Research Article **Open Access**

Effects of heat killed *Lactiplantibacillus plantarum* N1487-7 intake in humans with impaired liver function: A randomized, double-blind, placebo-controlled, parallel-group study

Eriko Uehara[1]*, Hideki Hokazono[1], and Naoki Miura[2]

[1]Research & Development Laboratory, Sanwa Shurui Co., Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan; [2]Miura Clinic, Medical Corporation Kanonkai, Higashitenma Building 9F, 1-7-17 Higashitenma, Kita-ku, Osaka 530-0044, Japan.

***Corresponding author:** Eriko Uehara, Ph.D., Research & Development Laboratory, Sanwa Shurui Co., Ltd., 2231-1 Yamamoto, Usa, Oita 879-0495, Japan.

Submission Date: December 6th, 2024, **Acceptance Date:** December 27th, 2024, **Publication Date:** January 2nd, 2025

Please cite this article as: Uehara E., Hokazono H., Miura N. Effects of heat killed Lactiplantibacillus plantarum N1487-7 intake in humans with impaired liver function: a randomized, double-blind, placebo-controlled, parallel-group study. *Functional Foods in Health and Disease* 2025; 15(1): 1-18. DOI: https://doi.org/10.31989/ffhd.v15i1.1529

ABSTRACT

Background: The liver is an organ that performs important functions such as nutrient synthesis and detoxification, and liver health is greatly affected by lifestyle habits. Inappropriate lifestyle habits such as overeating, excessive alcohol intake, and lack of exercise cause inflammation in the liver. Serum levels of liver function markers such as aspartate aminotransferase and low-density lipoprotein cholesterol (LDL-c) increase as liver function declines. Recently, it has been suggested that heat-sterilized *Lactiplantibacillus plantarum* N1487-7 (N1487-7-HK) have an anti-inflammatory effect, and this effect may be useful in supporting liver function.

Objective: To investigate the effect of oral intake of N1487-7-HK on liver function in healthy subjects with elevated serum levels of liver function markers.

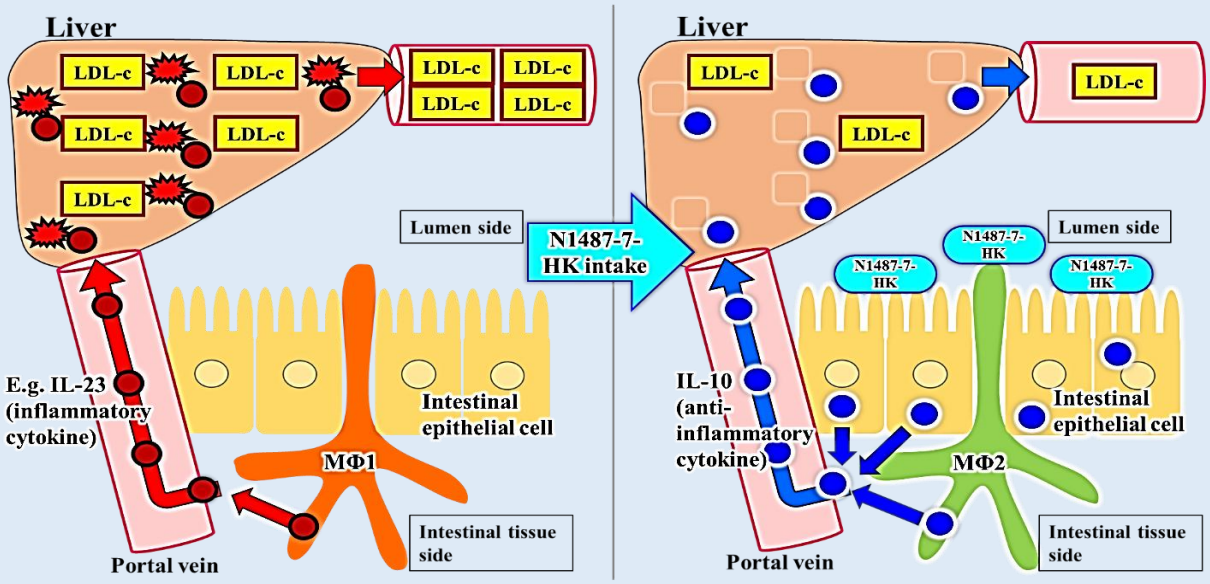
Methods: A randomized, double-blind, placebo-controlled, parallel-group comparative study was conducted. One hundred subjects (20–64 years old) were randomly assigned to receive N1487-7-HK (6×10^{10} cells/capsule/day) or a placebo, ingested once daily for 12 weeks. Efficacy endpoints included the serum levels of four liver function markers and of interleukin (IL)-10; safety endpoints included serum levels of LDL-c and total cholesterol (TC).

Results: There were no significant differences in serum levels of liver function markers or of IL-10 between the two groups. In safety evaluations, the LDL-c level change (p = 0.049 at Week 12) and the TC level changes (p = 0.047 at Week 6, p = 0.012 at Week 12) show a significantly greater reduction (improvement) in the N1487-7-HK group than in the placebo group. Notably, in a subgroup analysis of subjects with baseline serum TC levels below the median (228.5 mg/dL), serum IL-10 levels at study completion were significantly higher in the N1487-7-HK intake group than in the placebo group (p = 0.003 at Week 12).

Conclusions: These results suggested that N1487-7-HK ingestion has an anti-inflammatory effect and improves LDL-c and TC metabolism in subjects with impaired liver function. Therefore, N1487-7-HK supplementation may be effective in preventing or improving arteriosclerosis, myocardial infarction, cerebral infarction, and cholelithiasis.

Trial registration number: UMIN000051940.

Keywords: *Lactiplantibacillus plantarum*, liver function, interleukin-10, low density lipoprotein cholesterol, total cholesterol



Graphical Abstract: The anti-inflammatory effect of N1487-7 (N1487-7-HK reduces LDL cholesterol levels.

INTRODUCTION

The liver has multiple essential functions including the metabolism of major nutrients, detoxification of harmful substances, the production and secretion of bile (which aids in digestion and absorption), and maintenance of lipid and cholesterol homeostasis. [1] In the liver, dietary lipids and carbohydrates are broken down respectively into fatty acids and glucose, but any of these products that are not used are accumulated in the liver in the form of triglycerides. The accumulation of excess triglycerides

accumulates in the liver (e.g., due to the lack of exercise or excessive caloric intake) to exceed 5% of the liver weight results in non-alcoholic fatty liver disease (NAFLD). [2] Worldwide, the prevalence of this condition has increased in recent years and has been shown to correlate positively with mortality. [3] However, in 2020, an international expert panel revised the term and definition of NAFLD in adults and proposed the use of the term metabolic dysfunction-associated steatotic liver disease (MASLD). Furthermore, in 2023, three large multinational liver societies proposed replacing the NAFLD designation with MASLD. [4] MASLD is currently the most common chronic liver disease, leading to cirrhosis and hepatocellular carcinoma. [5] MASLD has been estimated to affect 25% of adults worldwide. [6]

A high-fat diet significantly increases lipid synthesis in the liver, leading to inflammation and increased levels of reactive oxygen species (ROS), accelerating fatty liver disease. [7] Aspartate aminotransferase/glutamic oxaloacetic transaminase (AST/GOT), alanine aminotransferase/glutamic pyruvate transaminase (ALT/GPT), gamma-glutamyl transpeptidase (γ-GTP), and alkaline phosphatase (ALP) are markers of liver function that accumulate in this organ as liver function declines. [8–10] In patients with NAFLD, inflammatory cells infiltrate liver tissue, and the AST and ALT present in liver cells are released into the blood, resulting in a significant increase in serum levels of these enzymes. [11] Patients with NAFLD are in a chronic inflammatory state, and γ-GTP, which normally is present in hepatocytes, is released into the blood with concomitant increases in the serum level increases of this marker. [12] When inflammation occurs in the liver, bile stagnates in the hepatic bile duct and is released into the blood, similarly leading to increases in the serum level of ALP. [13]

In MASLD, de novo lipid synthesis in the liver is increased while fatty acid oxidation and the export of low-density lipoprotein cholesterol (LDL-c) are decreased; this pattern which may promote migration of inflammatory cells into the liver and lead to inflammation. Lipids such as free cholesterol also may promote the production of inflammatory cytokines, leading to liver inflammation. [4] The liver synthesizes cholesterol and bile acids, which then are secreted into bile to facilitate the absorption of dietary fat and fat-soluble vitamins. [14] In one study of 163 patients with NAFLD, 49 (30.1%) had cholestatic syndrome, with significant hepatocellular inflammation and impaired lipid metabolism. [15]

Lactic acid bacteria are Gram-positive microbes that convert carbohydrates into lactic acid by fermentation.[16] The lactic acid bacterium *Lactiplantibacillus* (*Lpb.*) *plantarum* is listed in the Qualified Presumption of Safety (QPS) of the EFSA (European Safety Authority) and is generally regarded as safe (GRAS). Intriguingly, extracts of the multi-strain mixture probiotics containing *Lpb*. *plantarum* EMRO 009 have been used as anti-inflammatory agents for the treatment of chronic diseases. [17] Separately, *Lbp*. *plantarum* HOKKAIDO has been shown to promote the production of both pro- and anti-inflammatory cytokines by $CD14^+$ monocytes and to exert antiviral effects; notably, these antiviral activities are stronger when using killed bacteria than when using live bacteria. [18] In other experiments, *Lpb.* plantarum N1487-7 (N1487-7) was isolated from rice bran pickles, a traditional Japanese fermented food. Heat-sterilized N1487-7 (N1487-7-HK) was confirmed to be safe in bacterial reverse mutation tests and acute oral toxicity tests in mice. [19]

The Functional Food Center defines "functional foods" as follows: natural or processed foods that have proven clinically to have health benefits (as measured by specific biomarkers), to reduce the risk of disease, to be safe, and to contain biologically active compounds [20–23]. In previous work, we reported that N1487-7-HK is a functional food that promotes the anti-inflammatory

cytokine interleukin (IL)-10 by intestinal epithelial cells and macrophage cells; this strain also has been shown to enhance skin barrier function [19]. Since the increase serum levels of liver function markers is caused by liver inflammation, it may be possible to improve liver function by suppressing inflammation. Therefore, we investigated the effect of N1487-7-HK intake on liver function in otherwise healthy men and women aged 20 to 64 years, with elevated serum levels of liver function markers; specifically, we conducted a placebo-controlled, double-blind, randomized, parallel-group comparative study.

METHODS

Study design and ethical statement: This research followed a randomized, double-blind, placebo-controlled, parallel-group design. The research was conducted according to the study protocol, which received approval from the ethics review committee of the Kanonkai Miura Clinic (Osaka, Japan) on June 22, 2023 (approval number: R2215). The study adhered to the principles outlined in the "Declaration of Helsinki" (revised October 2013) and the "Ethical Guidelines for Medical and Biological Research Involving Human Subjects" (issued by the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Japanese Ministry of Health, Labour and Welfare on March 23, 2021, with partial revisions on March 10, 2022).

This trial was registered (before initiation) with the University Hospital Medical Information Network (UMIN) Clinical Trial Registry managed by the UMIN Center (ID No. UMIN000051940). Each subject was provided with an explanatory document and a consent form, both of which had been approved by the ethics review board; and the study details were thoroughly explained in these documents. The medical director obtained voluntary written consent from each individual prior to his/her entry onto the study.

The study was conducted at the Miura Clinic, Medical Corporation Kanonkai (a study site, Osaka, Japan), in collaboration with BML Co., Ltd. (a laboratory, Osaka, Japan), Eurofins GeneticLab Co., Ltd. (a laboratory, Sapporo, Japan), and Nikken Seil Co., Ltd. (a laboratory, Shizuoka, Japan).

Recruitment began on August 21, 2023. The study commenced on September 12, 2023, and follow-up was completed on December 31, 2023.

Subjects: Once informed consent was obtained from the subjects, these individuals were asked to provide information regarding their sex, age, medical history, allergies, alcohol and tobacco use, lifestyle and exercise routines, use of medications and health foods, and involvement in other clinical trials. This information was compiled as demographic data. The principal investigator reviewed the demographic data along with the results of pre-dose Week 0 and determined eligibility based on the inclusion and exclusion criteria. To be enrolled, subjects had to satisfy all the inclusion criteria and none of the exclusion criteria.

Inclusion criteria: Subjects were healthy males and females aged 20–64 years who fully understood the purpose and content of the study, voluntarily provided written consent to participate in the study, and met one of the following three conditions: AST, 31–50 U/L; ALT, 31–50 U/L; or γ-GTP, 61–100 U/L (for males) or 31–65 U/L (for females).

Exclusion criteria: Subjects excluded from this study were those with medical conditions that had the potential to interfere with the study's endpoints. Exclusions included individuals with a history of cardiovascular, hepatic, or renal disease (including cases involving secondary complications); with circulatory disorders or diabetes, or currently receiving medical treatment; with known allergies to food or medication, as well as those

experiencing symptoms of anemia; receiving treatment with pharmaceuticals or Chinese herbal remedies (even on an as-needed basis); engaged in high-intensity sports or on weight-reduction diets; reporting significantly irregular eating habits; expecting to alter their lifestyle during the study period (such as changes in diet, exercise, smoking, alcohol consumption, or sleep patterns); unwilling to discontinue the use of health foods (including functional foods or quasi-drugs); with an average weekly alcohol intake exceeding 60 g of pure alcohol; or who smoked 21 or more cigarettes per day. Females who were pregnant, breastfeeding, planning to become pregnant during the study, or potentially pregnant also were excluded. Lastly, subjects involved in other clinical trials at the time of enrollment, or whom the principal investigator deemed unsuitable, also were excluded.

Target sample size and rationale: No studies have evaluated the effects of N1487-7-HK on liver function markers. Therefore, the required sample size was determined with a 5% significance level and 80% statistical power. To accommodate potential dropouts, the sample size per group was increased, resulting in a final target of 50 subjects per group, with a total of 100 subjects.

Randomization: The allocation manager generated a randomization table using random numbers and then assigned allocation codes to the study foods. This allocation table was sealed by the allocation manager and remained unopened until the predetermined time for unblinding. Once the subjects for analysis were confirmed and their data finalized, the allocation manager unsealed the table and revealed the allocation details. In the event of a serious adverse event requiring emergency intervention, the table would have been partially unsealed to disclose only the necessary information. However, no such serious adverse events occurred during the study.

Study food: The active food was provided in the form of a hard capsule containing 6×10^{10} N1487-7-HK cells. The raw materials were supplied by Sanwa Shurui Co., Ltd. (Oita, Japan). [19] Additional ingredients in the capsule included microcrystalline cellulose (VIVAPUR 301; JRS Pharma, Rosenberg, Germany) and calcium stearate (Ca-St; Go Yen Chemical Industrial Co., Ltd., Kaohsiung, Taiwan). The placebo food, which visually matched the active food, contained dextrin (Pinedex #2; Matsutani Chemical Industry Co., Ltd., Hyogo, Japan) in place of N1487-7-HK.

Blinding: Since this was a double-blind study, both subjects and investigators were unaware of the treatment assignments. The packaging of the supplements was numbered by the manufacturer, and neither the subjects nor the investigators knew which numbers corresponded to the active or placebo food.

Interventions: Following the baseline assessment at pre-dose Week 0, subjects were instructed to take one assigned capsule daily with water or lukewarm water within 30 minutes after dinner for 12 consecutive weeks. Missed doses were not to be carried over to the next day.

Assessments and interviews were conducted 6 and 12 weeks after the start of the intervention, according to the study protocol.

Throughout the study period, subjects were required to maintain daily diaries (either online or on paper) from the beginning of intake until the day before the Week-12 assessment. The diary included the following: confirmation of study food intake, physical condition, medication intake, alcohol consumption, and exercise status.

Study period: Subjects were instructed to maintain their

regular lifestyle habits, including their diet, alcohol consumption, exercise, sleep schedule, and smoking patterns, before enrolling in the trial. Individuals were advised to avoid intense physical activity, restrictive diets, and overeating. Additionally, subjects were asked not to consume health products or supplements that could influence the study endpoints, as well as to refrain from taking medications. If medication use became necessary due to illness or other reasons, subjects were required to consult with the research team in advance. From the day before each test until the test's completion, subjects were instructed to abstain from alcohol and strenuous physical activity. Individuals were also required to stop eating and drinking by 10 p.m. on the night before the test and to fast until the test concluded, although water or lukewarm water was permitted. On the test day, subjects were asked to arrive at the test site without consuming the assigned study food.

Test items and timing: After obtaining informed consent from subjects, we administered a lifestyle questionnaire and measured their height exclusively at the pre-dose Week 0. To assess the efficacy of the test food, evaluations were conducted at three time points: pre-dose Week 0, Week 6, and Week 12.

Primary endpoints included the measurement of serum levels of AST, ALT, γ-GTP, and ALP. Secondary endpoints comprised serum levels of interleukins (IL)-10 and myeloperoxidase (MPO) levels, along with assessments using the second edition of the Profile of Mood States (POMS2) and the Visual Analog Scale (VAS) questionnaire for fatigue. For each evaluation, approximately 23.5 mL of blood was drawn from each subject. The serum levels of AST, ALT, γ-GTP, and ALP were analyzed using an automated system. IL-10 levels were quantified using the Human IL-10 ProQuantum Immunoassay Kit (Thermo Fisher Scientific, Waltham, MA, USA), while MPO levels were determined with an MPO enzyme-linked immunosorbent assay (ELISA) Kit (Immundiagnostik AG, Bensheim, Germany). The POMS2 (https://www.kanekoshobo.co.jp/book/b190469.html, in Japanese) questionnaire measured emotional states across seven domains: "anger-hostility," "confusion-bewilderment," "depression-dejection," "fatigue-inertia," "tension-anxiety," "vigor-activity," and "friendliness." The questionnaire also provided a Total Mood Disturbance (TMD) score, which aggregates "negative" mood aspects. T-scores were calculated according to the POMS2 (Japanese version) manual (Kaneko Shobo Co., Ltd.). The VAS questionnaire consisted of seven items related to fatigue, such as levels of "concentration" and "motivation." Subjects indicated their subjective fatigue level by placing an "×" on a 100-mm horizontal line. "Positive" statements were written on the left side and "negative" statements on the right side. The distance from the left edge to the participant's mark was measured in millimeters, with lower scores indicating less fatigue and higher scores indicating more fatigue. To assess the safety of the test food, several evaluations were performed at pre-dose Week 0, Week 6, and Week 12. These assessments included health monitoring, a daily diary (documenting subjective symptoms), measurements of body weight and body mass index, physical examinations (blood pressure and pulse), and laboratory tests (blood and urine analyses). The principal investigator recorded any subjective symptoms and objective adverse events, noting details such as onset and resolution dates, symptom severity, treatment, endpoints, and any potential links to the test food. Follow-up actions were taken as necessary until the adverse events either resolved or showed signs of improvement. Subjects used an online diary to log their consumption of the test food, any changes in physical condition, and the use of medications throughout the study period.

Analysis set and statistical analysis methods*:* Once the final tests were completed, the analysis set was confirmed, and the dataset was locked. Following the data lock confirmation, the allocation manager, under the principal investigator's instructions, accessed the study food allocation table.

In principle, all subjects, including those who discontinued or withdrew from the study, were included in both the efficacy and safety analyses. However, for the analysis of physical measurements, medical examinations, and laboratory tests, subjects who completed the study protocol were excluded from the analysis if those individuals met any of following exclusion criteria: displayed behavior that could significantly affect the reliability of the results, such as failing to maintain diary entries; were later identified as meeting exclusion criteria or failed to comply with study requirements during the research period; or had a study food intake rate below 70%. For adverse events, subjects who consumed the study food at least once were included in the evaluation.

Data management and statistical analyses were performed using IBM SPSS Statistics version 28 (International Business Machines Corporation, Armonk, NY, USA).

Subject characteristics (age, height, weight, body mass index, systolic and diastolic blood pressure, and pulse rate) at pre-dose Week 0 were summarized as mean ± standard deviation (SD) for each group. Group comparisons were conducted with an unpaired *t*-test, while comparisons between sexes utilized the chi-squared test.

For primary and secondary endpoints, as well as for physical and clinical examinations assessing the safety of the test food, the actual values at three time points and changes from Week 0 to Week 6 and from Week 0 to Week 12 were calculated (mean ± SD) for each group. Intergroup comparisons of parameters at each time point, except for those parameters obtained using the VAS questionnaire, were conducted using an unpaired *t*-test. For the VAS questionnaire, the Wilcoxon signed-rank test was employed for intergroup comparisons. Intragroup comparisons from pre-dose Week 0 to subsequent time points were performed using a Bonferroni-corrected paired *t*-test for all endpoints except for those obtained via the VAS questionnaire. For data obtained using the VAS questionnaire, the Wilcoxon signed-rank test with Bonferroni correction was applied. All statistical tests were two-tailed, with a 5% significance level. The number of adverse events in each group was recorded, and a list was compiled. If the same adverse event occurred more than once in a subject, this observation was counted as a single event. Outliers in test and measurement data were included in the analysis. Missing values were neither imputed nor included in the analysis. The intake rates of study food, of medications, and of health supplements, were recorded and assessed. The intake rate for the study food was calculated by dividing the number of times the food was consumed by the total number of scheduled intakes. No deviations from the original study protocol were identified.

RESULTS

Demographics: The study flow is outlined in Figure 1. A total of 163 subjects who provided written informed consent underwent screening, resulting in the exclusion of 63 individuals. The remaining 100 subjects who met the inclusion criteria were selected for the study. After beginning the study food intake, one male subject from each group withdrew for personal reasons.

The datasets used for each evaluation also are detailed in Figure 1. All 100 subjects who consumed the study food at least once were included in the safety analysis (Full Analysis Set, FAS). Prior to unblinding the allocation table, subjects eligible for the efficacy analysis were determined. Two male subjects in the active food

intake group (Group A) were excluded from the efficacy analysis because the test food intake rate was below 70%; these cases were treated as missing data. Consequently, the efficacy analysis (Per Protocol Set, PPS) included 47 subjects (30 males, 17 females) from Group A and 49 subjects (31 males, 18 females) from the placebo food intake group (Group P).

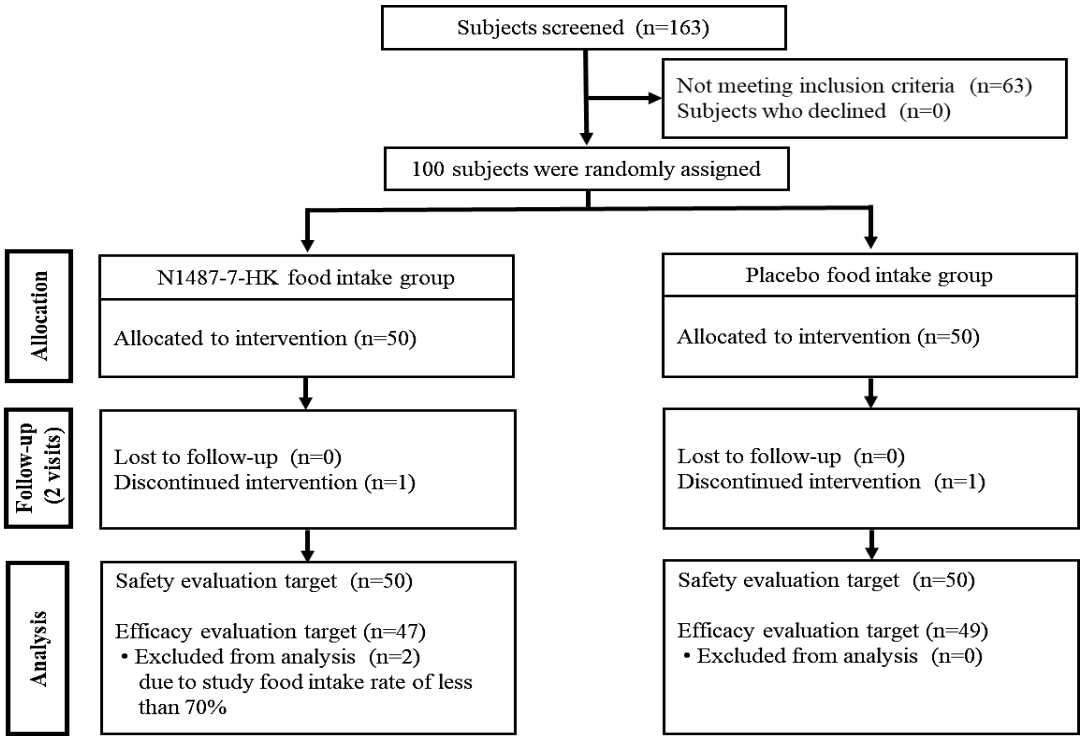


Figure 1. Flowchart of the study.

Tables 1 and 2 show the subject demographics for the FAS and PPS, respectively. No significant differences were observed between the two groups in either data set.

Table 1. Subject demographics (Full Analysis Set).

	Unit	Group A			Group P			Group comparisons
Number of subjects			50			50		NS
Male			33			32		NS
Female			17			18		NS
Age	years	49.9	±	8.6	49.9	±	9.3	NS
Height	cm	168.0	±	9.0	166.2	±	8.1	NS
Body weight	kg	70.0	±	12.3	67.6	±	12.2	NS
Body Mass Index	kg/m^2	24.7	±	3.1	24.3	±	3.1	NS
Systolic blood pressure	mmHg	124.5	±	15.3	125.5	±	11.9	NS
Diastolic blood pressure	mmHg	79.9	±	12.5	76.7	±	10.5	NS
Pulse rate	beats/min	74.4	±	9.9	71.6	±	11.7	NS

Table 2. Subject demographics (Per Protocol Set).

	Unit	Group A			Group P			Group comparisons
Number of subjects			47			49		NS
Male			30			31		NS
Female			17			18		NS
Age	years	49.9	±	8.9	50.0	±	9.4	NS
Height	cm	167.8	±	9.2	166.2	±	8.2	NS
Body weight	kg	70.0	±	12.6	67.8	±	12.2	NS
Body Mass Index	kg/m^2	24.7	±	3.1	24.4	±	3.1	NS
Systolic blood pressure	mmHg	125.7	±	14.9	125.4	±	12.0	NS
Diastolic blood pressure	mmHg	80.6	±	12.6	76.6	±	10.5	NS
Pulse rate	beats/min	74.5	±	10.2	71.3	±	11.6	NS

Tables 1 and 2 present the demographic characteristics of subjects in the Full Analysis Set and the Per Protocol Set, respectively. All measurements were taken at pre-dose Week 0. "Group A" corresponds to the N1487-7-HK food intake group, while "Group P" represents the placebo food intake group. Data are shown as mean ± standard deviation, except for the number of subjects and sex distribution.

Group comparisons were conducted via two-tailed unpaired *t*-tests, while comparisons between sexes utilized two-tailed chi-squared tests. "NS" indicates no significant difference at $p \geq 0.05$.

Study food intake rate: The mean intake rate of study foods was 94.8 ± 10.7% in Group A and 97.0 ± 11.3% in Group P.

Primary endpoints (measurement of serum AST, ALT, γ-GTP, and ALP levels): Table 3 provides data for serum markers of liver function in the two groups at multiple time points. No significant differences were detected for intergroup comparisons. For the intragroup comparisons between time points, ALP levels were significantly increased in both Group A and Group P at Week 12 (compared to Week 0).

Data are presented as mean ± standard deviation. The abbreviations for the test items are as follows: AST, Aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase. "Group A" represents the N1487-7-HK food intake group, while "Group P" denotes the placebo food intake group. Group comparisons were analyzed using two-tailed unpaired *t*-tests. Intragroup changes from pre-dose Week 0 were evaluated using two-tailed Bonferroni-corrected paired *t*-tests. A statistically significant difference is indicated by * ($p < 0.05$). The number of subjects was 47 in Group A and 49 in Group P.

Secondary endpoints and other evaluation items (measurement of serum IL-10 and MPO levels, POMS2, and VAS questionnaire on fatigue)

Serum IL-10 levels: No significant differences in serum IL-10 levels were detected by intergroup comparisons. The intragroup comparisons to pre-dose Week 0 data (21.5 ± 25.43 pg/mL, mean ± SD), revealed significant decreases in IL-10 levels in Group P at Week 6 (16.3 ± 14.3 pg/mL; $p = 0.0086$) and Week 12 (15.5 ± 14.2 pg/mL; $p = 0.0070$); in contrast, Group A showed no significant changes from Week 0 to Week 12.

Table 3. Primary endpoints.

	Unit		**Pre-dose**			**Week 6**			**Week 12**			**ΔWeek 6**			**ΔWeek 12**			***p*-value (intragroup)**	
			Week 0															Week 6	Week 12
AST	U/L	Group A	27.9	±	8.9	28.9	±	9.1	29.3	±	9.6	1.0	±	9.2	1.4	±	8.5	0.920	0.500
		Group P	29.1	±	7.1	29.5	±	8.6	30.4	±	10.8	0.4	±	6.2	1.3	±	9.3	1.000	0.630
		p-value (between groups)	0.470			0.740			0.600			0.710			0.960			-	-
ALT	U/L	Group A	33.9	±	13.6	34.8	±	14.0	35.3	±	16.2	0.9	±	13.0	1.4	±	14.0	1.000	0.960
		Group P	35.0	±	14.6	36.1	±	14.4	37.2	±	24.3	1.1	±	10.3	2.2	±	19.8	0.930	0.880
		p-value (between groups)	0.690			0.640			0.650			0.940			0.830			-	-
γ-GTP	U/L	Group A	55.3	±	21.2	57.5	±	28.4	59.3	±	29.8	2.2	±	26.9	4.0	±	25.7	1.000	0.590
		Group P	59.1	±	24.2	63.1	±	32.6	61.6	±	28.1	4.0	±	21.3	2.6	±	22.7	0.380	0.870
		p-value (between groups)	0.420			0.370			0.690			0.710			0.780			-	-
ALP	U/L	Group A	77.7	±	21.6	81.2	±	26.6	84.1	±	25.6	3.6	±	14.5	6.4	±	12.8	0.200	0.002 *
		Group P	79.2	±	23.6	81.9	±	24.1	83.3	±	24.3	2.7	±	8.3	4.1	±	8.7	0.052	0.004 *
		p-value (between groups)	0.750			0.900			0.870			0.730			0.300			-	-

Serum MPO levels, POMS2*:* No significant differences or changes in MPO levels and POMS2 scores were detected, by either inter- or intragroup comparisons.

VAS questionnaire on fatigue*:* No significant differences in VAS scores were detected by intergroup comparisons. The intragroup comparisons to pre-dose Week-0 data revealed significant decreases (improvements) at Week 12 in Group A for the categories “concentration” (Week 0: 34.3 ± 21.7; Week 12, 30.1 ± 18.6; p = 0.023) and "motivation" (Week 0: 36.9 ± 21.9; Week 12: 30.4 ± 19.3; p = 0.0092). Other intragroup comparisons did not show significant differences.

Exploratory analysis of serum LDL-c and total cholesterol (TC) levels: Because liver function markers and serum cholesterol levels have been reported to be closely related [24–26], additional exploratory analyses of serum levels of both LDL-c and TC levels were also conducted, although these parameters constituted safety endpoints rather than efficacy endpoints. Table 4 presents the measured values and changes for LDL-c and TC levels. Group A showed a significantly greater reduction (improvement) than Group P in the LDL-c level change from pre-dose Week 0 to Week 12. Similarly, Group A showed a significantly greater reduction (improvement) than Group P in the TC level change from pre-dose Week 0 both to Week 6 and to Week 12 (at the respective time points).

Intragroup analysis revealed a significant increase (decline) in TC levels in Group P at Week 12 compared those at pre-dose Week 0 (Table 4).

Data are presented as mean ± standard deviation. LDL, low-density lipoprotein. "Group A" represents the N1487-7-HK food intake group, while "Group P" denotes the placebo food intake group. Group comparisons were analyzed using a two-tailed unpaired t-test. Intragroup changes from pre-dose Week 0 were evaluated using a two-tailed Bonferroni-corrected paired t-test with statistically significant differences indicated by * (p < 0.05). The number of subjects was 47 in Group A and 49 in Group P.

Post hoc subgroup analysis based on TC levels: To examine the correlation between TC levels and IL-10 levels, a subgroup analysis was performed on subjects whose TC levels were below the median (228.5 mg/dL) at pre-dose Week 0 (Table 5). In intergroup comparisons of these subgroups, IL-10 levels in Group A were significantly higher than in Group P at Week 12. By intragroup comparisons, IL-10 levels in Group P at Week 6 were significantly lower than at pre-dose Week 0

Table 4. Exploratory analysis.

	Unit		**Pre-dose**			**Week 6**			**Week 12**			**ΔWeek 6**			**ΔWeek 12**			***p*-value (intragroup)**	
			Week 0															**Week 6**	**Week 12**
LDL-cholesterol	mg/dL	Group A	146.1	±	42.5	140.7	±	43.4	142.4	±	40.4	-5.5	±	24.9	-3.7	±	22.4	0.270	0.520
		Group P	137.8	±	40.1	139.4	±	37.3	143.7	±	39.1	1.6	±	21.7	5.9	±	24.7	1.000	0.210
		p-value (between groups)	0.320			0.880			0.880			0.140			0.049 *			-	-
Total cholesterol	mg/dL	Group A	232.0	±	43.0	225.6	±	42.5	229.4	±	40.7	-6.4	±	28.8	-2.6	±	26.7	0.270	1.000
		Group P	226.3	±	43.4	230.5	±	42.1	237.5	±	44.1	4.2	±	22.4	11.2	±	26.3	0.380	0.009 *
		p-value (between groups)	0.520			0.570			0.350			0.047 *			0.012 *			-	-

Table 5. Post hoc subgroup analysis by total cholesterol levels.

	Unit		**Pre-dose**			**Week 6**			**Week 12**			**ΔWeek 6**			**ΔWeek 12**			***p*-value (intragroup)**	
			Week 0															Week 6	Week 12
IL-10	pg/mL	Group A	20.3	±	12.5	19.4	±	12.4	15.8	±	4.3	-0.9	±	4.5	-4.5	±	9.9	0.237	0.167
		Group P	17.6	±	4.3	14.2	±	4.8	11.9	±	4.4	-3.3	±	4.6	-5.7	±	4.8	0.047 *	0.122
		p-value (between groups)	0.332			0.0710			0.003 *			0.0660			0.604			-	-

Post hoc subgroup analyses were conducted on subjects with total cholesterol (TC) levels below the median value of 228.5 mg/dL. Data are presented as mean ± standard deviation. IL-10, interleukin 10. "Group A" represents the N1487-7-HK food intake group, while "Group P" denotes the placebo food intake group. Group comparisons were analyzed using a two-tailed unpaired *t*-test. Intragroup changes from pre-dose Week 0 were evaluated using a two-tailed Bonferroni-corrected paired *t*-test with statistically significant differences indicated by * ($p < 0.05$). The number of subjects was 23 in Group A and 25 in Group P.

Safety test: In the hematology, blood biochemistry, and urinalysis assessments, several significant changes were observed compared to pre-dose Week 0; however, all changes remained within reference intervals. Some subjects tested positive for protein (qualitative) and occult blood (qualitative) in the urinalysis. These findings were attributed to menstruation or natural physiological variations, leading the principal investigator to conclude that there were no clinical concerns for these parameters.

Adverse events were recorded, including diarrhea, headache, and muscle pain, with a total of 51 cases in group A and 20 in group P. The diarrhea was attributed to excessive beverage intake, and symptoms like headache and diarrhea were temporary, resolving the next day. The muscle pain was linked to exercise. As mentioned above, there was no causal relationship with the study food in any of the cases, and the principal investigator determined that all these events were "not related" to the study food.

DISCUSSION

This study aimed to investigate the effects of N1487-7-HK intake on healthy subjects with high serum liver function markers. Based on cellular studies and animal experiments, N1487-7-HK cells, which were used as the test food in the present study, have been suggested to have anti-inflammatory effects. [19] According to an open-label study (unpublished), this bacterium could improve liver function. Given that impaired liver function is thought to be caused by inflammation, we conducted a randomized, double-blind, placebo-controlled, parallel-group study to evaluate the effect of liver function improvement through the anti-inflammatory action of this strain. Although subjects with elevated serum levels of liver function markers consumed N1487-7-HK cells, no significant improvements in these markers were observed. However, ALP levels increased significantly in both groups from pre-dose Week 0 to Week 12, which may reflect a decrease in vitamin D levels. Notably, this study was conducted in Japan during a time of year when daylight hours gradually decrease (September–December); as daylight hours decrease, vitamin D levels also are known to decrease. [27] Furthermore, as vitamin D levels decrease, ALP levels are known to increase ($p = 0.022$). [28] Therefore, the increase in ALP levels in both groups was likely attributable to seasonal variation. The reference values for serum markers of liver function, which were the primary endpoints of this study, vary depending on the testing institution in Japan. According to the Japan Society of Ningen Dock and Preventive Medical Care (https://www.ningen-dock.jp/public_method/#pm11, in Japanese), individuals with serum ALT levels between 31 and 50 U/L are considered at risk for hepatitis, fatty liver, and liver cancer. Similarly, those with γ-GTP levels between 51 and 100 U/L are considered at risk for bile duct obstruction and drug-induced liver injury in addition to hepatitis, fatty liver, and liver cancer. In the present study, the pre-dose Week-0 serum ALT levels were 33.9 ± 13.6 U/L in Group A and 35.0 ± 14.6 U/L in Group P, and serum γ-GTP levels were 55.3 ± 21.2 U/L in Group A and 59.1 ± 24.2 U/L in Group P. These values indicated that subjects were within the risk interval for these diseases. On the other hand, in

our unpublished open-label study (which aimed to examine the effects of N1487-7-HK on immunity), serum levels of liver function markers were measured as a test item to evaluate safety. The inclusion criteria for that unpublished study were individuals with a slightly weakened immune system. In that unpublished study, a low-dose group ingested the same amount of N1487-7-HK cells as in the present study, and a high-dose group ingested twice the amount of N1487-7-HK cells. The serum γ-GTP levels in the low- dose group were 34.4 ± 5.4 U/L at pre-dose Week 0 and 30.3 ± 3.9 U/L after 8 weeks of intake, which were within the reference interval, and represented a significant ($p < 0.05$) decrease over time. Furthermore, the serum γ-GTP levels in the high-dose group were 59.1 ± 14.0 U/L at pre-dose Week 0 and 52.0 ± 11.2 U/L after 8 weeks of intake, which coincidentally were within the risk interval for liver dysfunction and represented a nominal ($p < 0.10$) but not statistically significant decrease over time. Those results suggested that, among subjects exhibiting baseline γ-GTP levels within the reference interval, the intake of N1487-7-HK cells has the effect of lowering the serum level of this marker; in contrast, among subjects exhibiting elevated baseline γ-GTP levels within the risk interval for liver disease, efficacy may require ingestion of this bacterium at more than twice the dose used in the present study.

The liver synthesizes, metabolizes, and excretes cholesterol; serum levels of liver function markers and serum cholesterol levels are closely related. [24–26] According to the Japan Society of Ningen Dock and Preventive Medical Care (https://www.ningen-dock.jp/public_method/#pm11, in Japanese), individuals with serum LDL-c levels between 120 and 179 mg/dL are considered at risk for the progression of atherosclerosis, which increases the likelihood of myocardial infarction and cerebral infarction. In the present study, LDL-c levels at Week 0 were 146.1 ± 42.5 mg/dL in Group A and 137.8 ± 40.1 mg/dL in Group P, indicating that the subjects were within the at-risk interval for these conditions. Individuals whose serum levels of liver function markers were classified as "risk level" as indicated by the Japan Society of Ningen Dock and Preventive Medicine were recruited for the present study, but their LDL-c levels also happened to be within the “risk level” interval. Two articles reported that individuals with NAFLD had elevated serum levels of both liver function markers and LDL-c. [25, 26] Therefore, an exploratory analysis of the serum levels of both LDL-c and TC levels also was conducted. Compared to Group P, Group A demonstrated a significant reduction (improvement) in both LDL-c and TC levels at 12 weeks. Another study has shown that the administration of the *Lacticaseibacillus casei* Shirota strain, a type of lactic acid bacteria, to apoE-deficient mice, improves lipid profiles, attenuates cholesterol accumulation in the liver and aorta, and inhibits the development of atherosclerotic lesions. [29]

The close connection and strict cooperation between the gut and the liver give rise to a functional entity known as the "gut-liver axis." This connection involves cellular and molecular interactions among the gut, gut microbiota, and liver, which are influenced by various factors such as diet, genetics, and the environment. MASLD has been linked to alterations in the gut microbiota and the integrity of the intestinal barrier, suggesting that the gut-liver axis plays a crucial role in the development of this disease [30]. N1487-7-HK has been suggested to promote IL-10 production in intestinal epithelial cells and macrophages. [19] Macrophages are present throughout the body and are known to exist in two types: pro-inflammatory M1 type (M1) and anti-inflammatory M2 type (M2). [31] Orally administered 5-aminosalicylic acid promotes intestinal M1 to M2 polarization and reduces inflammatory responses [32]. Since N1487-7-HK also has been suggested to polarize M1 to M2 [19], orally administered N1487-7 may polarize intestinal M1 to M2, thereby inducing the release of IL-10

and attenuating the inflammatory response. These results suggest that orally administered N1487-7-HK promotes the release of IL-10 from intestinal epithelial cells and intestinal M2 cells, suppresses inflammation in the liver, and promotes LDL-c and TC metabolism.

The Japanese Committee for Clinical Laboratory Standards currently defines the reference interval for serum TC levels as 142 to 248 mg/dL. [33] Elevated TC levels are associated with a risk of arteriosclerosis and myocardial infarction. [34] The median serum TC level observed in the present study was 228.5 mg/dL, indicating that many subjects had TC levels near the upper limit of this reference interval. In a subgroup analysis of subjects with serum TC levels below the median (228.5 mg/dL), Group A demonstrated significantly higher serum IL-10 levels than did Group P at Week 12. Group P at Week 6 showed a significant decrease in serum IL-10 levels compared to pre-dose Week 0. IL-10 is known to ameliorate tissue damage and has been reported to exert anti-inflammatory effects and to promote hepatocyte regeneration in the liver. [35, 36] Our findings suggest that ingestion of N1487-7-HK cells may have an anti-inflammatory effect and may contribute to the attenuation of serum levels of LDL-c and TC levels in individuals with impaired liver function who present with elevated serum LDL-c and TC levels. However, it is possible that these anti-inflammatory effects are less pronounced or absent in individuals with serum TC levels exceeding the upper limit of the reference interval.

This study may have been affected by selection bias. Subjects were recruited from those with high serum levels of liver function markers, not from those with high serum cholesterol levels. Furthermore, serum cholesterol levels were not set as the primary endpoint in present study but instead were included as safety evaluation endpoints. To further validate our findings, future studies will need to be conducted as double-blind trials recruiting subjects with high serum cholesterol levels, and with serum LDL-c and TC levels as primary endpoints.

The functional components of N1487-7-HK cells may include cell membrane elements altered by heat during sterilization, as well as heat shock proteins synthesized instantly during the process. However, these components remain unidentified, necessitating further research [19]. Therefore, we propose that the functional components of N1487-7 are generated through heating and serve to promote the production of anti-inflammatory cytokines in intestinal epithelial cells and macrophages, contributing to a decrease in serum LDL-c and TC levels. Our findings indicate that the consumption of N1487-7-HK may help prevent or improve conditions such as atherosclerosis, myocardial infarction, cerebral infarction, and cholelithiasis.

Regarding the safety of N1487-7-HK cells, no problematic test values or fluctuations were obtained by physical examinations or clinical tests. No adverse events thought to be causally related to the test food were found in the interviews and diaries, and no safety issues were found with this test food. Given the above information, we concluded that foods containing N1487-7-HK cells are safe for consumption within the endpoints established by this study. The effectiveness of N1487-7-HK in improving LDL-c and TC metabolism and relieving bile obstruction was previously unknown, as was its 12-week safety. Therefore, the findings of this study are highly novel. A future double-blind study with serum LDL-c and TC levels as the primary endpoints is planned to verify the reproducibility of the present results.

CONCLUSIONS

In this study, 12 weeks of consumption of N1487-7-HK by healthy males and females aged 20–64 years with elevated serum levels of liver function markers, LDL-c, and TC resulted in significant decreases in serum levels of LDL-c and TC compared to a placebo group. In a subgroup analysis of subjects exhibiting serum TC levels that were

below the median (228.5 mg/dL) at pre-dose Week 0, IL-10 levels at 12 weeks were significantly higher in the N1487-7-HK intake group than in the control group. These results indicated that orally ingested N1487-7-HK has anti-inflammatory properties and is effective in improving LDL-c and TC metabolism and in relieving bile obstruction. Since elevated serum LDL-c and TC levels are an indicator of arteriosclerosis, myocardial infarction, cerebral infarction, and cholelithiasis, we propose that N1487-7-HK might serve as a functional food ingredient that may be useful in preventing and improving these diseases.

Abbreviations: AST, Aspartate aminotransferase/glutamic oxaloacetic transaminase; ALT, alanine aminotransferase/glutamic pyruvate transaminase; γ-GTP, gamma-glutamyl transpeptidase; ALP, alkaline phosphatase; LDL-c, Low Density Lipoprotein cholesterol; *Lpb.*, *Lactiplantibacillus*; N1487-7-HK, heat-killed *Lactiplantibacillus plantarum* N1487-7; IL, Interleukin; Pre-dose Week 0, Tests performed before starting intake; Active food, N1487-7-HK cells-containing food; Placebo food, N1487-7-HK cells-free food; Week 6, Test conducted 6 weeks after starting intake; Week 12, Test conducted 12 weeks after starting intake; MPO, myeloperoxidase; POMS2, profile of mood states 2nd edition; VAS, visual analog scale; FAS, Full Analysis Set; Group A, active food intake group; PPS, Per Protocol Set; Group P, placebo food intake group; xx ± yy, mean (or least squares mean value) ± standard deviation (or standard error); TC, Total cholesterol.

Funding: The present study was funded by Sanwa Shurui Co., Ltd.

Competing Interests: Uehara and Hokazono are employees of Sanwa Shurui Co., Ltd. Miura is a doctor at Miura Clinic. Sanwa Shurui Co., Ltd. concluded a contract with Oneness Support Co., Ltd. and commissioned this study. Oneness Support Co., Ltd. concluded contracts with Miura Clinic, Kannonkai Medical Corporation (study site), BML Co., Ltd., Eurofins Genetic Lab Co., Ltd., and Nikken Zaile Co., Ltd. (laboratory) to conduct this study. Remuneration based on these contracts is legitimate remuneration for conducting this study and does not affect the results of this study.

Author Contributions: Conceptualization: E.U. and H.H. Data curation: N.M. Formal analysis: N.M. Funding acquisition: E.U. and H.H. Investigation N.M. Methodology: E.U. and H.H. Project administration: E.U. and H.H. Resources: E.U. Supervision: H.H. Visualization: E.U. Writing-original draft E.U. Writing-review and editing: H.H., and N.M

REFERENCES

1. Trefts E, Gannon M, Wasserman DH: The liver. Curr Biol 2017, 27: R1147–R1151. DOI: https://doi.org/10.1016/j.cub.2017.09.019
2. Pelusi S, Valenti L: Hepatic fat as clinical outcome and therapeutic target for nonalcoholic fatty liver disease. Liver Int 2019, 39: 250–256. DOI: https://doi.org/10.1111/liv.13972
3. Tang SY, Tan JS, Pang XZ, Lee GH: Metabolic dysfunction associated fatty liver disease: the new nomenclature and its impact. World J Gastroenterol 2023, 29: 549–560. DOI: https://doi.org/10.3748/wjg.v29.i3.549
4. Targher G, Byrne CD, Tilg H: MASLD: a systemic metabolic disorder with cardiovascular and malignant complications. Gut 2024, 73: 691–702. DOI: https://doi.org/10.1136/gutjnl-2023-330595
5. Chan WK, Petta S, Noureddin M, Goh GBB, Wong VWS: Diagnosis and non-invasive assessment of MASLD in type 2 diabetes and obesity. Aliment Pharmacol Ther 2024, 59: S23–S40. DOI: https://doi.org/10.1111/apt.17866
6. Harrison SA, Browne SK, Suschak JJ, Tomah S, Gutierrez JA, Yang J, Robertset MS, et al.: Effect of pemvidutide, a GLP-1/glucagon dual receptor agonist, on MASLD: a randomized, double-blind, placebo-controlled study. J Hepatol 2024, 1–11. DOI: https://doi.org/10.1016/j.jhep.2024.07.006
7. Rajendran R, Suman S, Divakaran SJ, Swatikrishna S, Tripathi P, Jain R, Sagar K, et al.: Sesaminol alters phospholipid

metabolism and alleviates obesity-induced NAFLD. FASEB J 2024, 38: e23835.
DOI: https://doi.org/10.1096/fj.202400412RR

8. Antona ME, González PM, Ramos C, Cabrera J, Olano C, Morales C, Zago V, et al.: Curcumin exerts a protective effect against obesity and liver injury induced by an atherogenic diet. Functional Foods in Health and Disease 2021, 11: 673–689. DOI: https://doi.org/10.31989/ffhd.v11i12.862
9. Ikai E, Noborizaka Y, Tsuritani I, Honda R, Ishizaki M, Yamada Y: Serum γ-glutamyl transpeptidase levels and hypertension in non-drinkers: a possible role of fatty liver in the pathogenesis of obesity related hypertension. Obes Res 1993, 1: 425–515.
DOI: https://doi.org/10.1002/j.1550-8528.1993.tb00029.x
10. Kalas MA, Chavez L, Leon M, Taweesedt PT, Surani S: Abnormal liver enzymes: a review for clinicians. World J Hepatol 2021, 13: 1688–1698.
DOI: https://doi.org/10.4254/wjh.v13.i11.1688
11. Tian J, Cai M, Jin S, Chen Q, Xu J, Guo Q, Yan Z, et al.: JianPi-QingHua formula attenuates nonalcoholic fatty liver disease by regulating the AMPK/SIRT1/NF-κB pathway in high-fat-diet-fed C57BL/6 mice. Pharm Biol 2023, 61: 647–656.
DOI: https://doi.org/10.1080/13880209.2023.2188549
12. Johansson HE, Haenni A, Zethelius B: Platelet counts and liver enzymes after bariatric surgery. J Obes 2013, 567984.
DOI: https://doi.org/10.1155/2013/567984
13. Wang Z, Liu Z, Zheng J, Huang L, Jin R, Wang X, Chen D, et al.: The effects of low-dose IL-2 on Th17/Treg cell imbalance in primary biliary cholangitis mouse models. BMC Gastroenterol 2024, 24: 87.
DOI: https://doi.org/10.1186/s12876-024-03176-0
14. Moriles KE, Zubair M, Azer SA: Alanine aminotransferase (ALT) test. StatPearls [Internet] Last update: February 27, 2024.
DOI: https://www.ncbi.nlm.nih.gov/books/NBK559278/
15. Shipovskaya AA, Dudanova OP: Intrahepatic cholestasis in nonalcoholic fatty liver disease. Ter Arkh 2018, 90: 69–74.
DOI: https://doi.org/10.26442/terarkh201890269-74
16. Brenner DJ, Staley JT, Krieg NR: Classification of procaryotic organisms and the concept of bacterial speciation. Bergey's Manual® of Systematic Bacteriology 2001, 1: 27–31.
DOI: https://doi.org/10.1007/978-0-387-21609-6_4
17. Nyotohadi D, Kok T: Potential of multi-strain probiotics extract as an anti-inflammatory agent through inhibition of macrophage migration inhibitory factor activity. Functional Foods in Health and Disease 2023, 13: 1–10.
DOI: https://doi.org/10.31989/ffhd.v13i1.1033
18. Ikehata M, Konnai S, Okagawa T, Abe K, Honma M, Kitamura T, Maekawa N, et al.: In vitro evaluation of *Lactiplantibacillus plantarum* HOKKAIDO strain, effective lactic acid bacteria for calf diarrhea. Front Vet Sci 2023, 10: 1145445.
DOI: https://doi.org/10.3389/fvets.2023.1145445
19. Uehara E, Hokazono H: Heat-killed *Lactiplantibacillus plantarum* N1487-7 induces IL-10 production and enhances skin barrier function. Functional Foods in Health and Disease 2024, 14: 856–870.
DOI: https://doi.org/10.31989/ffhd.v14i11.1483
20. Martirosyan D, Kanya H, Nadale C: Can functional foods reduce the risk of disease? Advancement of functional food definition and steps to create functional food products. Functional Foods in Health and Disease 2021, 11: 213–221.
DOI: https://doi.org/10.31989/ffhd.v11i5.788
21. Martirosyan D, Lampert T, Lee M: A comprehensive review on the role of food bioactive compounds in functional food science. Functional Foods Science 2022, 2: 64–78.
DOI: https://doi.org/10.31989/ffs.v2i3.906
22. Martirosyan D, Stratton S: Quantum and tempus theories of function food science in practice. Functional Foods Science 2023, 3: 55–62.
DOI: https://www.doi.org/10.31989/ffs.v3i5.1122
23. Williams K, Oo T, Martirosyan D: Exploring the effectiveness of lactobacillus probiotics in weight management: A literature review. Functional Foods Science 2023, 3: 45–54.
DOI: https://doi.org/10.31989/ffs.v3i5.1115
24. Ginsberg HN: Lipoprotein physiology. Endocrinol Metab Clin North Am 1998, 27: 503–519.
DOI: https://doi.org/10.1016/S0889-8529(05)70023-2
25. Marin-Alejandre BA, Abete I, Cantero I, Monreal JI, Elorz M, Herrero JI, Benito-Boillos A, et al.: The metabolic and hepatic impact of two personalized dietary strategies in subjects with obesity and nonalcoholic fatty liver disease: the fatty liver in obesity (FLiO) randomized controlled trial. nutrients 2019, 11: 2543.
DOI: https://doi.org/10.3390/nu11102543
26. Bo' CD, Perna S, Allehdan S, Rafique A, Saad S, AlGhareeb F, Rondanelli M, et al.: Does the mediterranean diet have any effect on lipid profile, central obesity and liver enzymes in non-alcoholic fatty liver disease (NAFLD) subjects? A systematic review and meta-analysis of randomized control trials. nutrients. 2023, 9: 2250.
DOI: https://doi.org/10.3390/nu15102250
27. Stalgis-Bilinski KL, Boyages J, Salisbury EL, Dunstan CR,

Henderson SI, Talbot PL: Burning daylight: balancing vitamin D requirements with sensible sun exposure. Med J Aust 2011, 194: 345–348.
DOI: https://doi.org/10.5694/j.1326-5377.2011.tb03003.x

28. Seamans KM, Hill TR, Scully L, Meunier N, Andrillo-Sanchez M, Polito A, Hininger-Favier I, et al.: Vitamin d status and indices of bone turnover in older European adults. Int J Vitam Nutr Res 2011, 81: 277–285.
DOI: https://doi.org/10.1024/0300-9831/a000075
29. Tang Z, Ma J, Zeng Z, Wu S, Hou M: Inhibition of atherosclerotic plaque formation in ApoE-deficient mice by dietary supplementation with *Lactobacillus casei.* Functional Foods in Health and Disease 2014, 4: 147–158.
DOI: https://doi.org/10.31989/ffhd.v4i4.19
30. Benedé-Ubieto R, Cubero FJ, Nevzorova YA: Breaking the barriers: the role of gut homeostasis in Metabolic-Associated Steatotic Liver Disease (MASLD). Gut Microbes 2024, 16: 2331460.
DOI: https://doi.org/10.1080/19490976.2024.2331460
31. Zhang K, Guo J, Yan W, Xu L: Macrophage polarization in inflammatory bowel disease. Cell Commun Signal 2023, 21: 367. DOI: https://doi.org/10.1186/s12964-023-01386-9
32. Park B, Han G, Jin DY, Gil KC, Shin D, Lee J, Park JY, et al.: Mucoadhesive mesalamine prodrug nanoassemblies to target intestinal macrophages for the treatment of inflammatory bowel disease. ACS Nano 2024, 18: 16297–16311.DOI: https://doi.org/10.1021/acsnano.4c05544
33. Ichihara K, Yomamoto Y, Hotta T, Hosogaya S, Miyachi H, Itoh Y, Ishibashi M, et al.: Collaborative derivation of reference intervals for major clinical laboratory tests in Japan. Ann Clin Biochem 2016, 53: 347–356.
DOI: https://doi.org/10.1177/0004563215608875
34. Zhang G, Jiang W, He F, Fu J, Xu X, Luo X, Cao Z: LDL-C and TC mediate the risk of PNPLA3 inhibition on cardiovascular diseases. J Clin Endocrinol Metab 2024, dgae264.
DOI: https://doi.org/10.1210/clinem/dgae264
35. Hammerich L, Tacke F: Interleukins in chronic liver disease: lessons learned from experimental mouse models. Clin Exp Gastroenterol 2014, 7: 297–306.
DOI: https://doi.org/10.2147/CEG.S43737
36. Lan L, Chen Y, Sun C, Sun Q, Hu J, Li D: Transplantation of bone marrow-derived hepatocyte stem cells transduced with adenovirus-mediated IL-10 gene reverses liver fibrosis in rats. Transpl Int 2008, 21: 581–592.
DOI: https://doi.org/10.1111/j.1432-2277.2008.00652.x