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Original article

Safety and efficacy of nanoparticulated brown rice germ extract on reduction of body fat mass and improvement of fuel metabolism in both pre-obese and mild obese subjects without excess of visceral fat accumulation

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Abstract

Beneficial effects on obesity and dysmetabolism of a variety of functional ingredients contained in brown rice have attracted academic as well as clinical attention. On the other hand, it is noteworthy that the absorption efficiency of some of the functional ingredients derived from brown rice from the digestive tract is significantly low. Based on these backgrounds, we developed the “nanoparticulated brown rice germ extract” by encapsulating brown rice germ extract containing γ-oryzanol, one of the functional ingredients abundantly and specifically in brown rice (rice bran), in nanoparticles, and conducted safety and efficacy tests in humans. The present study was performed appropriately after the approval from the ethics committee of Hokkaido Information University. Japanese men and women whose BMI is 23 kg/m^2 or more and less than 25 kg/m^2, or 25 kg/m^2 or more and less than 30 kg/m^2, and whose visceral fat area is less than 100 cm^2 when measured by bioelectric-impedance methods were recruited for the study. We conducted a placebo-controlled, randomized, double-blind, parallel-group comparative study targeting approximately 78 people who met the eligible criteria, and examined metabolic improvements when the "nanoparticulated brown rice germ extract" tablets were ingested once-a-day continuously for 12 weeks. Although a decreasing trend was observed in the abdominal visceral fat area, which is a primary endpoint, there were no significant differences among the ingestion groups of test food (low dose group: 5 mg, and medium-dose group: 10 mg). Among the secondary endpoints, plasma LDL-cholesterol (LDL-C) level showed a marginally significant decrease in the medium and low dose (5 mg) groups compared to the control group (placebo ingestion) after 8 weeks of ingestion ($p = 0.078$, $p = 0.098$). The plasma lipid ratio (LDL-C/HDL-C) showed a significant decrease in the low dose group compared to the control group after 4 and 8 weeks of ingestion ($p = 0.016$, $p = 0.018$). The plasma non-HDL-C level showed a marginally significant or significant decrease in the medium and low dose groups compared to the control group after 8 weeks of ingestion ($p = 0.099$, $p = 0.031$). Blood level of HbA1c also showed a significant decrease in the medium-dose group compared to the control group after 4, 8, and 12 weeks of ingestion ($p = 0.029$, $p = 0.011$, $p = 0.011$). Furthermore, plasma level of adiponectin, which has potent anti-atherosclerotic actions, showed a marked increase in the high, medium, and low dose groups ($p = 0.012$, $p = 0.039$, $p = 0.017$) after 4 weeks of ingestion compared to the control group, and also showed a marginally significant or significant increase after 8 and 12 weeks of ingestion. During the hospital visit, problematic changes were not observed in blood pressure, pulse rate, general blood tests, liver and kidney functions, and urinalysis, and adverse events suggesting a causal relationship with the test food were not observed. This clinical intervention study confirmed the safety of ingestion of the “nanoparticulated brown rice germ extract”, which was also proven to improve the atherogenic lipid profile, reduce HbA1c level, and increase plasma adiponectin concentration. Results obtained tempt us to expect that nanoparticulated brown rice germ extract is well eligible for practical use.

***KEY WORDS*:** brown rice, γ-oryzanol, nanoparticulated brown rice germ extract, obesity disease, metabolically-beneficial impact, adiponectin

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Introduction

Beneficial effects on obesity and dysmetabolism of a variety of functional ingredients contained in brown rice have attracted academic as well as clinical attention [1]. For the past several years, we have focused on the mechanism of action of γ-oryzanol, one of the functional ingredients present abundantly and specifically in brown rice among a wide variety of grains, and have elucidated that γ-oryzanol reduces the dependence on animal fat by acting on the hypothalamus and reward system in the brain [2, 3], improves glucose metabolism by working on the pancreatic endocrine cells such as α and β cells to increase glucose-responsive insulin secretion [4, 5], and further, improves the intestinal flora imbalance associated with obesity in mice [6].

It should be noted that, due to the extreme lipid-soluble properties, the absorption efficiency of various kinds of functional ingredients in brown rice from the digestive tract is significantly low [1, 7]. In this context, we developed a new material in which γ-oryzanol is encapsulated in PLGA (poly (DL-lactide-co-glycolide)) nanoparticles, and reported that there is a remarkable improvement in glucose, and lipid metabolism is observed at a dose as low as approximately 1/100th of the commonly administered γ-oryzanol when the material was orally administered to genetically obese diabetic model, ob/ob mice [6]. If similar effects could be reproduced in humans, we expected that it could be developed as an excellent dietary supplement that would help prevent lifestyle-related metabolic diseases and progression of obesity.

Based on this background, in the present study, we developed the "nanoparticulated brown rice germ extract," by encapsulating brown rice germ extract containing highly amounts of γ-oryzanol in nanoparticles, and conducted clinical trials for a placebo-controlled randomized, double-blind, parallel-group comparative study to validate the safety and efficacy in improving metabolism in humans.

Subjects and Methods

Summary of the Test Food and Placebo Food

Based on our previous studies in mice [6], we developed a test food in which brown rice germ extract, which is an active ingredient, was encapsulated in nanoparticles to improve the absorption rate in the digestive tract. The nanoparticles used in the present study are manufactured as "edible nanoparticles," and the surface structure is made of HPC (hydroxypropyl cellulose), which is used for food worldwide. Brown rice germ extract, which is an active ingredient, is encapsulated inside the nanoparticles, and rice bran oil, which has long been used as food by the Japanese, is used as a raw material for the brown rice germ extract.

The safety of the nanoparticulated brown rice germ extract was confirmed with animal tests (single-dose oral toxicity test, reverse mutation test, chromosomal aberration test, micronucleus test, and repeated dose oral toxicity test, respectively). Quality was maintained by manufacturing in a factory with a well-established quality and hygiene management system, and safety for its use as food was sufficiently ensured with the pre-shipping inspection. The placebo food used in the present study was obtained by mixing foods and food additives conforming to the standards of the Food Sanitation Act, which are widely distributed and sold, and have been extensively used as raw material for food. It was also manufactured in a factory with a well-established quality and hygiene management system, and safety for its use as food was sufficiently ensured with the pre-shipping inspection.

Basic Information on the Test Food and Placebo Food

Three doses of nanoparticulated brown rice germ extract (tablets), high-dose (containing 20 mg), medium-dose (containing 10 mg), and low-dose (5 mg) were prepared, and when examining the efficacy, such as the reduction of body fat mass and improvement in dysmetabolism, the study was conducted by allocating one of the three doses to each of the subject groups.

Hydroxypropyl cellulose, brown rice germ extract, maltitol, and sodium bicarbonate are used as raw materials for the nanoparticles; maltitol, sodium bicarbonate, starch, crystalline cellulose, and citric acid are used for the tablet excipients, and highly dispersed silicon dioxide and sucrose fatty acid esters are used for the tablet lubricants. None of the raw materials correspond to food allergens that require labeling, or for which labeling is recommended.

In the case of high dose, properties of nanoparticulated brown rice germ extract (tablets) were: 8 mm, packed in aluminum pouches (2 tablets/pouch), containing one tablet of 200 mg x 2 tablets = 400 mg (containing nanoparticulated brown rice germ extract 10 mg/tablet x 2 tablets), and were stored at room temperature avoiding direct sunlight, high temperature, and humidity. The subjects had to ingest 2 tablets once daily in the morning (after breakfast) (in case they forget, then they have to consume the tablets either after lunch or dinner) with cold or hot water. The expiry date was one year from the date of manufacture (Manufactured by Kanehide Bio Co., Ltd., Okinawa, Japan).

Placebo food tablets were: 8 mm, packed in aluminum pouches (2 tablets/pouch), containing one tablet of 200 mg x 2 tablets = 400 mg (placebo tablets x 2 tablets), which were stored at room temperature avoiding direct sunlight, high temperature, and humidity. The raw materials used were maltitol, sodium bicarbonate, starch, crystalline cellulose, and citric acid for the tablet excipient, and highly dispersed silicon dioxide and sucrose fatty acid esters for the tablet lubricant. None of the raw materials correspond to food allergens that require labeling, or for which labeling is recommended. The method of ingestion was the same as for the test food. The expiry date was also one year from the date of manufacture (Manufactured by Kanehide Bio Co., Ltd.).

The testing agency stored the test food appropriately, and after the test ended, the remaining test food was disposed appropriately with the responsibility of the testing agency. The test food (3 doses) and placebo food were visually indistinguishable, and each test food was assigned an allocation identification code, which was printed on the corresponding test food package. The assignment information of the allocation identification codes was stored securely by the allocation manager to prevent disclosure. Delivery of the test food for 12 weeks was couriered to the subjects

before the start date of ingestion. All subjects were notified to return the remaining test food when visiting the laboratory for examination after 4, 8 and 12 weeks of ingestion.

Safety Evaluation on the Excessive Ingestion of Nanoparticulated Brown Rice Germ Extract

For safety evaluation, the subjects were given thrice the highest recommended daily dose (20 mg) of the nanoparticulated brown rice germ extract that was planned to be used for efficacy validation described below, and was to be ingested daily for 4 weeks. An open-label, uncontrolled study was conducted, initially planned with 30 subjects, but finally, the analysis was performed for 24 subjects. The subject profile of the safety confirmation test are shown in ***Table 1***. As the primary endpoint, the types, severity, and frequency of occurrence of adverse effects were tabulated to evaluate the safety of the test food. Type, severity, and frequency of adverse events were evaluated as the secondary endpoint.

Volunteers registered at the Health Information Science Research Center, Hokkaido Information University, who fully understood the significance, contents, and purpose of this test and prior written consent was obtained for participation in the present study were selected as subjects. The following selection criteria were used to recruit the subjects: 1) Japanese men and women between the ages of 20 and 65, and 2) Subjects whose body mass index (BMI) and serum triglyceride level meets any of the criteria (1) to (3); (1) BMI of less than 25.0 kg/m^2 and serum triglyceride level of less than 150 mg/dL, (2) BMI ranging from high-normal to obese class I (23.0 kg/m^2 or more and less than 30.0 kg/m^2) (3) Serum triglyceride level is normal to high (120 mg/dL or more and less than 150 mg/dL), and subjects who did not meet the following exclusion criteria were screened.

Exclusion criteria: 1) Subjects who were under physician's advice, treatment and/or medication for obesity, dyslipidemia or diabetes, 2) Subjects with familial hypercholesterolemia, 3) Subjects with severe cerebrovascular diseases, heart diseases, liver diseases, kidney diseases, gastrointestinal diseases, or infectious diseases requiring notification, 4) Subjects with a history of major gastrointestinal surgery, such as gastrectomy, gastrointestinal suture, or intestinal resection, 5) Subjects with significant abnormalities in blood pressure and blood test, 6) Subjects with severe anemia, 7) Pre- and post-menopausal women with substantial changes in physical condition, 8) Subjects who may be allergic to drugs or foods (especially from the grass family), 9) Subjects who regularly

Table 1. Profile of subjects in safety confirmation test on the excessive ingestion of nanoparticulated brown rice germ extract.

		Control	HD group	p-value	MD group	p-value	LD group	p-value
Sex	Male subject (number of people)	3	3	1.000	4	1.000	4	1.000
	Male subject proportion (%)	15.00	15.00		20.00		20.00	
Age	Number	20	20		20		20	
	Mean (age)	49.9	50.9	0.714	52.7	0.351	51.5	0.584
	SD	10.3	7.4		8.3		7.8	
Stature	Number	20	20		20		20	
	Mean (cm)	158.33	158.47	0.955	157.64	0.801	158.63	0.912
	SD	9.35	6.61		7.76		7.99	
Body weight	Number	20	20		20		20	
	Mean (kg)	61.25	60.73	0.825	60.87	0.876	61.63	0.880
	SD	8.85	5.49		6.20		7.08	
BMI	Number	20	20		20		20	
	Mean (kg/m^2)	24.33	24.16	0.712	24.46	0.788	24.44	0.823
	SD	1.61	1.26		1.43		1.32	
Body fat percentage	Number	20	20		20		20	
	Mean (%)	32.98	33.76	0.653	33.24	0.877	32.88	0.948
	SD	4.80	5.94		5.68		5.30	
Abdominal visceral fat area (Impedance method)	Number	20	20		20		20	
	Mean (cm^2)	87.20	86.85	0.948	90.40	0.630	83.70	0.562
	SD	19.31	13.60		22.30		18.54	
ingestion rate	Number	20	20		20		20	
	Mean (%)	99.23	98.75	0.862	99.46	0.883	96.73	0.201
	SD	1.35	2.90		0.82		14.36	

Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Independent t-tests were performed for anthropometric measurements (stature, weight, BMI, body fat percentage) and abdominal visceral fat area. Fisher's exact test was performed for gender. The Mann-Whitney U test was performed for the ingestion rate. Test food, nanoparticulated brown rice germ extract; BMI, body mass index; SD, standard deviation.

use medicines, health foods, and supplements (including ingredients such as polyphenols such as catechin and quercetin, licorice extracts containing glabridin, chitoglucan, and dietary fibers such as indigestible dextrin) that may affect body weight and body fat mass, 10) Individuals who regularly use medicines, health foods, and supplements that affect plasma lipids (γ-oryzanol, eicosapentaenoic acid [EPA], docosahexaenoic acid [DHA], medium-chain fatty acids, plant sterols, sesamin, turmeric, polyphenols, indigestible dextrin dietary fibers or other ingredients), 11) Subjects who regularly use medicines, health foods, and supplements (including dietary fibers such as indigestible dextrin, and products containing ingredients such as polyphenol) that may affect blood glucose level, 12) Excessive smokers and alcohol users, and subjects with extremely irregular diets, 13) Women who had donated 400 mL blood within 16 weeks or men who had given 400 mL blood within 12 weeks before starting the ingestion. Also, subjects who have given 200 mL blood within 4 weeks or blood components within 2 weeks before the start of ingestion, 14) Women who are pregnant, possibly pregnant, or lactating, 15) Subjects who are currently participating in other clinical trials or have participated within the past 4 weeks, 16) Subjects determined to be ineligible by the principal investigator.

Excessive smokers are defined as those smoking 21 or more cigarettes per day. Alcohol addicts are defined as those who drink 20 g of pure alcohol per day (180 mL of sake, 500 mL of beer, 60 mL of whiskey double, 90 mL of shochu (a traditional Japanese distilled spirit) or 2 glasses of wine (about 200 mL)) more than 6 days a week. After screening, the principal investigator selected 30 individuals who were deemed appropriate to participate in the present study, but ultimately only 27 completed the study.

Evaluation Items in Safety Evaluation

The main evaluation items for safety evaluation are as follows. (1) Interview with physician, (2) Anthropometric measurement (height, weight, body fat percentage, BMI, and visceral fat area by bioelectric impedance [BEI] method), (3) Vital check (blood pressure and pulse rate during hospital visit), (4) Lifestyle questionnaire, (5) Dietary questionnaire, (6) Blood tests (blood collection volume: Approx. 13 mL for blood collection tubes: 3): General blood tests (white blood cell [WBC], red blood cell [RBC], hemoglobin [Hb], hematocrit [Ht], platelet [Plt]), liver functions (aspartate transaminase [AST], Alanine transaminase [ALT], γ-glutamyl transpeptidase [γ-GTP], alkaline phosphatase [ALP], lactate dehydrogenase [LDH]), renal functions (blood urea nitrogen [BUN], creatinine [CRE], uric acid [UA]), lipid metabolism (total cholesterol [TC], low-density-lipoprotein-cholesterol [LDL-C], high-density-lipoprotein-cholesterol [HDL-C], triglyceride [TG]), glucose metabolism (fasting blood sugar (FBS), HbA1c).

Furthermore, on the first day of ingestion, after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion, all items excluding Lifestyle and Dietary questionnaires were implemented among the above items. The waist circumference was measured at the navel level in the standing position with both feet aligned and arms down on both sides. Digital automatic blood pressure monitor HEM-7080IC (manufactured by OMRON Corporation, Kyoto, Japan) was used to measure blood pressure and pulse rate. Body composition analyzer DC-320 (manufactured by Tanita Corporation, Tokyo, Japan) was used to measure body weight, body fat percentage and BMI.

Overview of Efficacy Validation for the Ingestion of Nanoparticulated Brown Rice Germ Extract

Volunteers registered at the Health Information Science Research Center, Hokkaido Information University, who fully understood the significance, contents, and purpose of this test and prior written consent was obtained for participation in this study were targeted for the present study.

During the recruitment, subjects had to report following selection criteria: 1) Japanese men or women between the ages of 20 and 65, 2) BMI of 23 kg/m^2 or more and less than 25 kg/m^2 or 3) BMI of 25 kg/m^2 or more and less than 30 kg/m2, and the visceral fat area, evaluated with the BEI method, is less than 100 cm2, and the following exclusion criteria, that is, 1) Subjects who are under physician's advice, treatment and/or medication for obesity, dyslipidemia or diabetes, 2) Subjects judged to have familial hypercholesterolemia, 3) Subjects with severe cerebrovascular diseases, heart diseases, liver diseases, kidney diseases, gastrointestinal diseases, or infectious diseases requiring notification, 4) Subjects using a pacemaker or defibrillator, 5) Subjects with a history of major gastrointestinal surgery, such as gastrectomy, gastrointestinal suture, or intestinal resection, 6) Subjects with significant abnormalities in blood pressure and blood test, 7) Subjects with severe anemia, 8) Pre- and post-menopausal women with significant changes in physical condition, 9) Subjects who may be allergic to drugs or foods (especially from the grass family), 10) Subjects who regularly use medicines, health foods, and supplements (including ingredients such as polyphenols such as catechin and quercetin, licorice extracts containing glabridin, chitoglucan, and dietary fiber such as indigestible dextrin) that may affect body weight and body fat mass, 11) Subjects who regularly use medicines, health foods, and supplements that may affect plasma lipid profile (γ-oryzanol, EPA, DHA, medium-chain fatty acids, plant sterols, sesamin, turmeric, polyphenols, indigestible dextrin dietary fibers, or other ingredients), 12) Subjects who regularly use medicines, health foods, and supplements (including dietary fiber such as indigestible dextrin, and products containing ingredients such as polyphenol) that may affect plasma glucose level, 13) Excessive smokers and alcohol users, and subjects with extremely irregular diets, 14) Women who had donated 400 mL blood within 16 weeks or men who had donated 400 mL blood within 12 weeks before starting the ingestion. Also, those who donated 200 mL blood within 4 weeks or blood components within 2 weeks before the start of ingestion, 15) Women who are pregnant, possibly pregnant or lactating, 16) Subjects who are currently participating in other clinical trials or have participated within the past 4 weeks, 17) Subjects judged by the principal investigator to be ineligible. Those who were not in conflict with the above 17 items and were judged by the principal investigator to be appropriate for participation in the present study were selected as subjects. The definitions of excessive smokers and alcohol users were the same as in safety evaluation.

Study Design and Evaluation Items for Efficacy Validation

The present study was set as a placebo-controlled randomized, double-blind, parallel-group comparative study. The planned test group was, 1) 20 subjects ingesting the test food (nanoparticulated brown rice germ extract 20 mg) at a high dose (high-dose [HD] group), 2) 20 subjects in the test food medium-dose (10 mg) group (medium-dose [MD] group), 3) 20 subjects in the test food low-dose (5 mg) group (low-dose [LD] group), and 4) Placebo food ingestion group 20 subjects (control group), but the final number of subjects was 78 with 19～20 in each group.

An allocation manager from a third-party refers to the subject list, randomizes the subjects with the stratified permutation block method based on gender, age, BMI, and abdominal visceral fat area (using the BEI method) as stratification factors and assigns the subjects to four groups. Two independent t-tests were performed for the age, physical measurements (height, weight, BMI, and body fat percentage), and abdominal visceral fat area among the 4 groups. Fisher's exact test was performed for gender. When judged at a significance level of 5% per test, a significant difference was not observed between the groups in age, height, weight, BMI, body fat percentage, and visceral fat area. The subject profiles are shown in ***Table 2***. The allocation manager strictly stored the documents or password-protected electronic media related to the allocation in a place that can be locked. After that, the personal information executive notified the subjects of the date, time, and location of the clinical study based on the assignment results.

The test food ingestion period is 12 weeks, with 2 tablets of the test food or placebo food to be ingested once a day after breakfast with water or hot water. The tests were performed for each subject on the screening day, the ingestion start date of test food, and 4, 8, and 12 weeks after ingestion, respectively. A washout period of one week was set before starting the ingestion of the test food. The subjects were asked to record and submit daily changes in physical condition, use of medicines that they do not generally use and ingestion of test food in a diary for 13 weeks from one week before the start of ingestion up to the final examination 12 weeks after ingestion.

The examination items on the screening day were, (1) Interview with physician, (2) Anthropometric measurements (height, weight, body fat percentage, BMI, and waist circumference), (3) Measurement of visceral fat area by BEI method, (4) Vital check (blood pressure and pulse rate during hospital visit), (5) Lifestyle questionnaire, (6) Dietary questionnaire, and (7) Blood tests (blood collection volume: Approx. 13 mL for blood collection tubes: 3): General blood tests (WBC, RBC, Hb, Ht, and Plt), liver function (AST,

Table 2. Profile of the subjects in the effective validation study on the ingestion of nanoparticulated brown rice germ extract.

		Control	HD group	p-value	MD group	p-value	LD group	p-value
Sex	Male subject (number of people)	2	2	1.000	4	0.661	4	0.66
	Male subject proportion (%)	10.53	10.53		20.00		21.05	
Age	Number	19	19		20		19	
	Mean (age)	50.16	51.16	0.738	52.65	0.416	51.79	0.591
	SD	10.52	7.56		8.33		7.85	
Stature	Number	19	19		20		19	
	Mean (cm)	157.53	157.64	0.962	157.64	0.968	158.72	0.669
	SD	8.88	5.63		7.76		8.20	
Body weight	Number	19	19		20		19	
	Mean (kg)	60.59	60.23	0.875	60.87	0.909	61.73	0.660
	SD	8.58	5.15		6.20		7.26	
BMI	Number	19	19		20		19	
	Mean (kg/m^2)	24.32	24.22	0.827	24.46	0.780	24.44	0.807
	SD	1.65	1.27		1.43		1.36	
Body fat percentage	Number	19	19		20		19	
	Mean (%)	33.60	34.49	0.551	33.24	0.822	32.93	0.667
	SD	4.03	5.07		5.68		5.44	
Abdominal visceral fat area (Impedance method)	Number	19	19		20		19	
	Mean (cm^2)	84.84	86.79	0.698	90.40	0.385	84.58	0.964
	SD	16.62	13.97		22.30		18.62	
ingestion rate	Number	19	19		20		19	
	Mean (%)	0.99	0.99	0.817	0.99	0.923	1.00	0.163
	SD	0.01	0.02		0.01		0.00	

Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Independent t-tests were performed for anthropometric measurements (stature, weight, BMI, body fat percentage) and abdominal visceral fat area. Fisher's exact test was performed for gender. The Mann-Whitney U test was performed for the ingestion rate. Test food, nanoparticulated brown rice germ extract; BMI, body mass index; SD, standard deviation.

ALT, γ-GTP, ALP, and LDH), renal functions (BUN, CRE, and UA), lipid metabolism profile (TC, LDL-C, HDL-C, and TG), and glucose metabolism (FBS, HbA1c).

The following were performed on ingestion start date, and after 4, 8, and 12 weeks of ingestion: (1) Interview with physician, (2) Anthropometric measurements (weight, body fat percentage, BMI, and waist circumference), (3) Measurement of visceral fat area by BEI method (not performed after 4 weeks) (4) Vital check (blood pressure and pulse rate during hospital visit), (5) Personality questionnaire (Japanese version TIPI-J) (only on ingestion start date), and (6) Blood tests (blood collection volume: Approx. 13 mL, blood collection tubes: 3): General blood tests (WBC, RBC, Hb, Ht, and Plt), liver function (AST, ALT, γ-GTP, ALP, and LDH), renal functions (BUN, CRE, and UA), lipid metabolism profile (TC, LDL-C, HDL-C, TG, LDL-C/HDL-C, and non-HDL), glucose metabolism (FBS, HbA1c, insulin, and homeostasis model assessment for insulin resistance [HOMA-IR]), adipokines (blood levels of adiponectin and leptin), urinalysis (volume of urine collected: approx. 10 mL, urine sample tube 1 No.), and urinalysis (pH, protein, sugar, urobilinogen, bilirubin, ketone bodies, and occult blood).

Statistical Analysis

The result was indicated as the mean value ± standard deviation. For the safety test, 2 independent t-tests were performed for age and physical measurements (height, weight, BMI, and body fat percentage). Fisher's exact test was performed for gender. For the efficacy test, 2 independent t-tests were performed for the age, physical measurements (height, weight, BMI, and body fat percentage), and abdominal visceral fat area, and a Fisher's exact test was performed for gender. A Mann-Whitney U test was performed for comparison between groups. The abdominal visceral fat area was evaluated by performing 2 independent t-tests for the change in the amount from before the start of ingestion to that at each time point of evaluation. The significance level in the statistical hypothesis test was 5% with the two-sided test.

Ethical Guidelines

- Benefits and measures to minimize the disadvantage and risks of a subject during safety evaluation

The benefits to the subjects are that regular blood tests will be conducted and results can be immediately obtained from the physicians. The disadvantages include, test food has to be ingested daily, time and effort are required to make entries in the diary, regular visits to the hospital cause time constraints, and blood collections and various tests cause a burden. As measures to minimize risks, safety was further ensured with the exclusion criteria, and we worked in cooperation with the medical institutions conducting the tests to prevent health hazards during the onset of adverse events. For ensuring that subjects obtain medical examination when required due to poor physical conditions, the name of the medical institution and name of the concerned physician will be communicated to the consultation office, and a system is established so that the principal investigator can promptly take appropriate measures when necessary.

- Protection of subject rights and acquisition of informed consent for safety evaluation

The present study was conducted after the deliberation by the Ethics Committee of Hokkaido Information University, and before starting the study, the following content was explained to the subjects, and informed consent for participation in the present study was obtained in writing. That is, 1) Name of the study, Ethics Committee has reviewed and the Committee President has approved the study, 2) Name, affiliation and contact information of the principal investigator, consultation office for the tests and medical institutions conducting the tests, 3) The purpose and method of the study, 4) Expected benefits and potential disadvantages of participating in the study, 5) Subjects can withdraw from the study at any time, 6) Subjects will not be adversely affected by not participating in or withdrawing from the study, 7) Methods for anonymizing, storing, and disposing of samples and information to maintain confidentiality of the subjects, 8) Matters concerning compensation for health hazards requiring medical treatment, 9) Matters relating to the cost of the study, 10) Matters concerning the research funding and conflicts of interest, and 11) Methods with which the study information will be disclosed. We respected the protection of the privacy of the subjects and stored the information with the strict compliance.

- Handling of data and storage of records during safety evaluation

When handling data of subjects and materials obtained during the present study, the personal information executive will carefully anonymize the information to protect the privacy of the subjects so that just the information cannot be used to identify individuals. The information was anonymized by deleting personal information that could identify individual subjects and assigning an anonymous number not related to personal information. The tables relating the personal information and the anonymous number was properly managed and kept at a location that can be locked under the direction of the personal information manager. When information is stored as electronic data, a password is set for the electronic media and kept at a location that can be locked.

The data and materials will be retained for 5 years after the completion of the study or 3 years after the final publication of results, whichever is later. Even when the materials are disposed of, personal information will be strictly anonymized to prevent disclosure of personal information. Subject data obtained in this test was anonymized to protect personal information before using the data for comparison and correlation analysis with other studies, publication in papers, presenting at academic conferences, management of registered volunteers, and quality improvement of subsequent studies.

- Ethical review

The present study was conducted in compliance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects (February 28, 2017, partially revised by the Ministry of Education, Culture, Sports, Science, and Technology and Ministry of Health, Labour, and Welfare) and the Helsinki Declaration (amended at the WMA General Assembly in Fortaleza held in October 2013).

We obtained the approval for the above ethics review to carry out the study.

Safety Evaluation for Excessive Ingestion of Nanoparticulated Brown Rice Germ Extract

Ethics Review Number: 2017-22 (approved on October 30, 2017), 2017-23 (change approved on November 24, 2017) in the ethics committee of Hokkaido Information University.

Dose Determination Study of Nanoparticulated Brown Rice Germ Extract on Body Fat Reduction by Daily Ingestion

Ethics Review Number: 2017-18 (approved on September 25, 2017), 2017-19 (approved on October 27, 2017).

- Pre-registration

The UMIN test ID for the excessive ingestion of the test food is UMIN000030323, Receipt No.: R000034628, Test name: Safety Evaluation for Excessive Ingestion of Nanoparticulated Brown Rice Germ Extract, Date of disclosure of the study information: December 8, 2017; modified on July 20, 2018. The UMIN test ID for efficacy validation is UMIN000029511, Receipt No.: R000033721, Test name: Dose Determination Study of Nanoparticulated Brown Rice Germ Extract on Body Fat Reduction by Daily Ingestion, Date of disclosure of the study information: October 13, 2017; modified on July 20, 2018.

Results

Safety Evaluation on the Excessive Ingestion of Nanoparticulated Brown Rice Germ Extract

An open-label, uncontrolled study was conducted, initially planned with 30 subjects, but finally, the analysis was performed for 27 subjects. As the primary endpoint, the types, severity, and frequency of occurrence of adverse effects were tabulated to evaluate the safety of the test food. The result of tabulating and evaluating the types, severity, and frequency of occurrence of adverse events as secondary endpoint, showed that no noticeable adverse effects or clinically problematic adverse events occurred for any of the study items described under the section "Subjects and Method," even if thrice the recommended daily intake for nanoparticulated brown rice germ extract is continued to be ingested for 4 weeks. Of the initial 30 subjects, 2 subjects were excluded because they were unable to ingest the test food properly, while one subject accidentally happened to develop the symptoms of the influenza virus during the study period; thus, the study was stopped for the subject. Though the subject was excluded, it was determined that there was no causal relationship with the ingestion of the test food. From the above, it was confirmed that there was no problem in the safety of this test food within the framework of the evaluation system of daily ingestion for at least 4 weeks.

Regarding the body composition, a significant decrease in body weight, body fat mass, and BMI was observed at every point in time compared to the day the ingestion was started. In particular, the body fat percentage showed a significant decrease after 4 weeks of ingestion and 2 weeks after the end of ingestion ($p = 0.014$, $p = 0.017$, respectively). Of note, as a result of a stratified analysis of body fat mass, the decrease in body fat mass was more noticeable in the subject group with higher BMI. In detail, the subject group with normal BMI and TG showed a marginally significant decrease 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.084$). Meanwhile, the subject group ranging from high-normal BMI to obese class I showed a marginally significant or significant decrease after 4 weeks of ingestion and 2 weeks after the end of ingestion compared to before starting ingestion ($p = 0.057$, $p = 0.022$, respectively).

Regarding blood lipids profile, both TC and LDL-C levels showed a significant decrease at every point in time compared to the day ingestion was started. TC values showed a significant reduction after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting ingestion ($p = 0.001$, $p = 0.001$, $p = 0.037$, respectively). Stratified analysis also showed a significant decrease after 2 weeks of ingestion compared to before starting ingestion for the subject group with normal BMI and TG ($p = 0.029$). Furthermore, the subject group ranging from high-normal BMI to obese class I showed a significant or marginally significant decrease after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.033$, $p = 0.001$, $p = 0.098$, respectively). LDL-C values showed a significant decrease after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.003$, $p = 0.009$, $p = 0.002$). Stratified analysis showed a marginally significant decrease after 2 weeks of ingestion compared to before starting ingestion for the subject group with normal BMI and TG ($p = 0.082$). Notably, the subject group ranging from high-normal BMI to obese class I showed a significant decrease after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.029$, $p = 0.004$, $p = 0.011$, respectively). On the other hand, no significant differences or marginal significant differences were observed at any time in plasma HDL-C and TG levels.

Importantly, fasting blood glucose level in the early morning showed a significant decrease after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting ingestion ($p = 0.001$, $p = 0.026$, $p = 0.001$, respectively). In the stratified analysis, the subject group ranging from high-normal BMI to obese class I showed a significant decrease after 2 and 4 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.001$, $p = 0.010$, $p = 0.004$, respectively). The subject high-normal TG group showed a significant decrease after 2 weeks of ingestion, and 2 weeks after the end of ingestion compared to before starting the ingestion ($p = 0.029$, $p = 0.029$). However, the blood HbA1c level unexpectedly showed a significant increase after 2 weeks of ingestion and 2 weeks after the end of the ingestion compared to before starting the ingestion ($p < 0.001$, $p < 0.001$, respectively).

In humans and rodents, it has been reported that γ-oryzanol contained in brown rice germ extract exemplifies a line of metabolically-beneficial impact such as anti-obesity, lipid-lowering, and glucose-lowering effects [1-6]. In agreement with these notions, the present "safety evaluation" study also suggests the test food may exert anti-obesity, lipid-lowering, and glucose-lowering effects, especially in subjects with high BMI.

- Efficacy Validation for the Ingestion of Nanoparticulated Brown Rice Germ Extract

The abdominal visceral fat area before the start of ingestion, after 8 and 12 weeks of ingestion was 82.1 ± 18.0 kg, 89.1 ± 24.1 kg, 82.2 ± 23.1 kg in the control group, and 86.8 ± 14.8 kg, 90.9 ± 18.4 kg in the HD group, 90.1 ± 15.3 kg, 90.4 ± 23.3 kg, 89.4 ± 22.6 kg, 87.3 ± 23.0 kg in the MD group, 86.0 ± 24.6 kg, 90.1 ± 22.9 kg, 84.2 ± 19.7 kg in the LD group. The amount of change from before the start of ingestion to after 4 weeks of ingestion, the amount of change from before the start of ingestion to after 8 weeks of ingestion, the amount of change from before the start of ingestion to after 12 weeks of ingestion, respectively, were 6.6 ± 16.0 kg and 0.1 ± 16.1 kg in the control group, 4.5 ± 18.3 kg and 3.3 ± 17.6 kg in the HD group, 0.0 ± 19.8 kg and −3.1 ± 10.9 kg in the MD group, and 4.1 ± 14.4 kg and −1.8 ± 12.1 kg in the LD group.

As a result of the statistical analysis, a significant increase is observed in the percentage of body fat of the control group after 8 weeks of ingestion as compared to the day of the screening ($p < 0.001$). On the other hand, HD group (1), MD group (2), and LD group (3) somehow showed slight but a significant increase after 8 and 12 weeks of ingestion as compared to the day of screening ((1): $p = 0.026$, $p = 0.007$, (2): $p = 0.057$, $p = 0.054$, (3) $p = 0.021$, $p = 0.050$). Consequently, the abdominal visceral fat area, which is the primary endpoint, showed a slight decrease in the test food intake group compared to the control group, but dose-dependence or a statistically significant difference was not observed (***Fig. 1***). Significant differences and marginally significant differences were not observed among groups at any time.

Regarding the secondary endpoints, such as body weight and BMI, significant differences, marginally significant differences, and dose-dependence were not observed among groups at any time (***Fig. 2***). After 8 weeks of ingestion, the plasma LDL-C level showed a marginally significant decrease in the MD and LD groups compared to the control group ($p = 0.078$, $p = 0.098$), but a significant difference and dose-dependency of the effects were not observed. Significant differences and marginally significant differences were not observed among groups at any time for HDL-C. The LDL-C/HDL-C ratio showed a significant decrease in the LD group compared to the control group after 4 and 8 weeks of ingestion ($p = 0.016$, $p = 0.018$, respectively). After 8 weeks of ingestion, the serum non-HDL-C level, which is an indicator of atherogenic lipids, showed a trend of decrease or a significant decrease in the MD and LD groups compared to the control group ($p = 0.099$, $p = 0.031$, respectively).

Significant differences and marginally significant differences were not observed among the groups at any point of time in the fasting blood glucose level, and dose-dependency was also not observed (***Fig. 3-a***). In contrast, blood HbA1c levels were significantly reduced in the MD group compared to the control group after 4, 8, and 12 weeks of ingestion ($p = 0.029$, $p = 0.011$, $p = 0.011$, respectively, ***Fig. 3-b***).

Noticeably, plasma concentration of adiponectin showed a significant increase in the HD, MD, and LD groups compared to the control group after 4 weeks of ingestion ($p = 0.012$, $p = 0.039$, $p = 0.017$, respectively, ***Fig. 4-a***). The HD group showed a significant increase compared to the control group ($p = 0.028$, ***Fig. 4-a***) after 8 weeks of ingestion and HD and LD groups showed a marginally significant increase or significant increase compared to the control group ($p = 0.064$, $p = 0.021$, respectively, ***Fig. 4-c***) after 12 weeks of ingestion.

Body composition (weight, BMI, body fat percentage), vital signs (blood pressure and pulse rate during hospital visit), general blood testing (WBC, RBC, Hb, Ht, and Plt), liver functions (AST, ALT, γ-GTP, ALP, and LDH), renal functions (BUN, CRE, and UA) and urinalyses (protein, sugar, urobilinogen, bilirubin, ketone bodies, and occult blood) did not show clinically problematic findings and adverse events having a causal relationship with the test food were not observed at all. From a line of results above, it was concluded that there were no particular safety issues in the present "efficacy validation" study.

Discussion

In Japan, the proportion of obese people due to overnutrition and lack of physical exercise has increased in recent years. According to the reports in the 2015 National Health and Nutrition Survey by the Ministry of Health, Labor, and Welfare, 29.5% of men and 19.2% of women have been reported to fall in the obese category with a BMI of 25 kg/m^2 or more [8]. Because obesity is closely associated with the development of a series of lifestyle-related diseases such as type 2 diabetes, dyslipidemia, hypertension, a variety of arteriosclerotic diseases, and non-alcoholic fatty liver disease (NAFLD), prevention and amelioration of obesity disease are important challenges worldwide.

Based on this background, there is a growing interest in foods that can be ingested daily and easily as well as are effective in preventing and improving obesity disease without causing adverse effects. Of note, a wide variety of impact on metabolic of food ingredients such as brown rice germ extract (γ-oryzanol) have been intensively reported in recent years [1, 7]. Brown rice is unpolished rice that is rich in functional ingredients such as vitamins, minerals, dietary fibers, and γ-oryzanol, compared to white rice [1]. In particular, γ-oryzanol, which is specifically and abundantly in brown rice, is an ester mixture of ferulic acid and several kinds of phytosterols. In addition to its previously known beneficial effects for dyslipidemia and dysregulation of autonomic nervous system [1], our recent research in mice has elucidated that γ-oryzanol reduces the dependence on animal fat by acting on the hypothalamus and reward system in the brain [2, 3], improves glucose metabolism by working on pancreatic endocrine cells including α and β cells to increase glucose-responsive insulin secretion [4, 5], and further, improves the imbalance of gut microbiota associated with chronic feeding of high fat diet and obesity [6]. Furthermore, in a crossover interventional study targeting 30- to 60-year-old men with metabolic syndrome living in Okinawa, the staple food (white rice) was replaced by brown rice with the same amount of calories in one meal daily without changing the side dish menu, and consequently, post-prandial hyperglycemia and obesity-associated metabolic derangement were considerably improved (Brown Rice and Visceral Fat Obesity in Okinawa (BRAVO Study) [7]. However,

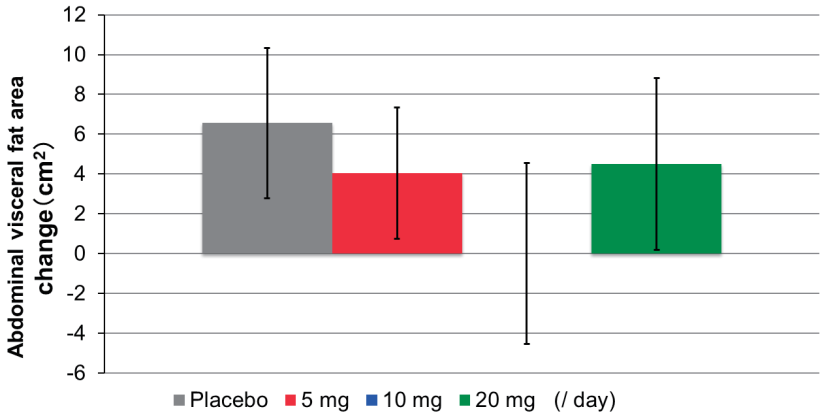

Fig. 1. **Changes in abdominal visceral fat area 8 weeks after the ingestion of nanoparticulated brown rice germ extract in each dose group.**

Results are expressed as mean ± SE. Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Test food, nanoparticulated brown rice germ extract; SE, standard error.

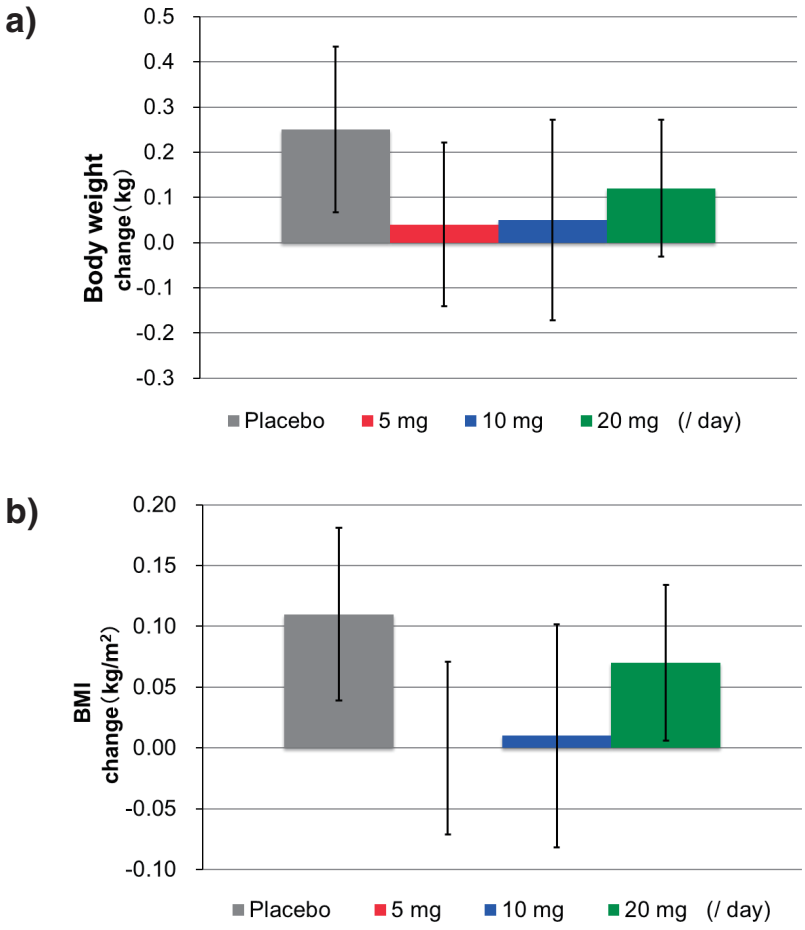

Fig. 2. **Changes in body weight and BMI 4 weeks after the ingestion of nanoparticulated brown rice germ extract in each dose group.**

a) Body weight. **b)** BMI. Results are expressed as mean ± SE. Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Test food, nanoparticulated brown rice germ extract; BMI, body mass index; SE, standard error.

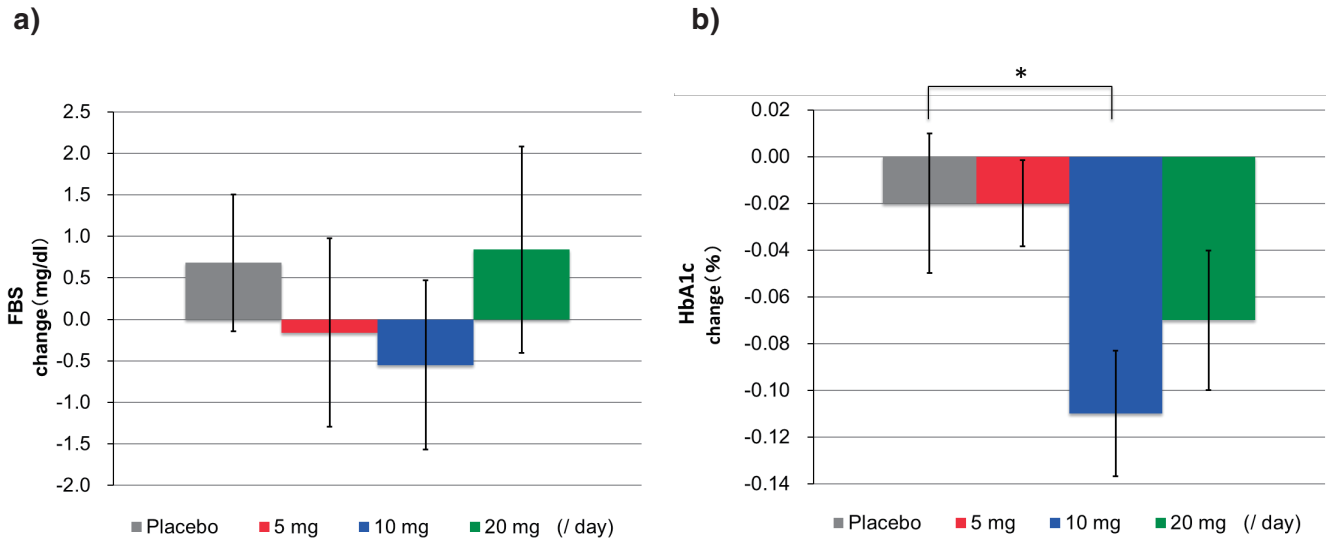

Fig. 3. **Changes in FBS level and HbA1c level 4 weeks after the ingestion of nanoparticulated brown rice germ extract in each dose group.**

a) FBS. **b)** HbA1c. Results are expressed as mean ± SE. *p < 0.05, by t-test. Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Test food, nanoparticulated brown rice germ extract; FBS, fasting blood glucose; SE, standard error.

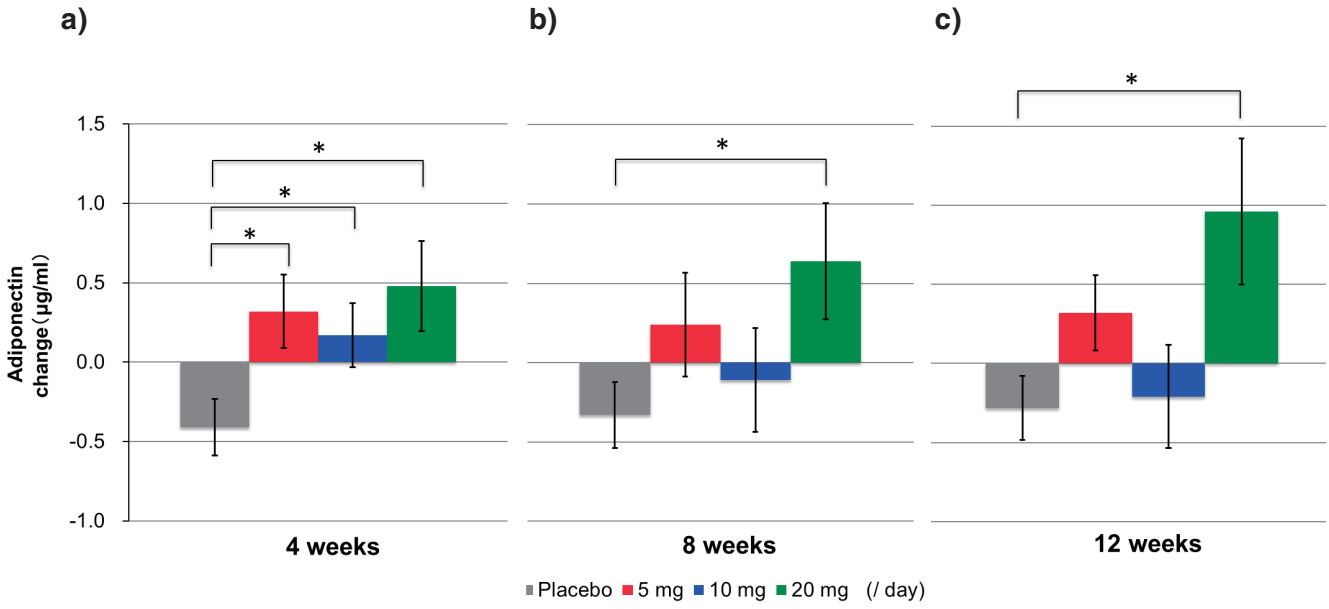

Fig. 4. **Adiponectin changes after the ingestion of nanoparticulated brown rice germ in each dose group.**

a) 4 weeks. **b)** 8 weeks. **c)** 12 weeks. Results are expressed as mean ± SE. *p < 0.05, by t test. Control (n = 20): Ingestion group of placebo food. HD group (n = 20): High dose of test food (20 mg). MD group (n = 20): Medium dose of test food (10 mg). LD group (n = 20): Low dose of test food (5 mg). Test food, nanoparticulated brown rice germ extract; SE, standard error.

there has long been concern that the absorption efficiency of extremely-lipophilic γ-oryzanol is markedly diminished [1,7]. It is therefore considered essential to devise innovative ways to enable γ-oryzanol to be more efficiently absorbed by intestinal tract in human clinics.

Based on this background, we developed a new material in which γ-oryzanol is encapsulated in PLGA nanoparticles, and reported that there was a remarkable improvement in dysmetabolism of glucose and lipids at a dose as low as about 1/100th of the commonly administered dose of γ-oryzanol when the material was orally administered to genetically obese diabetic ob/ob mice [6]. In this context, aiming for the application in humans, we developed the brand-new "nanoparticulated brown rice germ extract" in which brown rice germ extract is encapsulated in nanoparticles. From the results of oral administration of nanoparticulated brown rice germ extract in mice, it was elucidated that the absorption rate was exaggerated by approximately 5 times compared to non-nanoparticulated brown rice germ extract. If similar effects would be reproduced in humans, we expect that it could be developed as an excellent dietary supplement that would help prevent lifestyle-related metabolic diseases.

The safety evaluation of the present study showed that no noticeable adverse effects or clinically problematic adverse events occurred for any of the study items described under the section **"Subjects and Method,"** even when thrice the recommended daily intake for nanoparticulated brown rice germ extract is continued to be ingested for 4 weeks. It should be noted that the nanoparticles used in the present study are manufactured as "edible nanoparticles", and the surface structure is made of HPC (hydroxypropyl cellulose), which is widely used for food. On the other hand, the PLGA nanoparticles used in our mouse experiments have not been approved to be used as food additives for humans, and in the present human trial, this point was technically improved, and the present study was conducted with utmost attention to safety.

As described in the section **"Results"**, all the adverse events in clinical findings were mild, and none of the cases were clinically problematic, and the causal relationship with the test food was determined to be negative as the cases recovered or became less severe during the ingestion period of the test food. The adverse events (abnormal fluctuations) in the laboratory evidence were considered to be within the physiological range of fluctuations in daily life, and the causal relationship was determined to be negative. From the notion above, it was confirmed that there was no particular problem in the safety of this test food within the framework of the evaluation system of daily ingestion for at least 4 weeks.

The efficacy validation test of the ingestion of the nanoparticulated brown rice germ extract, showed a slight decrease in the abdominal visceral fat area, which is the primary endpoint, in the test food group compared to the control group, but regrettably, dose-dependency of the effects or statistically-significant difference were not observed (***Fig. 1***). Among the secondary endpoints of body weight and BMI, significant differences, marginally significant differences, and dose-dependency were not also observed among groups at any time (***Fig. 2***). The plasma LDL-C level showed a marginally significant decrease in the test food group compared to the control group, however, no significant difference and dose-dependency were observed.

On the other hand, the LDL-C/HDL-C ratio, a clinical marker for atherogenic lipid profile, showed a significant decrease in the LD group compared to the control group. In the same way, after 8 weeks of ingestion, the plasma non-HDL-C level, which is also an indicator for atherosclerotic lipids, showed a trend to decrease or a significant decrease in the MD and HD groups compared to the control group.

Significant differences and marginally significant differences were not observed among the groups at any point in time in the fasting blood glucose level, and dose-dependency was also not observed. On the other hand, the blood HbA1c level after 4, 8, and 12 weeks of ingestion showed a significant decrease in the MD group compared to the control group. Notably, plasma adiponectin concentration showed a significant increase in the HD, MD, and LD groups compared to the control group after 4 weeks of ingestion. Adiponectin, a potent anti-arteriosclerotic humoral mediator, is a physiologically active substance and specifically secreted from adipocyte [9]. Since plasma adiponectin concentration is well known to decrease sensitively by reflecting the dysfunction and accumulation of visceral fat [9], it is reasonable to speculate that an apparent increase in the plasma adiponectin concentration by ingestion of the nanoparticulated brown rice germ extract may indicate an improvement in visceral fat function before the actual decrease in the visceral fat mass.

During the hospital visit by the subjects, problematic changes were not observed in blood pressure and pulse rate, general blood tests, liver and kidney functions, and urinalyses, and no adverse events having a causal relationship with the test food were observed. From these observations, the safety of daily ingestion of the "nanoparticulated brown rice germ extract" for 12 weeks was confirmed in the present study. Functionally, results obtained tempt us to expect that the extract may contribute, at least partly, to prevent the occurrence or progression of arteriosclerotic and metabolic diseases by improving the atherogenic lipid profile, reducing the HbA1c levels and increasing the plasma adiponectin concentration. However, a clear correlation between the dose and effect was not necessarily observed in the present study. In this context, the determination of an appropriate dose remains to be a challenge for future studies. The reason for such ambiguities of the results may also be that the number of subjects for each dose was few at 19～20, due to which a statistically significant difference could not be detected, and further, the 12-week study period may have been too short to represent a significant improvement in various parameters for subjects without apparent metabolic or arteriosclerotic diseases. Since beneficial effects for obesity, dyslipidemia, and hyperglycemia were pronounced in subjects having higher BMI in the safety evaluation study in which as much as thrice the middle dose (10 mg) used during efficacy validation was ingested daily for 4 weeks, detailed examination of the adequate ingestion amount by clinical studies is warranted in the next step.

Based on results of our basic and clinical research, as well as those from other studies worldwide, brown rice can be expected not only to reduce body fat mass and ameliorate fuel metabolism but to improve cognitive dysfunction, a series of addictive behaviors and to exert anti-aging impact [10]. We named the nanoparticulated brown rice germ extract

used in the present study as Brown Rice Foodicle® (Foodicle is a combination of Food and Particle, which is the registered trademark of Sentan Pharma Co., Ltd). We are currently developing materials for practical use that can bring out a promising potential for a variety of health promotion of brown rice.

Conclusion

This clinical intervention study confirmed the safety of the ingestion of the "nanoparticulated brown rice germ extract," which was also proven to improve the atherogenic lipid profile, reduce HbA1c level, and increase plasma adiponectin concentration. Results obtained tempt us to expect that nanoparticulated brown rice germ extract is well eligible for practical use.

Conflict of Interest

This research received support from Sentan Pharma Inc.

Acknowledgement

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