.9 ± 1.9		0.393	21.8 ± 1.9		0.085
Systolic blood pressure (mmHg)	100 - 139	LC-Plasma	20	114.7 ± 15.6	0.603	119.9 ± 15.1	0.953	**0.013**	118.3 ± 18.1	0.503	0.083	114.0 ± 16.9	0.845	0.757
		Placebo	20	117.0 ± 11.8		119.7 ± 11.1		0.263	114.9 ± 12.8		0.259	114.8 ± 9.4		0.311

Continued

Diastolic blood pressure (mmHg)	50 - 89	LC-Plasma	20	70.0 ± 13.8	0.320	72.8 ± 12.1	0.450	0.123	70.3 ± 13.9	0.265	0.878	66.3 ± 12.7	0.788	**0.034**
		Placebo	20	66.2 ± 9.3		70.2 ± 9.7		**0.037**	66.1 ± 8.6		0.951	65.4 ± 7.7		0.703
Pulse rate (bpm)	40 - 80	LC-Plasma	20	78.3 ± 15.9	0.742	78.1 ± 16.0	0.903	0.930	73.7 ± 10.7	0.505	0.206	72.8 ± 10.1	0.478	0.093
		Placebo	20	76.9 ± 10.2		78.7 ± 12.2		0.319	75.8 ± 9.4		0.603	75.2 ± 11.0		0.508

p value 1: unpaired t-test (LC-Plasma group vs placebo group). *p* value 2: paired t-test (vs 0 W).

Table 3. Hematological parameters at the start of intake, during the intake period and during follow up observations of all subjects.

Parameter	Reference range	Study foods	n	0 W	2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
White blood cell count (/μL)	3300 - 9000	LC-Plasma	20	5270 ± 1025	0.105	5360 ± 1161	0.275	0.605	5420 ± 1363	0.220	0.548	5205 ± 1025	0.255	0.676
		Placebo	20	5860 ± 1212		5865 ± 1677		0.985	6095 ± 2000		0.346	5680 ± 1526		0.430
Red blood cell count ($\times 10^4$/μL)	M: 430 - 570 F: 380 - 500	LC-Plasma	20	472.6 ± 38.7	0.692	477.7 ± 37.7	0.223	0.209	466.8 ± 35.1	0.745	0.203	466.9 ± 39.8	0.836	0.189
		Placebo	20	468.1 ± 32.3		463.7 ± 33.4		0.161	463.1 ± 35.4		0.344	469.3 ± 34.4		0.774
Hemoglobin (g/dL)	M: 13.5 - 17.5 F: 11.5 - 15.0	LC-Plasma	20	14.3 ± 1.4	0.730	14.5 ± 1.3	0.523	0.118	14.2 ± 1.3	0.907	0.709	14.2 ± 1.4	0.865	0.360
		Placebo	20	14.4 ± 1.3		14.2 ± 1.3		**0.023**	14.2 ± 1.4		0.141	14.2 ± 1.3		0.074
Hematocrit (%)	M: 39.7 - 52.4 F: 34.8 - 45.0	LC-Plasma	20	44.4 ± 3.7	0.634	44.4 ± 3.2	0.285	0.919	44.9 ± 3.3	0.840	0.279	44.4 ± 3.9	0.937	0.881
		Placebo	20	43.8 ± 3.3		43.3 ± 3.2		0.056	44.7 ± 3.5		0.082	44.5 ± 3.6		0.106
Platelet count ($\times 10^4$/μL)	14.0 - 34.0	LC-Plasma	20	27.5 ± 5.8	0.977	28.1 ± 5.1	0.377	0.244	26.9 ± 4.8	0.920	0.282	27.9 ± 4.6	0.980	0.492
		Placebo	20	27.6 ± 3.8		26.9 ± 3.8		0.220	26.8 ± 4.0		0.106	28.0 ± 4.2		0.382
MCV (fl)	85 - 102	LC-Plasma	20	94.0 ± 4.1	0.791	93.3 ± 4.8	0.840	**0.036**	96.3 ± 4.8	0.877	**0.000**	95.3 ± 4.5	0.744	**0.000**
		Placebo	20	93.7 ± 2.9		93.5 ± 2.7		0.428	96.5 ± 3.1		**0.000**	94.9 ± 3.1		**0.000**
MCH (pg)	28.0 - 34.0	LC-Plasma	20	30.3 ± 1.7	0.238	30.4 ± 1.7	0.495	0.207	30.5 ± 1.7	0.841	**0.010**	30.3 ± 1.6	0.974	0.557
		Placebo	20	30.8 ± 1.3		30.7 ± 1.2		0.070	30.6 ± 1.2		**0.020**	30.3 ± 1.2		**0.000**
MCHC (%)	30.2 - 35.1	LC-Plasma	20	32.2 ± 1.0	**0.016**	32.6 ± 1.1	0.522	**0.000**	31.7 ± 0.8	0.915	**0.001**	31.8 ± 0.8	0.645	**0.017**
		Placebo	20	32.9 ± 0.9		32.8 ± 0.8		0.276	31.7 ± 0.9		**0.000**	31.9 ± 0.7		**0.000**
NEUT/leukocyte fractionation (%)	40.0 - 75.0	LC-Plasma	20	54.4 ± 8.3	0.661	54.7 ± 7.1	0.559	0.860	56.6 ± 8.4	0.774	0.290	55.2 ± 6.2	0.662	0.594
		Placebo	20	55.4 ± 6.5		56.0 ± 7.2		0.666	55.9 ± 8.2		0.761	56.3 ± 8.5		0.647
LYMPH/leukocyte fractionation (%)	18.0 - 49.0	LC-Plasma	20	36.0 ± 6.9	0.798	36.1 ± 6.6	0.632	0.936	34.8 ± 7.9	0.998	0.518	35.6 ± 5.8	0.898	0.756
		Placebo	20	35.4 ± 6.6		35.0 ± 7.7		0.709	34.8 ± 7.6		0.632	35.3 ± 8.6		0.925
MONO/leukocyte fractionation (%)	2.0 - 10.0	LC-Plasma	20	6.0 ± 1.0	0.193	5.8 ± 1.4	0.170	0.341	5.2 ± 1.5	0.786	**0.011**	5.7 ± 1.2	**0.018**	0.200
		Placebo	20	5.4 ± 1.4		5.3 ± 1.1		0.488	5.3 ± 1.4		0.723	4.9 ± 0.9		**0.045**
EOSINO/leukocyte fractionation (%)	0.0 - 8.0	LC-Plasma	20	3.1 ± 2.1	0.929	3.0 ± 2.0	0.759	0.379	3.0 ± 2.2	0.649	0.577	3.0 ± 1.6	0.986	0.582
		Placebo	20	3.1 ± 2.1		3.2 ± 2.2		0.710	3.3 ± 2.2		0.222	3.0 ± 2.0		0.580
BASO/leukocyte fractionation (%)	0.0 - 2.0	LC-Plasma	20	0.5 ± 0.2	0.278	0.5 ± 0.2	0.326	1.000	0.4 ± 0.3	**0.018**	0.064	0.6 ± 0.4	0.384	0.838
		Placebo	20	0.6 ± 0.3		0.6 ± 0.4		0.852	0.7 ± 0.4		0.269	0.6 ± 0.3		0.759

p value 1: unpaired t-test (LC-Plasma group vs placebo group), *p* value 2: paired t-test (vs 0 W).

Table 4. Hematological parameters at the start of intake, during the intake period and during the follow up observations for male and female subjects.

Male														
Parameter	Reference range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
Red blood cell count ($\times 10^4/\mu L$)	M: 430 - 570	LC-Plasma	10	496.3 ± 38.8	0.724	502.5 ± 29.4	0.242	0.203	488.4 ± 32.2	0.941	0.187	493.8 ± 32.0	0.948	0.602
		Placebo	10	491.1 ± 24.4		488.0 ± 23.9		0.079	487.4 ± 26.7		0.573	492.9 ± 29.1		0.772
Hemoglobin (g/dL)	M: 13.5 - 17.5	LC-Plasma	10	15.4 ± 0.9	0.897	15.6 ± 0.7	0.330	0.285	15.2 ± 0.7	0.960	0.247	15.2 ± 0.7	1.000	0.254
		Placebo	10	15.5 ± 0.8		15.3 ± 0.7		**0.000**	15.2 ± 1.0		0.245	15.2 ± 0.9		0.140
Hematocrit (%)	M: 39.7 - 52.4	LC-Plasma	10	46.9 ± 3.1	0.610	46.7 ± 2.2	0.397	0.642	47.2 ± 2.4	0.868	0.728	47.2 ± 2.4	0.953	0.546
		Placebo	10	46.3 ± 2.4		45.8 ± 2.3		**0.048**	47.3 ± 2.4		0.098	47.1 ± 2.8		0.188
Female														
Parameter	Reference Range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
Red blood cell count ($\times 10^4/\mu L$)	F: 380 - 500	LC-Plasma	10	448.8 ± 19.9	0.679	452.8 ± 27.6	0.242	0.563	445.1 ± 22.9	0.563	0.614	439.9 ± 26.6	0.591	0.240
		Placebo	10	445.0 ± 20.5		439.4 ± 21.6		0.367	438.8 ± 24.9		0.476	445.7 ± 20.4		0.916
Hemoglobin (g/dL)	F: 11.5 - 15.0	LC-Plasma	10	13.2 ± 0.7	0.456	13.4 ± 0.8	0.550	0.267	13.3 ± 0.9	0.764	0.596	13.1 ± 1.1	0.735	0.781
		Placebo	10	13.4 ± 0.8		13.2 ± 0.7		0.290	13.2 ± 0.9		0.377	13.2 ± 0.8		0.334
Hematocrit (%)	F: 34.8 - 45.0	LC-Plasma	10	41.9 ± 2.2	0.667	42.2 ± 2.3	0.158	0.652	42.7 ± 2.6	0.571	0.258	41.7 ± 3.1	0.833	0.786
		Placebo	10	41.4 ± 2.1		40.8 ± 1.6		0.257	42.0 ± 2.2		0.427	41.9 ± 2.3		0.390

p value 1: unpaired t-test (LC-Plasma group vs placebo group). *p* value 2: paired t-test (vs 0 W).

Table 5. Biochemical parameters at the start of intake, during the intake period and during follow up observations for all subjects.

Parameter	Reference range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
AST (GOT) (U/L)	10 - 40	LC-Plasma	20	18.5 ± 5.3	0.241	18.2 ± 4.3	0.877	0.739	18.6 ± 5.9	0.357	0.852	17.1 ± 5.2	0.710	0.127
		Placebo	20	16.8 ± 3.2		18.0 ± 3.8		0.183	17.2 ± 3.7		0.728	17.7 ± 4.9		0.441
ALT (GPT) (U/L)	5 - 45	LC-Plasma	20	16.5 ± 8.5	0.762	15.7 ± 7.8	0.621	0.319	16.3 ± 8.4	0.836	0.813	14.3 ± 8.0	0.485	**0.027**
		Placebo	20	15.7 ± 6.9		16.8 ± 6.0		0.427	16.8 ± 6.7		0.554	16.3 ± 9.8		0.796
LD (LDH) (U/L)	120 - 240	LC-Plasma	20	166.1 ± 25.4	**0.027**	168.6 ± 18.4	0.119	0.570	168.4 ± 22.4	**0.047**	0.645	164.1 ± 20.4	0.431	0.671
		Placebo	20	150.1 ± 18.1		157.6 ± 24.7		**0.005**	155.2 ± 18.1		0.071	159.0 ± 19.7		**0.008**
Total bilirubin (mg/dL)	0.2 - 1.2	LC-Plasma	20	0.81 ± 0.29	0.951	0.85 ± 0.20	0.483	0.433	0.77 ± 0.21	0.626	0.487	0.80 ± 0.24	0.756	0.825
		Placebo	20	0.82 ± 0.22		0.90 ± 0.24		0.070	0.81 ± 0.24		0.825	0.78 ± 0.27		0.418

Continued

ALP (U/L)	100 - 325	LC-Plasma	20	169.0 ± 41.6	**0.048**	174.8 ± 38.6	**0.045**	0.113	167.7 ± 40.9	**0.020**	0.715	162.9 ± 39.8	**0.019**
		Placebo	20	200.0 ± 53.2		206.7 ± 57.1	**0.032**		206.4 ± 58.0		0.123	204.6 ± 57.6	**0.011**
γ-GT (γ-GTP) (U/L)	M: ≤80 F: ≤30	LC-Plasma	20	19.3 ± 9.4	0.954	18.7 ± 7.8	0.517	0.323	17.3 ± 7.7	0.332	0.059	17.8 ± 6.8	0.304	0.091
		Placebo	20	19.1 ± 6.5		20.2 ± 6.7		0.346	20.2 ± 10.4		0.565	21.6 ± 15.1		0.435
CK (CPK) (U/L)	M: 60 - 270 F: 40 - 150	LC-Plasma	20	117.6 ± 63.0	0.057	138.3 ± 127.1	0.421	0.516	173.4 ± 228.3	0.220	0.303	117.8 ± 66.1	0.573	0.984
		Placebo	20	86.2 ± 31.9		112.3 ± 66.0	**0.014**		106.3 ± 75.6		0.179	143.7 ± 192.5		0.175
Fasting blood glucose (mg/dL)	70 - 109	LC-Plasma	20	83.6 ± 7.2	0.468	82.1 ± 6.2	0.593	0.260	79.5 ± 9.0	0.370	**0.005**	79.5 ± 9.3	0.350	**0.025**
		Placebo	20	82.1 ± 6.2		81.0 ± 6.7		0.315	81.7 ± 6.1		0.704	81.8 ± 6.2		0.791
HbA1c (%)	4.6 - 6.2	LC-Plasma	20	5.4 ± 0.3	0.356	5.3 ± 0.3	0.859	**0.004**	5.4 ± 0.3	0.297	1.000	5.4 ± 0.3	0.613	0.104
		Placebo	20	5.3 ± 0.3		5.3 ± 0.3		0.481	5.3 ± 0.3		0.629	5.4 ± 0.3		**0.007**
Total protein (mg/dL)	120 - 219	LC-Plasma	20	196.4 ± 25.7	0.839	202.9 ± 23.8	0.612	0.120	193.7 ± 19.4	0.877	0.431	193.5 ± 20.5	0.896	0.433
		Placebo	20	198.3 ± 31.1		198.6 ± 29.7		0.938	195.0 ± 32.0		0.361	194.7 ± 33.3		0.275
LDL-cholesterol (mg/dL)	65 - 139	LC-Plasma	20	114.7 ± 24.1	0.617	118.1 ± 19.5	0.265	0.339	112.7 ± 16.8	0.488	0.482	112.5 ± 18.1	0.610	0.458
		Placebo	20	110.9 ± 24.2		110.5 ± 22.8		0.895	108.2 ± 23.3		0.332	109.0 ± 24.4		0.528
HDL-cholesterol (mg/dL)	M: 40 - 85 F: 40 - 95	LC-Plasma	20	63.4 ± 12.4	0.266	62.3 ± 14.0	0.349	0.374	61.1 ± 11.5	0.134	0.057	60.1 ± 12.0	0.055	**0.008**
		Placebo	20	68.8 ± 17.7		66.8 ± 15.9		0.176	67.6 ± 15.1		0.442	69.3 ± 16.9		0.805
Triglyceride (TG) (mg/dL)	30 - 149	LC-Plasma	20	74.7 ± 31.5	0.950	76.5 ± 25.4	0.639	0.706	74.0 ± 28.2	0.878	0.901	83.4 ± 39.8	0.398	0.188
		Placebo	20	74.0 ± 37.7		71.9 ± 34.8		0.649	75.8 ± 41.9		0.728	72.7 ± 39.0		0.892
Total protein (g/dL)	6.7 - 8.3	LC-Plasma	20	7.1 ± 0.4	0.058	7.2 ± 0.4	0.430	0.138	7.1 ± 0.3	0.473	0.938	7.1 ± 0.4	**0.001**	0.353
		Placebo	20	7.3 ± 0.4		7.3 ± 0.4		0.214	7.2 ± 0.5		0.056	7.5 ± 0.4		**0.034**
Albumin (g/dL)	3.8 - 5.2	LC-Plasma	20	4.5 ± 0.2	0.790	4.5 ± 0.3	0.521	0.356	4.5 ± 0.2	0.473	0.643	4.4 ± 0.3	0.385	**0.004**
		Placebo	20	4.5 ± 0.2		4.5 ± 0.3		0.761	4.4 ± 0.3		0.209	4.5 ± 0.3		1.000
Urea nitrogen (mg/dL)	8.0 - 20.0	LC-Plasma	20	13.6 ± 2.9	0.519	12.7 ± 2.7	0.610	0.089	13.2 ± 3.0	0.402	0.524	12.8 ± 3.5	0.469	0.244
		Placebo	20	13.0 ± 3.1		12.3 ± 2.4		0.133	14.0 ± 3.1		0.158	13.5 ± 2.0		0.431
Creatinine (mg/dL)	M: 0.61 - 1.04 F: 0.47 - 0.79	LC-Plasma	20	0.79 ± 0.17	0.618	0.79 ± 0.18	0.830	0.934	0.73 ± 0.16	0.965	**0.000**	0.77 ± 0.17	0.591	0.144
		Placebo	20	0.76 ± 0.14		0.77 ± 0.12		0.227	0.74 ± 0.11		0.123	0.74 ± 0.13		0.084
Uric acid (mg/dL)	M: 3.8 - 7.0 F: 2.5 - 7.0	LC-Plasma	20	5.6 ± 1.1	0.187	5.4 ± 1.4	0.416	0.287	5.7 ± 1.2	0.632	0.642	5.6 ± 1.3	0.345	0.750
		Placebo	20	5.1 ± 0.9		5.1 ± 0.8		0.756	5.5 ± 0.9		**0.001**	5.3 ± 0.9		0.200
Sodium (Na) (mEq/L)	137 - 147	LC-Plasma	20	140.6 ± 2.0	0.928	140.9 ± 1.4	0.151	0.554	140.8 ± 1.7	0.503	0.640	141.3 ± 2.1	0.486	**0.049**
		Placebo	20	140.6 ±		141.6 ± 1.4	**0.016**		141.2 ± 1.6		0.083	141.7 ± 1.5		**0.010**
Chloride (Cl) (mEq/L)	98 - 108	LC-Plasma	20	103.9 ± 1.3	1.000	104.6 ± 2.0	0.735	0.126	104.0 ± 2.3	0.096	0.913	105.2 ± 2.0	1.000	**0.006**
		Placebo	20	103.9 ± 1.7		104.8 ± 1.7	**0.028**		105.1 ± 1.8		**0.013**	105.2 ± 1.3		**0.001**
Potassium (K) (mEq/L)	3.5 - 5.0	LC-Plasma	20	4.3 ± 0.4	0.459	4.3 ± 0.3	0.166	0.605	4.3 ± 0.4	0.458	0.753	4.4 ± 0.2	0.408	0.217
		Placebo	20	4.3 ± 0.1		4.2 ± 0.2		0.470	4.3 ± 0.2		0.691	4.3 ± 0.1		**0.026**
Calcium (Ca) (mg/dL)	8.4 - 10.4	LC-Plasma	20	9.5 ± 0.3	0.816	9.5 ± 0.3	0.743	0.357	9.5 ± 0.3	0.251	0.597	9.5 ± 0.3	0.780	0.500
		Placebo	20	9.5 ± 0.3		9.4 ± 0.4		0.199	9.4 ± 0.4		0.087	9.5 ± 0.4		0.814

Continued

Inorganic phosphorus (mg/dL)	2.5 - 4.5	LC-Plasma	20	3.6 ± 0.5	0.257	3.5 ± 0.6	0.527	0.607	3.6 ± 0.5	0.384	0.834	3.4 ± 0.5	0.099	0.084
		Placebo	20	3.8 ± 0.5		3.6 ± 0.4		0.177	3.7 ± 0.4		0.496	3.7 ± 0.6		0.771
Magnesium (mg/dL)	1.9 - 2.5	LC-Plasma	20	2.1 ± 0.1	0.575	2.2 ± 0.1	0.350	**0.004**	2.1 ± 0.1	0.225	0.163	2.1 ± 0.1	0.770	1.000
		Placebo	20	2.1 ± 0.1		2.2 ± 0.1		**0.000**	2.2 ± 0.1		**0.001**	2.1 ± 0.1		0.772
Serum iron (μg/dL)	M: 50 - 200 F: 40 - 180	LC-Plasma	20	104.4 ± 42.9	0.812	101.6 ± 36.6	0.133	0.649	92.6 ± 35.2	0.172	0.132	105.7 ± 37.1	0.735	0.874
		Placebo	20	107.7 ± 44.3		119.4 ± 36.7		0.368	107.3 ± 31.5		0.965	101.8 ± 35.2		0.575

p value 1: unpaired t-test (LC-Plasma group vs placebo group). p value 2: paired t-test (vs 0 W).

Table 6. Biochemical parameters at the start of intake, during the intake period and during follow up observations for male and female subjects.

Male														
Parameter	Reference range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
γ-GT(g-GTP) (U/L)	M: ≤80	LC-Plasma	10	23.2 ± 10.9	0.712	21.7 ± 8.9	0.864	0.189	20.6 ± 8.7	0.574	0.203	20.8 ± 7.4	0.494	0.142
		Placebo	10	21.6 ± 7.9		21.1 ± 6.4		0.700	23.3 ± 12.1		0.616	25.5 ± 20.0		0.551
CK(CPK) (U/L)	M: 60 - 270	LC-Plasma	10	142.0 ± 66.7	0.087	192.6 ± 164.1	0.214	0.432	162.2 ± 119.0	0.606	0.495	150.2 ± 81.1	0.509	0.690
		Placebo	10	99.0 ± 30.0		122.0 ± 55.7		0.096	136.8 ± 96.1		0.191	208.5 ± 261.0		0.202
HDL-cholesterol (mg/dL)	M: 40 - 85	LC-Plasma	10	55.7 ± 8.5	0.475	53.6 ± 9.7	0.425	0.173	54.5 ± 7.5	0.350	0.496	53.9 ± 8.9	0.242	0.064
		Placebo	10	59.6 ± 14.6		58.5 ± 16.3		0.423	59.7 ± 15.4		0.882	61.3 ± 17.2		0.536
Creatinine (mg/dL)	M: 0.61 - 1.04	LC-Plasma	10	0.91 ± 0.11	0.279	0.92 ± 0.14	0.205	0.304	0.86 ± 0.12	0.518	**0.010**	0.88 ± 0.12	0.476	0.128
		Placebo	10	0.86 ± 0.10		0.86 ± 0.08		0.955	0.83 ± 0.08		0.216	0.85 ± 0.08		0.505
Uric acid (mg/dL)	M: 3.8 - 7.0	LC-Plasma	10	6.3 ± 1.0	0.081	6.3 ± 1.3	0.090	0.850	6.3 ± 1.2	0.312	0.732	6.5 ± 1.0	0.057	**0.030**
		Placebo	10	5.5 ± 0.9		5.5 ± 0.8		0.885	5.9 ± 0.8		**0.008**	5.6 ± 0.9		0.228
Serum iron (μg/dL)	M: 50 - 200	LC-Plasma	10	116.0 ± 39.3	0.680	113.8 ± 29.8	0.728	0.851	100.4 ± 23.2	0.542	0.177	118.7 ± 32.5	0.152	0.812
		Placebo	10	107.5 ± 50.7		120.0 ± 46.7		0.604	108.9 ± 36.5		0.883	93.1 ± 43.3		0.428
Female														
Parameter	Reference Range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
γ-GT (g-GTP) (U/L)	F: ≤30	LC-Plasma	10	15.3 ± 5.6	0.549	15.6 ± 5.2	0.217	0.496	14.0 ± 5.0	0.321	**0.039**	14.7 ± 4.7	0.270	0.452
		Placebo	10	16.6 ± 3.7		19.2 ± 7.2		0.159	17.0 ± 7.9		0.814	17.7 ± 6.9		0.382
CK (CPK) (U/L)	F: 40 - 150	LC-Plasma	10	93.1 ± 51.0	0.305	84.0 ± 24.6	0.477	0.498	184.5 ± 309.1	0.282	0.399	85.4 ± 17.3	0.542	0.650
		Placebo	10	73.4 ± 29.7		102.5 ± 76.8		0.091	75.7 ± 27.3		0.812	78.9 ± 28.2		0.561
HDL-cholesterol (mg/dL)	F: 40 - 95	LC-Plasma	10	71.0 ± 11.0	0.270	70.9 ± 12.5	0.442	0.961	67.7 ± 11.2	0.123	**0.048**	66.2 ± 11.9	0.064	**0.040**
		Placebo	10	78.0 ± 16.0		75.0 ± 10.8		0.288	75.5 ± 10.3		0.428	77.2 ± 13.0		0.758

Continued

Creatinine (mg/dL)	F: 0.47 - 0.79	LC-Plasma	10	0.66 ± 0.12	0.951	0.65 ± 0.10	0.293	0.476	0.61 ± 0.09	0.309	**0.022**	0.65 ± 0.12	0.683	0.566
		Placebo	10	0.67 ± 0.10		0.69 ± 0.09		**0.032**	0.65 ± 0.05		0.399	0.63 ± 0.06		0.118
Uric acid (mg/dL)	F: 2.5 - 7.0	LC-Plasma	10	4.9 ± 0.8	0.801	4.5 ± 0.6	0.372	0.141	5.0 ± 0.8	0.724	0.741	4.7 ± 0.8	0.529	0.630
		Placebo	10	4.8 ± 0.7		4.7 ± 0.7		0.793	5.1 ± 0.9		0.057	5.0 ± 0.8		0.418
Serum iron (μg/dL)	F: 40 - 180	LC-Plasma	10	92.7 ± 45.2	0.438	89.3 ± 40.1	0.067	0.511	84.8 ± 44.0	0.219	0.487	92.6 ± 38.3	0.230	0.994
		Placebo	10	107.8 ± 39.8		118.7 ± 25.7		0.378	105.7 ± 27.6		0.879	110.4 ± 24.2		0.827

p value 1: unpaired t-test (LC-Plasma group vs placebo group). *p* value 2: paired t-test (vs 0 W).

Table 7. Urinalysis parameters at the start of intake, during the intake period and during follow up observations for all subjects.

Parameter	Study Foods	n	0 W		2 W			4 W			6 W		
			mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
Urine protein	LC-Plasma	20	0.20 ± 0.70	0.534	0.10 ± 0.45	0.317	0.157	0.00 ± 0.00	0.317	0.180	0.00 ± 0.00	1.000	0.180
	Placebo	20	0.05 ± 0.22		0.00 ± 0.00		0.317	0.05 ± 0.22		1.000	0.00 ± 0.00		0.317
Urine glucose	LC-Plasma	20	0.00 ± 0.00	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000
	Placebo	20	0.00 ± 0.00		0.00 ± 0.00		1.000	0.00 ± 0.00		1.000	0.00 ± 0.00		1.000
Urobilinogen	LC-Plasma	20	0.00 ± 0.00	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000
	Placebo	20	0.00 ± 0.00		0.00 ± 0.00		1.000	0.00 ± 0.00		1.000	0.00 ± 0.00		1.000
Urine bilirubin	LC-Plasma	20	0.00 ± 0.00	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000	0.00 ± 0.00	1.000	1.000
	Placebo	20	0.00 ± 0.00		0.00 ± 0.00		1.000	0.00 ± 0.00		1.000	0.00 ± 0.00		1.000
Urine ketone body	LC-Plasma	20	0.00 ± 0.00	1.000	0.00 ± 0.00	1.000	1.000	0.20 ± 0.89	0.317	0.317	0.00 ± 0.00	1.000	1.000
	Placebo	20	0.00 ± 0.00		0.00 ± 0.00		1.000	0.00 ± 0.00		1.000	0.00 ± 0.00		1.000
Occult blood reaction	LC-Plasma	20	0.20 ± 0.89	0.317	0.15 ± 0.67	0.971	0.655	0.20 ± 0.89	0.971	1.000	0.00 ± 0.00	0.152	0.317
	Placebo	20	0.00 ± 0.00		0.10 ± 0.45		0.317	0.05 ± 0.22		0.317	0.10 ± 0.31		0.157

p value 1: Wilcoxon's rank sum test (LC-Plasma group vs placebo group) asymptotic significance probability. *p* value 2: Wilcoxon's signed rank test (vs 0 W) asymptotic significance probability.

Table 8. Urine pH and urine specific gravity at the start of intake, during the intake period and during follow up observations for all subjects.

Parameter	Reference range	Study foods	n	0 W		2 W			4 W			6 W		
				mean ± SD	*p* value 1	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2	mean ± SD	*p* value 1	*p* value 2
Urine pH	5.0 - 7.5	LC-Plasma	20	6.2 ± 0.6	0.895	6.5 ± 0.8	0.338	0.083	6.4 ± 0.8	0.914	0.367	6.3 ± 0.6	0.610	0.748
		Placebo	20	6.2 ± 0.6		6.3 ± 0.7		0.530	6.4 ± 0.6		0.167	6.2 ± 0.6		0.881
Urine specific gravity	1.006 - 1.030	LC-Plasma	20	1.019 ± 0.007	0.464	1.017 ± 0.009	0.213	0.328	1.020 ± 0.009	0.558	0.658	1.018 ± 0.009	0.257	0.692
		Placebo	20	1.021 ± 0.009		1.020 ± 0.008		0.713	1.022 ± 0.009		0.724	1.021 ± 0.008		0.875

p value 1: unpaired t-test (LC-Plasma group vs placebo group). *p* value 2: paired t-test (vs 0 W).

Table 9. Adverse events.

Group	Participant number	Event No.	Adverse event (symptom)	Date of Onset	Relationship between the onset and intake period	Severity	Treatment	Outcome	Outcome Date	Causal relation with the study food	Reason for causal judgment	Continuation or discontinuation of the trial	Judgment date on the trial continuation (or discontinuation)
LC-Plasma	KBR 13 - 517	1	Common cold	June 25, 2017	After the end of intake period	Moderate	Yes	Recovered	June 29, 2017	not related	It was an occasional event due to an infectious disease and was probably not associated with the trial food.	continued	June 25, 2017
Placebo	KBR 13 - 533	1	Gingival pain	May 23, 2017	During the intake period	Mild	No	Recovered	May 26, 2017	not related	It was an occasional event and was probably not associated with the trial food.	continued	May 23, 2017
Placebo	KBR 13 - 537	1	Common cold	June 3, 2017	During the intake period	Moderate	Yes	Recovered	June 7, 2017	not related	It was an occasional event due to an infectious disease and was probably not associated with the trial food.	continued	June 3, 2017
Placebo	KBR 13 - 540	1	Edema	June 18, 2017	After the end of intake period	Mild	No	Recovered	July 1, 2017	not related	It was an occasional symptom, which developed late after the ingestion start, and thus was probably not associated with the trial food.	continued	June 18, 2017
	KBR 13 - 540	2	Metrorrhagia	July 1, 2017	After the end of intake period	Mild	No	Recovered	July 5, 2017	not related	It was judged to be a transient symptom because it occurred during the period without intake of the atudy food, and the symptom disappeared without treatment, and thus was probably not associated with the study food	continued	July 1, 2017

differed between males and females (Table 4 and Table 6). Data for the qualitative evaluation parameters were scored and basic statistics were calculated. Statistical analysis was performed by Wilcoxon's signed rank test for comparison of the data obtained at the start of ingestion (in week 0), and those obtained at the examination in weeks 2, 4 (intake period) and 6 (at the end of the follow up). In addition, statistical analysis was conducted by Wilcoxon's rank sum test for comparison of the data at each examination between the LC-Plasma group and placebo group (Table 7). All statistical tests were conducted with a significance level of 0.05.

3. Results

Subject characteristics: The Consolidated Standards of Reporting Trials flow diagram for this trial is shown in Figure 1. A total of 103 healthy volunteers were initially recruited. Fifty eight subjects were excluded based on the exclusion criteria, three subjects refused to participate and two subjects were not selected.

Table 1(a) and Table 1(b) show the baseline characteristics of each group. A significant difference between the LC-Plasma group and the placebo group was observed in the levels of LD (LDH), however, this fluctuation in a single parameter is negligible since the primary purpose of the trial is to evaluate the overall safety of excessive intake of LC-Plasma capsules. The trial was conducted as illustrated in Table 1(a) and Table 1(b). None of the subjects met the predefined discontinuance criteria during the examination period, and thus data obtained from all 40 subjects were included in the analysis.

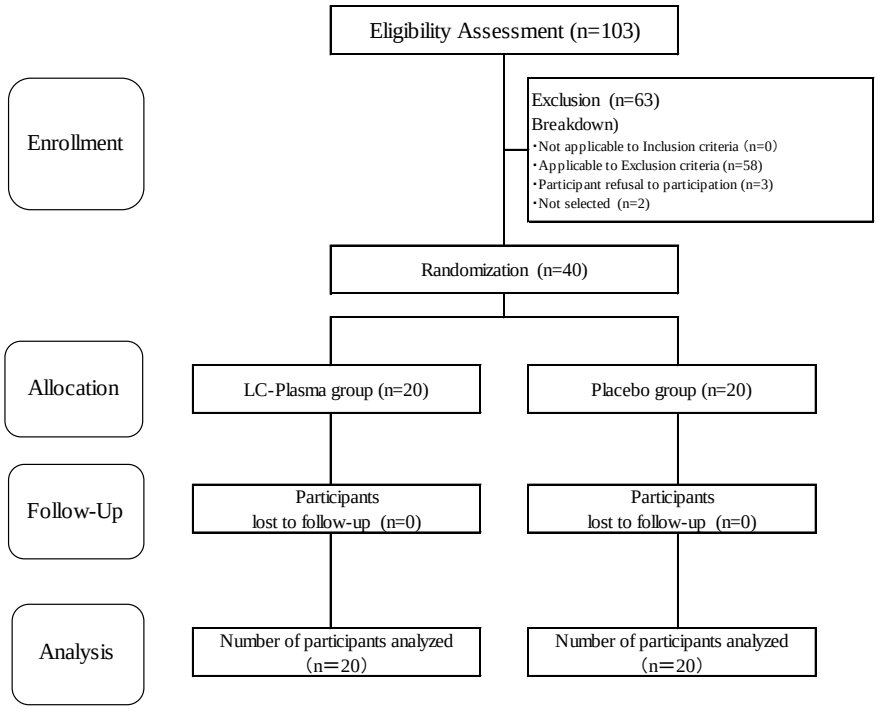

Figure 1. Consolidated standards of reporting trials diagram: Enrollment, random assignment, and follow-up of subjects.

Physical examinations: The results of the physical examinations are shown in Table 2. Comparing the values to week 0, significant changes were observed in the following parameters: in the LC-Plasma group, higher systolic blood pressure in week 2, lower diastolic blood pressure in week 6; in the placebo group, higher diastolic blood pressure in week 2. No significant changes were observed in the other parameters. Although significant changes were observed among several parameters in the physical examinations during the trial, the changes were within the range of physiological fluctuation and were deemed clinically irrelevant. Based on the physical measurements, this trial was judged to be of no safety concern by the principal investigator.

Hematological examinations: The results of the hematological examinations of all subjects are shown in Table 3. Comparing the values in week 0, significant changes were observed in the following parameters: in the LC-Plasma group, lower MCV in week 2, higher MCV in weeks 4 and 6, higher MCH in week 4, higher MCHC in week 2, lower MCHC in weeks 4 and 6, lower MONO/leukocyte fractionation in week 4; in the placebo group, lower hemoglobin in week 2, higher MCV in weeks 4 and 6, lower MCH in weeks 4 and 6, lower MCHC in weeks 4 and 6, lower MONO/leukocyte fractionation in week 6. Compared to the placebo group, significant changes were observed in the LC-Plasma group in the following parameters: lower MCHC at the start of ingestion; higher MONO/leukocyte fractionation in week 6; lower BASO/leukocyte fractionation in week 4. The results of the hematological examinations in male and female subjects are shown in Table 4. In the male group, compared to the values in week 0, significant changes were observed in the following parameters: in the placebo group, lower hemoglobin in week 2, lower hematocrit in week 2. No significant changes were observed in the female group. Although significant changes were observed among several parameters in the hematological examinations during the trial, these changes were within the range of physiological fluctuation and deemed clinically irrelevant. Based on the hematological measurements, this trial was judged to be of no safety concern by the principal investigator.

Biochemical examinations: The results of the biochemical examinations of all subjects are shown in Table 5. Compared to the values in week 0, significant changes were observed in the following parameters: in the LC-Plasma group, lower ALT (GPT) in week 6, lower ALP in week 6, lower fasting blood glucose in weeks 4 and 6, lower HbAlc in week 2, lower HDL-cholesterol in week 6, lower albumin in week 6, lower creatinine in week 4, higher sodium (Na) in week 6, higher chloride (Cl) in week 6, higher magnesium in week 2; in the placebo group, higher LD (LDH) in weeks 2 and 6, higher ALP in week 2, higher CK (CPK) in week 2, higher HbAlc in week 6, higher total protein in week 6, higher uric acid in week 4, higher sodium (Na) in week 2 and in week 6, higher chloride (Cl) in week 2, in week 4 and in week 6, higher potassium (K) in week 6, higher magnesium in weeks 2 and 4. Compared to the placebo group, significant changes were observed in the LC-Plasma group in the following parameters:

higher LD (LDH) at the start of ingestion and week 4; lower ALP at the start of ingestion and weeks 2, 4 and 6; lower total protein in week 6. The results of the biochemical examinations of male or female subjects are shown in Table 6. In the male group, compared to the values in week 0, significant changes were observed in the following parameters: in the LC-Plasma group, lower creatinine in week 4, higher uric acid in week 6; in the placebo group, higher uric acid in week 4. In the female group, compared to the values in week 0, significant changes were observed in the following parameters: in the LC-Plasma group, lower γ-GT (g-GTP) in week 4, lower HDL-cholesterol in weeks 4 and 6, lower creatinine in week 4; in the placebo group, higher creatinine in week 2. No significant changes were observed in the other parameters. Although significant changes were observed among several parameters in the biochemical examinations during the trial, these changes were within the range of physiological fluctuation and deemed clinically irrelevant. Based on the biochemical measurements, this was judged to be of no safety concern by the principal investigator.

Urinalysis examinations: The results of the urinalysis (quantitative test and scoring qualitative test) are shown in Table 7 and Table 8. No significant changes were observed in any of the parameters.

Adverse events: Five adverse events (one event in one subject in the LC-Plasma group and four events in three subjects in the placebo group) were observed during the trial period (Table 9). The principal investigator judged that all of the adverse events were "not related" to the trial capsules because the events were accidental or occurred outside of the intake period.

4. Discussion

Lactococcus lactis has been traditionally used in foods such as cheese and has been listed in "Qualified Presumption of Safety (QPS)-recommended biological agents intentionally added to food or feed" issued by the European Food Safety Authority [13]. Pharmacokinetics of LC-Plasma is thought that orally administrated LC-Plasma are taken up by immune cells in Peyer's patches of the intestinal tract and then induce expression of IFN-α [6]. LC-Plasma which are not taken up by immune cells are thought to be excreted in the feces. Previous pre-clinical studies in rats showed that single oral administration of 2000 mg/kg of LC-Plasma to 6-week-old Sprague-Dawley rats had no toxicity in general conditions, changes in body weight or necropsy, in addition that no toxicity was associated with repeated administration of 3.0×10^{10} or 3.0×10^{11} cfu/kg of LC-Plasma once a day for 28 days to 6-week-old Sprague-Dawley rats: measurements of body weight, urinalysis, hematological and blood biochemical examinations, histopathological examination of major organs and observations on the general condition of the rats were all judged to be within the range of physiological fluctuation. In micronucleus test, LC-Plasma was considered not to induce micronuclei in mouse peripheral blood with oral administrations of 500 mg/kg, 1000 mg/kg or 2000 mg/kg of LC-Plasma twice in consecutive 24-hour

intervals to 7-week-old ICR mice. Previous clinical trials also showed that the intake of a beverage containing 150 mg/day LC-Plasma (3 times the anti-viral infection effect dose) and 15 g/day indigestible dextrin for four weeks and intake of a beverage containing 50 mg/day LC-Plasma (anti-viral effective dose) and 5 g/day indigestible dextrin for twelve weeks were safe [14]. Moreover, products containing LC-Plasma have been marketed as soft drinks and yogurt from Kirin Beverage Co., Ltd. and Koiwai Milk Products Co., Ltd, and no health damage associated with them has been reported.

In this trial, the safety of ingesting LC-Plasma (250 mg/day) for four weeks was assessed in forty subjects. The examinations included physical, hematological, biochemical and urinary examinations and a medical interview. In the urinary examinations, there were no significant changes within the LC-Plasma group and the placebo group when compared to the reference values (week 0) and no significant changes between the LC-Plasma group and the placebo group. Significant differences and measurements outside the reference range were found in various parameters of the physical, hematological and biochemical examinations but none were considered to be a safety concern by the principal investigator. Significant differences in various parameters were also found between the LC-Plasma group and the placebo group, however, all of these differences were within the range of the reference values and within the range of physiological fluctuation. Thus, these differences were not considered to be a clinical safety concern attributable to the intake of the LC-Plasma capsules. The investigator determined that the adverse events noted in medical interviews by physicians and diary records by subjects were not related to the trial capsules because all of them were accidental or occurred outside of the intake period. Therefore, it was concluded that there are no clinical safety concerns associated with the excessive intake (5 times the anti-viral effective dose) of LC-Plasma.

5. Conclusion

There are no safety concerns associated with the consumption of heat-killed LC-Plasma (250 mg/day) for four weeks in the results obtained from physical, hematological, biochemical and urinary examinations and medical interviews in a randomized double-blind placebo-controlled parallel-group comparison method using 40 healthy adult males and females.

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