A placebo-controlled, parallel group, double-blind clinical study of the effectiveness of Sparassis crispa (LB-Scr) for treating patients with type 2 diabetes

Overview Report

Laborg Co., Ltd

Medical Fusion Co., Ltd.

Adaptgen Pharmaceutical Co., Ltd.

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1. Summary

The fungus Sparassis crispa (LB-Scr), also known as cauliflower mushroom or Hanabiratake, is reported to decrease blood sugar and total blood cholesterol levels, and to increase the blood concentration of total blood triglycerides. Another plant, the succulent Echevaria grauca B2, is a functional food ingredient that is thought to inhibit glucose absorption in the small intestine and to block elevation of blood sugar levels. The results of experiments using a rat model indicated that these functional food ingredients inhibit the elevation of blood sugar levels and show no toxicity or abnormal animal growth. These foods are expected to have protective and palliative effects against diabetes without causing adverse reactions.

In this explosive clinical study, we conducted a randomized, placebo-controlled, parallel group, double-blind clinical study design for evaluating the beneficial effects of ingestion of a test diet containing LB-Scr and E. grauca in normalizing elevated patient blood sugar levels and other palliative effects in patients with type 2 diabetes.

The test diet ingestion group (test diet group) showed a significantly decreased HbA1c concentration, the study primary endpoint, at day 168 (visit 6) compared with their baseline starting HbA1c concentration on day 0. In addition, no causal relationships were confirmed between ingestion of the test diet and any adverse events that developed during the 168-day study, and no interactions between the test diet and other medications taken by any subject during the study period were observed.

Based on these results, it was clear that ingestion of the test diet is safe in humans and free of any adverse reactions. The test materials evaluated in this study are an effective dietary supplement and are expected to have both protective and palliative effects against type 2 diabetes.

2. Background/Introduction

The number of diabetic patients is rapidly increasing in Japan, in accordance with lifestyle and social environmental changes. According to the 2008 “Patient Survey” from the Ministry of Health, Labor and Welfare (MHLW), which is published every 3 years, the total number of Japanese patients with diabetes (those who are presumed to receive continuous treatment) was estimated at 2,371,000 (1,312,000 males and 1,061,000 females).1) It is important to note that the total of male and female patient counts may not be equal to the total number of patients, since it is calculated on the basis of an average interval of medical examinations. In addition, according to the “Population Survey Report Outline” also published by MHLW, there were 14,644 deaths attributed to diabetes in 2011 that included 7,738 males and 6,926 females, with diabetes ranked as the 10th highest cause of death among all cause-specific deaths in females.

Diabetes is an incurable disease once it is

established, and it may cause numerous complications such as retinopathy, nephropathy, or neurological disorders unless effective treatment measures are initiated. It may eventually cause loss of vision and necessitate regular kidney dialysis treatments in the end stages of the disease. Diabetes can also initiate and promote cardiovascular diseases such as a stroke and ischemic heart disease. These serious complications put a great economic burden on social and medical welfare systems and a significant decrease in patient QOL.

The types of diabetes are broadly classified into the following categories: (1) type 1, which arises with the destruction of insulin-producing pancreatic ß cells, resulting in little or no insulin production and secretion; (2) type 2, which develops when glucose cannot be absorbed by target tissue cells due to a decrease in insulin secretion and insulin sensitivity; (3) diabetes in pregnancy, which is caused by a hormone imbalance during pregnancy; and (4) other infrequent types, such as diabetes caused by other diseases such as a genetic or immune abnormality, hepatic or pancreatic disease, or infection. Hereditary factors and environmental factors play important roles in diabetes initiation. In type 2 diabetes, lifestyle is a very important environmental factor that accounts for a large proportion of diabetes incidence in Japan.

Diabetes therapy is broadly classified into 3 categories: “diet therapy”, “exercise therapy”, and “medication therapy”. If the disease is at an early stage of development and symptoms are moderate, diabetes can possibly be controlled using a combination of “diet therapy”, with limited calorie ingestion and well-balanced diet, and “exercise therapy”, which seeks to activate insulin production with moderate exercise. However, “medication therapy” with an oral medicine and insulin injections becomes necessary when diabetes is more advanced and is administered in parallel and not in place of “diet therapy” and “exercise therapy”.

According to a study of Japanese immigrants living in the United States, the prevalence and morbidity of diabetes in a cohort of Americans of Japanese origin who had adopted a more Westernized lifestyle was 2 to 3 times higher than that in individuals still living in Japan, despite nearly identical hereditary backgrounds. If we consider the social environment in Japan, the current Japanese American is considered to represent potential future health concerns for the people of Japan. This sharp increase in diabetes incidence should be noted as a reason for taking immediate measures to detect, protect against, and treat diabetes in Japan.

To examine the effects of the ingredients contained in the test diet, a high-fat diet containing S. crispa was administered to rats and changes in blood sugar level were measured and compared between baseline
and the end of the study. This study showed the inhibitory effects of S. crispa on blood sugar levels. E. grauca, on the other hand, is a functional food ingredient, which was expected to inhibit the absorption of glucose in the small intestine and the elevation of blood sugar levels.

In a non-clinical trial evaluating a diet with the combined ingredients, rat glucose tolerance tests showed inhibition of the elevation of blood sugar level. Therefore, it was confirmed that the ingestion of the test diet is safe and expected to have protective and palliative effects against diabetes without causing any adverse reactions. This clinical study was performed in compliance with the "Ethical Guidelines for Clinical Studies" (Notification No. 225 from the Ministry of Labor and Welfare [MHLW]) since it is a clinical study evaluating food. If the interpretation of this guideline was unclear, we performed the study with reference to Good Clinical Practice (GCP) (MHLW Ordinance No. 28, issued in March, 1997).

3. Study methods

(1) Subjects

The study enrolled patients with type 2 diabetes who meet both inclusion and exclusion criteria (Table 2) from among the patients with well-defined type 2 diabetes (Table 1) according to the Practice Guideline for the Treatment for Diabetes issued by The Japan Diabetes Society in 2012 as documented during the observation or pre-observation period.

Table 1: Diabetes diagnostic criteria of The Japan Diabetes Society

a. Diabetes diagnostic criteria
(i) Blood sugar level
・Fasting blood sugar level is over 126 mg/dL.
・Blood sugar level at 2 hours after taking 75 mg oral glucose is over 200 mg/dL.
(ii) HbA1c level
・HbA1c (NGSP) level is over 6.5%
If any of the conditions in (i) were observed twice, or if (i) + (ii) was observed, the patient was diagnosed as having diabetes.

b. Type 2 diabetes
Approximately 95% of diabetic patients in Japan have type 2 diabetes, with the cumulative effects of the following factors causing a deficiency in insulin action and diabetes development.
(i) Hereditary factors
・Genetic abnormality related to the function of insulin-producing pancreatic β cells
・Genetic abnormality related to the transfer mechanism of insulin action
(ii)Environmental factors
・Overeating (especially high fat food)
・Lack of exercise
・Stress

Table 2: Subject inclusion and Exclusion criteria

a. Inclusion criteria
(i) Age: Japanese patients aged 20 to 65 years at the time that informed consent was provided
(ii) Sex: both males and females were included
(iii) Patients who were under treatment or desired to receive treatment at clinical trial sites as outpatients (outpatients only)
(iv) Patients with type 2 diabetes whose HbA1c level was between 6.5 and 9.5 % (NGSP) from among patients who had well-defined type 2 diabetes according to the Practice Guideline for the Treatment for Diabetes issued by The Japan Diabetes Society in 2012
(v) Patients who were taking antidiabetic drugs or who were able to control their diabetes without taking antidiabetic drugs
(vi) Patients who provided informed written consent of their own free will after receiving an adequate explanation and full understanding of the study before participation

b. Exclusion criteria
(i) Patients who violated any of the following criteria were excluded from this clinical study
・Patients who changed antidiabetic drugs during the time from the observation period to the date of test diet ingestion
・Patients who were suspected of having or were diagnosed with definite type 1 diabetes
・Patients who received insulin
・Patients who were allergic to mushroom (fungi) or who had a chronic food allergy
・Patients with a history of cardiac infarction
・Patients with a treatment history that included coronary arterial revascularization (PCI or CABG)
・Patients with the complications of unstable angina
・Patients with a history of cerebral vascular disease (excluding asymptomatic lacunar infarction)
・Patient who had been hospitalized due to heart failure
・Patients with serious hepatic disease
・Patients with serious renal disease
・Women during pregnancy or who wished to become pregnant during the study period
・Women who were breast-feeding
・Patients who participated in another study or who received a study drug during the 12 weeks before initiation of the test diet
・Patients who were under treatment for psychiatric disease or who needed such treatment by a doctor's judgment
・Patients who were judged inappropriate for participation by the attending investigator or subinvestigator

(2) Study design

We performed an exploratory clinical study by employing a randomized, placebo-controlled, parallel group, double-blind design. The study schedule is shown in Fig. 1. Subjects who met all of the inclusion and exclusion criteria during the observation period and who provided informed consent were given the test diet 3 times a day before meals (one pack of test diet (1800 mg/pack), a total of 3 packs a day) for 168 ± 7 consecutive days.

The patients had periodic hospital visits at regular intervals of 28 ± 7 days at Visit 1 (day 28), Visit 2 (day 56), Visit 3 (day 84), Visit 4 (day 112), Visit 5 (day 140), and Visit 6 (day 168) with the first day of ingesting the test diet as baseline (Visit 0). Each patient received prescribed check-ups and medical examinations.

The subjects were not allowed to take any food for at least 8 hours before receiving a fasting test on a prescribed visitation date, but were allowed to take water freely. Generally, the medical check-ups were scheduled in the morning if at all possible, but if the patients had afternoon appointments due to personal reasons, it was not treated as the deviation from protocol.

On the case report form, the date that informed consent was obtained, the subject identification code, the test diet allocation code, birth date (age), sex, pregnancy status, the year diagnosed with type 2 diabetes, treatment history, medical history / complications at the starting point of the ingestion of the test diet, current combination drugs and combination treatments, and all adverse events that developed during the period from the start of the test diet until the end of the study, in addition to the endpoints described in (3).

During the study period, changes or any increase/decrease in concurrent drug use, combination treatments, or exercise therapy were refrained from as completely as possible, to minimize any possible influence on study data. If the drug had not yet been prescribed, new prescriptions for antidiabetic drugs were delayed and insulin administration was prohibited during the duration from the observation period until the end of the clinical study.

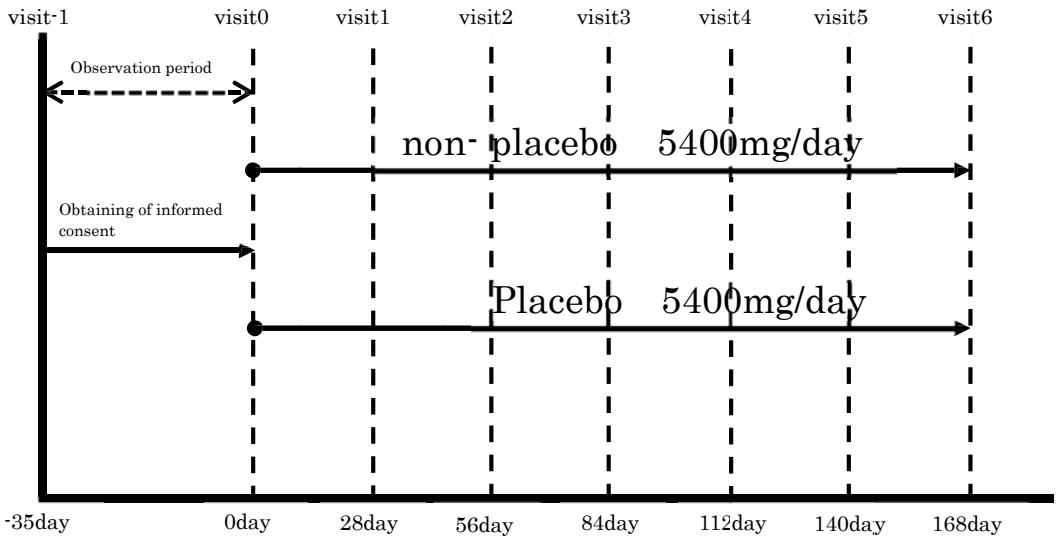

Fig. 1. Study schedule

(3) Endpoints

(i) Primary endpoint

As a confirmation endpoint of effectiveness on diabetes, we confirmed the change in HbA1c (NGSP) level on days 28, 56, 84, 112, 140, and 168, with the dietary start date (day 0) as baseline.

(ii) Secondary endpoint

1)We confirmed the variation in the following items on days 28, 56, 84, 112, 140, and 168 with the dietary start date (day 0) as baseline.

・General hematology tests: WBC, RBC, Hb, Ht, MCV, MCH, MCHC, PLT

・Biochemical tests: TP, COT, GPT, LDH, ALP, γ-GTP, Amy, BUN, CRE, UA, Na Cl, K, Ca, Fe, T-CHO, LDL, HDL, TG, GLU

・Urinalyses: pH, sugar, protein, occult blood, urobilinuria, ketone bodies

2) We confirmed the variation in the following items on days 56, 112 and 168, with the dietary start date (day 0) as baseline.

IRI, IRI antibody, GLU, and HOMA-β (insulin secretory capacity)

[*HOMA-β = 360 × fasting insulin level ÷ (fasting blood sugar level-63)]

3) We confirmed the variation in the following items on days 28, 56, 84, 112, 140, and 168 with the dietary start date (day 0) as baseline. A Robo Skin Analyzer RSA 50 manufactured by Inforward, Inc. was used as a measurement device, while Clinical Suite 2.1 from the same company was used for the analyses.

・moisture (moisture and oil contents)

・color (tone, clearness, brightness)

・pigmentation

・pore

・redness

・wrinkle (below one's eyes, corner of one's eye)

・texture

4) Survey using electronic questionnaire

In this clinical study, we administered an additional subject questionnaire utilizing iPads (Apple Corporation) to evaluate the

effectiveness of the test diet on diseases other than diabetes and how to best utilize it for electronic case report forms.

(iii) Safety endpoint

1) We confirmed all of the adverse events during the period from day 0 until the end of the study

2) We performed a standard 12-lead electrocardiogram at day 0 and at the end of the study

3) We performed a pregnancy test and oral survey only in a woman of childbearing potential

Pregnancy test: day 0

Oral confirmation: days 0, 28, 56, 84, 112, 140, and 168

(4) Test diet

We used LB-Scr "Laboag Sparassis crispa" (Laboag Co., Ltd), E. grauca B2 (Adaptgen Pharmaceutical Co., Ltd.) and Dextrin (Sandec#70; Sanwa Starch Co., Ltd.) for the test diet. (Formulation: 30–80 mesh granules, 3 consecutive aluminum packs (1800 mg/pack)). One divided package contained 750 mg of LB-Scr, 930 mg of E. grauca, and 120 mg of dextrin. Each placebo package contained 1680 mg of powder cellulose and 120 mg of dextrin. It was not possible to distinguish it from the test diet package by appearance. The study required study for 168 days, and since the number of the subjects who were able to comply with the requirements during the study was considered small, 70% ingestion of the test diet was regarded as the minimum ingestion necessary for inclusion in the effectiveness analysis.

(5) Statistical processing

Data were analyzed using SPSS (IBM corporation, Ver. 20.0) statistical software.

Two-way repeated measures ANOVA was used for comparison between the 2 groups and Friedman test was performed if the main efficacy was observed. Values below 5% ($P < 0.05$) were regarded as statistically significant.

4. Study results

(1) Subject backgrounds

We performed the effectiveness analysis in the subjects who met the requirement of more than 70 % of ingestion of the test diet.

The subject backgrounds for the 31 subjects included in the efficacy evaluation are shown in Table 3. The distribution chart of age and sex in these subjects is shown in Fig. 2. There were no significant differences between the placebo group and the test diet group and the groups were considered to be well balanced.

Table 3: A list of subject backgrounds

sex	pregnancy status
birth dates (age)	dates of obtaining informed consent
height/weight (BMI)	the first year of diabetes development
blood pressure /body temperature	treatment drugs
Diagnosis and treatment department	medical history
subject identification code	complications
test diet allocation code	concurrent combination drugs /treatments
observation and examination for efficacy evaluation	
survey, observation and examination for safety evaluation	

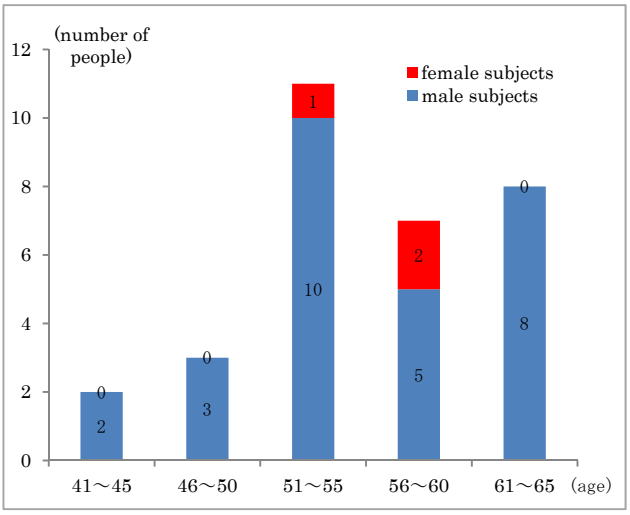

Fig. 2. Distribution chart of the efficacy evaluation for the 31 subjects by age group

(2) Effects on diabetes

① Effects on HbA1c (NGSP) level

The time course change in HbA1c levels for the 31 subjects in the efficacy evaluation is shown in Table 4 and Fig. 3. The average value at day 168 (6.78 ± 0.47%) in the test diet group was lower than the average value at day 0 (7.32 ± 0.70%) in the same group. On the contrary, the average value at day 168 (7.56 ± 1.31%) in the placebo group was higher than the average value at day 0 (7.26 ± 0.89%) in the same group.

The time-course change during the period from day 0 to day 168 in the test diet group is also shown in Table 5 and Fig. 4. The median value in the test diet group at day 168 (6.65%; IQR, 6.50%–7.00%) was significantly lower than the value at day 0 (7.10%; IQR, 6.90%–7.88%) (P = 0.005).

In addition, the number of people who showed an increase or decrease of HbA1c score at the end of the study (day 168) with the dietary start date (day 0) as baseline is shown in Table 6. A decrease was observed in 81.3% of subjects on the test diet, and 46.7% of subjects in the placebo group. These results show a tendency for a high rate of improvement in the test diet group.

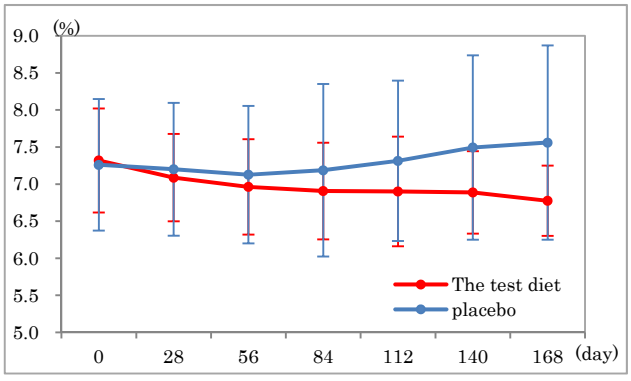

Fig. 3. Time course change in the HbA1c levels for 31 subjects in the efficacy evaluation (mean value ± standard variation)

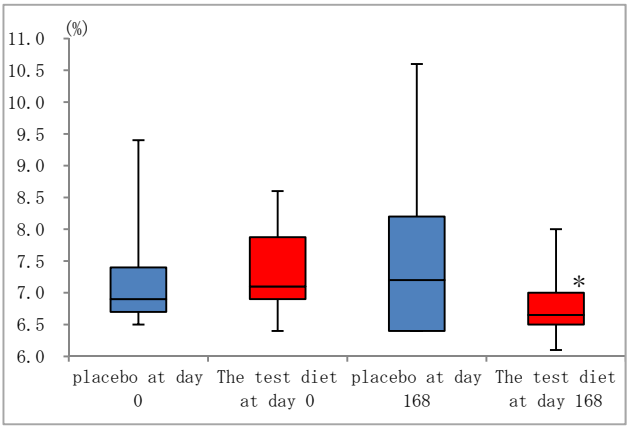

Fig. 4. Time course change in the HbA1c levels for 31 subjects in the efficacy evaluation from day 0 to day 168 (mean value and interquartile range[IQR])(*:P < 0.05)

Table 4: Time course change in HbA1c level (NGSP)
(mean value ± standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	7.26±0.89	7.20±0.90	7.13±0.93	7.19±1.16	7.31±1.08	7.49±1.24	7.56±1.31
The test diet	16	7.32±0.70	7.09±0.59	6.96±0.64	6.91±0.65	6.90±0.74	6.89±0.56	6.78±0.47

Unit: %

Table 5: Time course change in HbA1c level (NGSP) for 31 subjects in the efficacy evaluation
(mean value and interquartile range)

Group	SN	0day	168day
placebo	15	6.90(6.70-7.40)	7.20(6.40-8.20)
The test diet	16	7.10(6.90-7.88)	6.65(6.50-7.00)

Unit: %

Table 6: Change in HbA1c levels at the end of the study with the dietary start date as baseline for the 31 subjects in the efficacy evaluation

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	7	8	46.7%
The test diet	16	13	3	81.3%

② Effects on insulin (IRI) level

The time course change of IRI levels for the 31 subjects in the efficacy evaluation are shown in Table 7 and Fig. 5, while an increase or decrease in IRI levels at the end of the study (day 168) is shown in Table 7. The time course for change in IRI levels for patients whose IRI level was over the standard value (12.4 μU/mL) at the start of ingestion (day 0) (6 patients in the placebo group/4 patients in the test diet group) is shown in Table 8 and Fig. 6. The median value (9.35(7.53-13.50) μU/mL) at day 168 was significantly lower than the value at day 0 (14.40(13.05-16.88) μU/mL) in the test diet group (P = 0.046). The number of subjects who showed an increase or decrease in IRI at the end of the study (day 168) is shown in Tables 9 and 10. If we look at the IRI level in patients whose IRI level was over the standard value (12.4μU/mL) at the starting date of ingestion (day 0) (6 patients in the placebo group/4 patients in test diet group), 100.0% of patients showed a decrease in the test diet group, compared to 50.0% in the placebo group. These results showed a trend toward improved IRI levels in the test diet group.

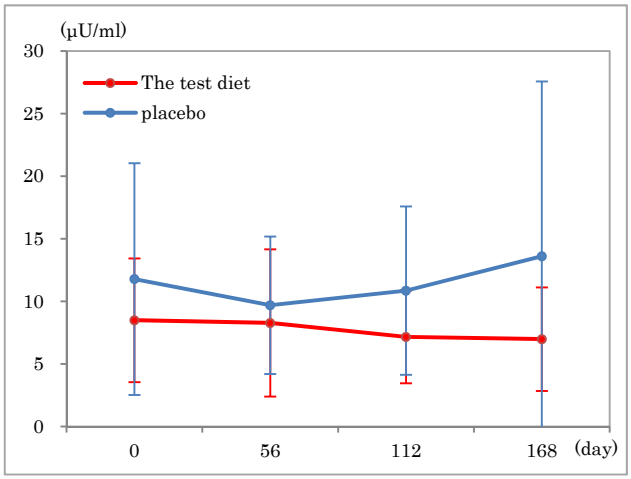

Fig. 5. Time course change in the IRI levels of the 31 subjects in the efficacy evaluation(mean value / interquartile range)

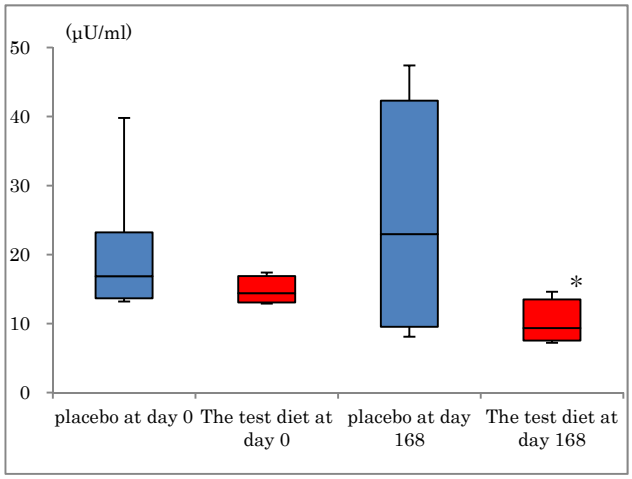

Fig. 6.: Time course change in the IRI levels of patients whose IRI level was over the standard value (12.4μU/mL) at the starting date of ingestion (day 0) (6 patients in the placebo group/4 patients in test diet group) and from day 0 to day 168 (mean value and interquartile range) (*:P < 0.05)

Table 7: Time course change in the IRI levels of the 31 subjects in the efficacy evaluation (mean value / interquartile range)

Group	SN	0day	56day	112day	168day
placebo	15	11.78±9.26	9.69±5.49	10.85±6.73	13.60±13.98
The test diet	16	8.49±4.95	8.27±5.89	7.16±3.71	6.98±4.14

Unit: μU/ml

Table 8: Time course change in the IRI levels of patients whose IRI level was over the standard value (12.4 μU/mL) at the starting date of ingestion (day 0) (6 patients in the placebo group/4 patients in test diet group) and from day 0 to day 168 (mean value and interquartile range)

Group	SN	0day	168day
placebo	4	16.85(13.65-23.23)	22.95(9.53-42.30)
The test diet	6	14.40(13.05-16.88)	9.35(7.53-13.50)

Unit: μU/ml

Table 9: Change in IRI levels for the 31 subjects in the efficacy evaluation at the end of the study from baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	9	6	60.0%
The test diet	16	9	7	56.3%

Table 10: Change in IRI levels at the end of the study in patients whose IRI level was over the standard value (12.4 μU/mL) at day 0 (6 patients in the placebo group/4 patients in test diet group)

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	4	3	3	50.0%
The test diet	6	4	0	100.0%

③ Effects on IRI antibody level

IRI antibody was measured at days 0, 56, 112, and 168 during the study. It was below 0.4% at all measurement time points in 30 of 31 subjects in the efficacy evaluation. This tendency to decrease was observed in one subject in the test diet group from the start of the study and it was decreased to 9.3% at day 168 from 14.1% at day 0. These values are presented in Table 11.

④ Effects on blood sugar level (GLU)

The time course change in GLU levels for all 31 subjects is shown in Table 12 and Fig. 7. No significant difference was observed in either the placebo or the test diet groups; however, if the average values between the placebo group and the test diet group are compared, there is an increasing trend in the placebo group and almost no change in the test diet group. This tendency was prominent, especially after day 112. The tendency for increasing GLU levels in the placebo group after 3 months is frequently observed in clinical studies of patients with diabetes due to the deterioration in the patients' adherence to dietary restriction. As this tendency was not observed in the test diet group, the possibility of inhibitory effects of the test diet on blood sugar elevation can be considered.

Table 11: The time course change in insulin antibody levels in one subject from the test diet group

Group	SN	0day	56day	112day	168day
The test diet	1	14.1	11.7	9.6	9.3

Unit: %

Table 12: The time course change in GLU levels for the 31 subjects in the efficacy evaluation (average value ± standard variation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
Placebo	15	155.67±42.88	136.00±32.50	138.07±29.27	145.40±32.25	146.13±32.95	171.33±80.09	161.60±66.86
The test diet	16	138.63±22.50	144.19±27.43	136.63±33.05	132.81±21.02	140.88±50.99	135.13±24.00	142.13±38.89

Unit:mg/dL

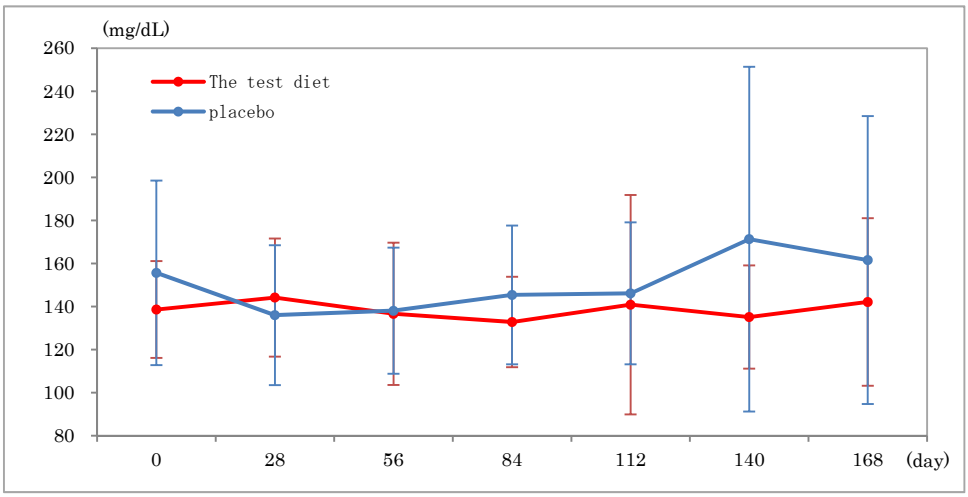

Fig. 7. The time course change in GLU levels for the 31 subjects in the efficacy evaluation (average value ± standard variation)

Table 13: Change in GLU levels for the 31 subjects in the efficacy evaluation considering the starting date of ingestion as baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	9	6	60.0%
The test diet	16	8	8	50.0%

⑤ Effects on HOMA-β level

The time course changes in HOMA-β levels for the 31 subjects in the efficacy evaluation are shown in Table 14 and Fig. 8. The number of subjects who showed a decrease or increase in HOMA-β level at the end of study (day 168) considering a starting date of the ingestion (day 0) as baseline is shown in Table 15. In addition, the time course change in HOMA-β at day 0 and day 168 is shown in Table 16 and Fig. 9. The median value in the test diet groups was significantly low at day 168 (27.81(18.96-47.52) compared to the median value at day 0 (36.15(22.10-54.81)) (P = 0.046).

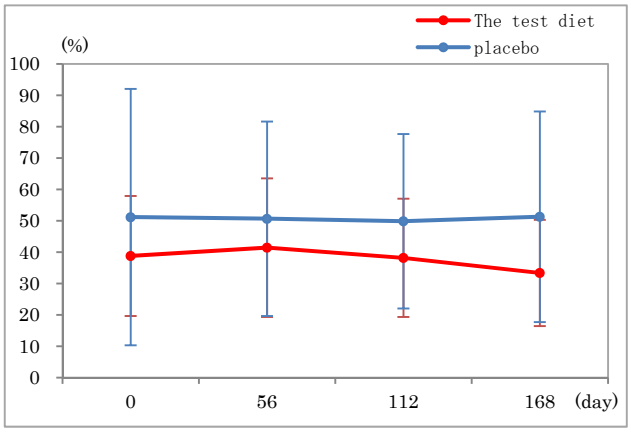

Fig. 8: Time course for HOMA-β levels for the 31 subjects in the efficacy evaluation (average value ± standard variation)

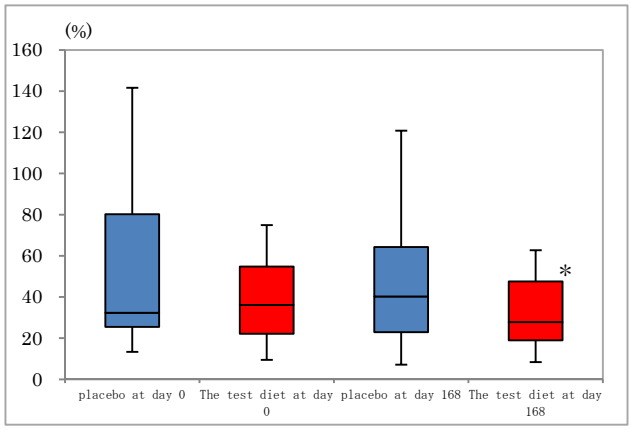

Fig. 9: Time course for HOMA-β levels for the 31 subjects in the efficacy evaluation from day 0 to day 168 (average value and interquartile range) (*:P < 0.05)

Table 14: Time course for HOMA-β levels for the 31 subjects in the efficacy evaluation (average value ± standard variation)

Group	SN	0day	56day	112day	168day
placebo	15	51.15±40.86	50.64±31.00	49.87±27.80	51.29±33.57
The test diet	16	38.78±19.14	41.44±22.09	38.18±18.84	33.37±16.92

Unit: %

Table 15: Change in HOMA-β levels for 31 subjects in the efficacy evaluation considering the starting date of ingestion as baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	8	7	53.3%
The test diet	16	9	7	56.3%

Table 16: Time course of changes in HOMA-β levels for 31 subjects during the efficacy evaluation from day 0 to day 168 (median value and interquartile range)

Group	SN	0day	168day
placebo	15	32.35(25.50-80.20)	40.24(22.91-64.29)
The test diet	16	36.15(22.10-54.81)	27.81(18.96-47.52)

Unit: %

(3) Effects on hepatic function

① Effects on γ-GTP level

The time course change in γ-GTP is shown in Table 17 and Fig. 10. The average value at day 168 in the test diet group (78.63 ± 75.29 IU/L) is lower than that at day 0 in the same group.

It data confirmed that the test diet reduces γ-GTP levels relative to earlier after the start of ingestion (day 56).

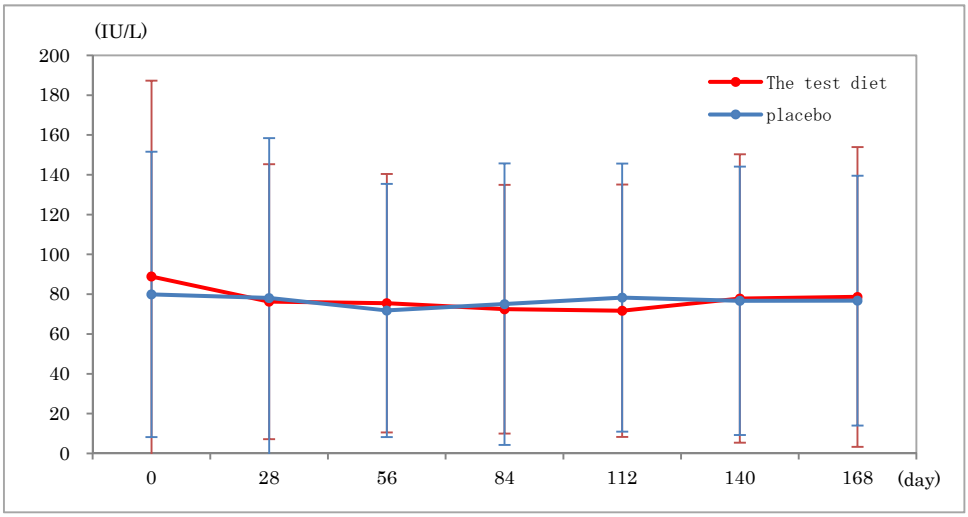

Fig. 10: Time course of changes in γ-GTP levels for 31 subjects during the efficacy evaluation (median value ±standard variation)

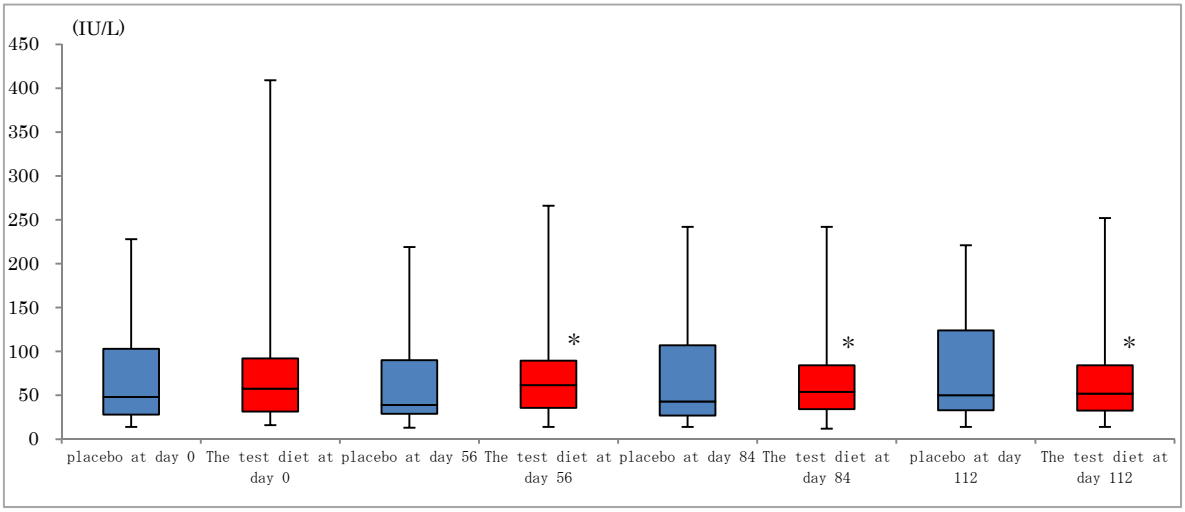

Fig. 11: Time course of changes in γ-GTP levels for 31 subjects during the efficacy evaluation at days 0-56, 84, and 112 (median value and interquartile range) (*:P < 0.05)

Table 17: Time course of changes in γ-GTP levels for 31 subjects during the efficacy evaluation (median value ±standard variation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	79.87±71.68	78.07±80.37	71.80±63.63	75.00±70.73	78.27±67.33	76.67±67.45	76.73±62.76
The test diet	16	88.88±98.42	76.25±69.09	75.44±64.93	72.44±62.47	71.69±63.38	77.81±72.45	78.63±75.29

Unit: IU/L

Table 18: Time course of changes in γ-GTP levels for 31 subjects during the efficacy evaluation at days 0-56, 84, and 112 (median value and interquartile range)

Group	SN	0day	56day	84day	112day
placebo	15	48.00 (28.00-103.00)	39.00 (29.00-90.00)	43.00 (27.00-107.00)	50.00 (33.00-124.00)
The test diet	16	57.50 (31.50-92.00)	61.50 (35.75-89.50)	54.00 (34.25-84.25)	52.00 (32.50-84.25)

Unit: IU/L

② Effects on GOT and GPT levels

The time course change in GOT levels is shown in Table 19 and Fig. 12. The average value at day 168 in the test diet group (28.69 ± 16.76 IU/L) decreased by 10.2% compared to the average value at day 0 in the same group (31.94 ± 30.12 IU/L), with a decreasing trend over time. On the contrary, GOT levels increased by 28.1% in the placebo group. This observation indicated that the test diet acts to decrease GOT levels more strongly than placebo. This tendency was most apparent at day 112.

Also, the number of subjects who showed an increase or decrease in GOT level at the end of study (day 168) compared to day 0 is shown in Table 20. Half of the subjects in the test diet group showed a decrease compared to 26.7% of subjects in the placebo group. These results demonstrated positive effects and improvements in the test diet group.

The time course change in GPT levels is shown in Table 21 and Fig. 13. The average value at day 168 (30.81 ± 25.67 IU/L) in the test diet group decreased by 17.3% compared to the average value at day 0 (37.25 ± 33.34 IU/L) in the same group, the result showing the tendency toward decrease over time. On the other hand, GPT level increased by 12.4% in the placebo group.

The median values in the test diet group at day 28 (P = 0.002), day 56 (P = 0.029), day 84 (P = 0.003), day 112 (P =0.003), day 140 (P = 0.012) and day 168 (P = 0.003) were significantly lower than the median value at day 0 (27.50 (20.50-35.50) IU/L) (refer to Table 22 and Fig. 14). When we compare the number of subjects who showed an increase or decrease in GPT level at day168 relative to their baseline value (day 0), 87.5 % of subjects in the test diet group showed a decrease compared to 40.0% in the placebo group (Table 23). These results showed that the test diet decreases GPT levels.

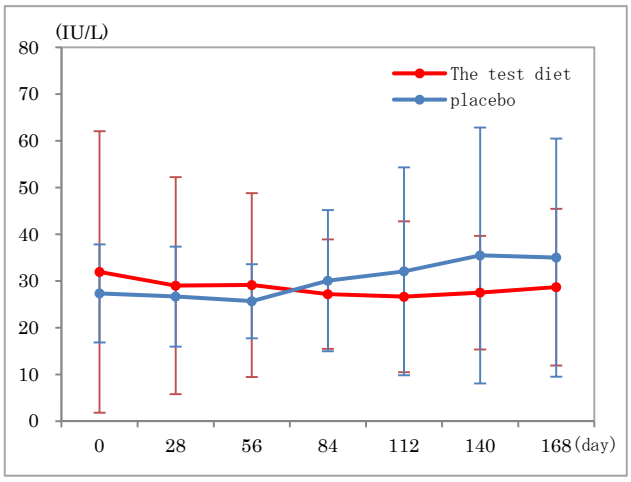

Fig. 12: Time course change in GOT levels for 31 subjects in the efficacy evaluation (average value±standard deviation)

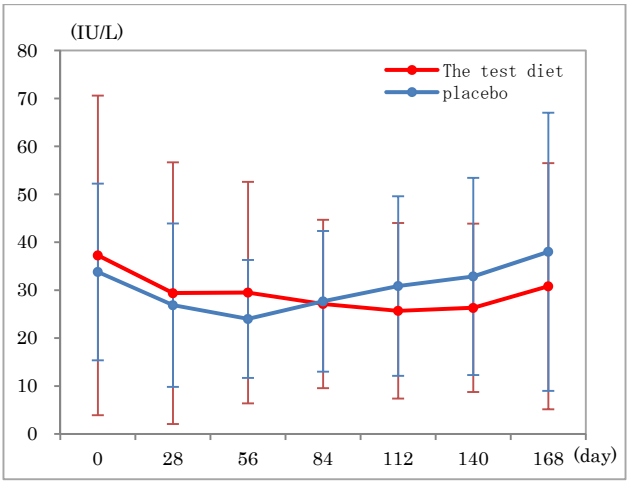

Fig. 13: Time course change in GPT levels for 31 subjects in the efficacy evaluation (average value ± standard deviation)

Table 19: Time course change in GOT levels for 31 subjects in the efficacy evaluation (average value±standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	27.33±10.49	26.67±10.70	25.67±7.93	30.07±15.10	32.07±22.23	35.47±27.39	35.00±25.48
The test diet	16	31.94±30.12	29.00±23.23	29.13±19.67	27.19±11.71	26.63±16.16	27.50±12.16	28.69±16.76

Unit: IU/L

Table 20: Changes in GOT levels for 31 subjects in the efficacy evaluation at the end of study compared to baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	4	11	26.7%
The test diet	16	8	8	50.0%

Table 21: Time course change in GPT levels for 31 subjects in the efficacy evaluation (average value ± standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	33.80±18.42	26.87±17.05	24.00±12.30	27.67±14.67	30.87±18.73	32.87±20.57	38.00±29.02
The test diet	16	37.25±33.34	29.38±27.29	29.50±23.11	27.13±17.56	25.69±18.31	26.31±17.57	30.81±25.67

Unit: IU/L

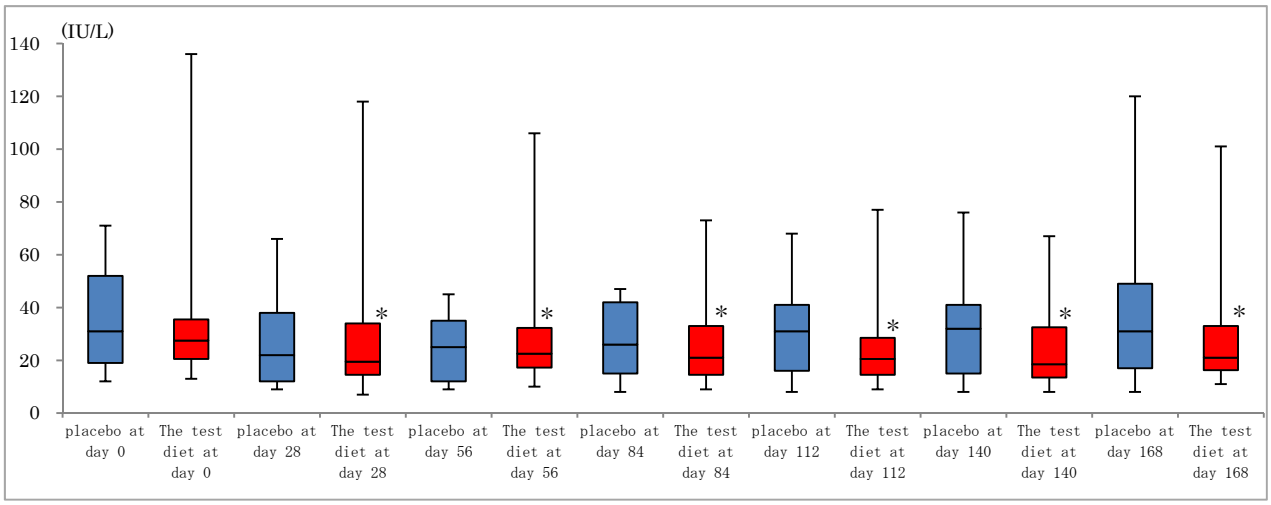

Fig. 14: Time course change in GPT levels for the 31 subjects in the efficacy evaluation (median value and interquartile range) (*:P < 0.05)

Table 22: Time course change in GPT levels for the 31 subjects in the efficacy evaluation (median value and interquartile range)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	31.00 (19.00-52.00)	22.00 (12.00-38.00)	25.00 (12.00-35.00)	26.00 (15.00-42.00)	31.00 (16.00-41.00)	32.00 (15.00-41.00)	31.00 (17.00-49.00)
The test diet	16	27.50 (20.50-35.50)	19.50 (14.50-34.00)	22.50 (17.25-32.25)	21.00 (14.50-33.00)	20.50 (14.50-28.50)	18.50 (13.50-32.50)	21.00 (16.25-33.00)

Unit: IU/L

Table 23: Changes in GPT levels for 31 subjects in the efficacy evaluation at the end of study compared to baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	6	9	40.0%
The test diet	16	14	2	87.5%

(4) Effects on skin

① Effects on pigmentation

The time course changes in frontal pigmentation scores are reported in Table 24 and Fig. 15. The average value at day 168 (32.1 ± 13.3) decreased by 5.0% compared to the average value at day 0 (33.8 ± 13.3) in the placebo group. Conversely, the average value at day 168 (31.8 ± 13.8) decreased by 9.9% compared to the average value day 0 (35.3 ± 13.9) in the test diet group, with the frontal pigmentation score decreasing more over time in the test diet group than in the placebo group. In addition, the median values at day 140 (28.0 (21.3–37.5) were significantly lower than the median value at day 0 (32.5 (25.8–45.8)) (P = 0.008) in the test diet group (refer to Table 25 and Fig. 16).

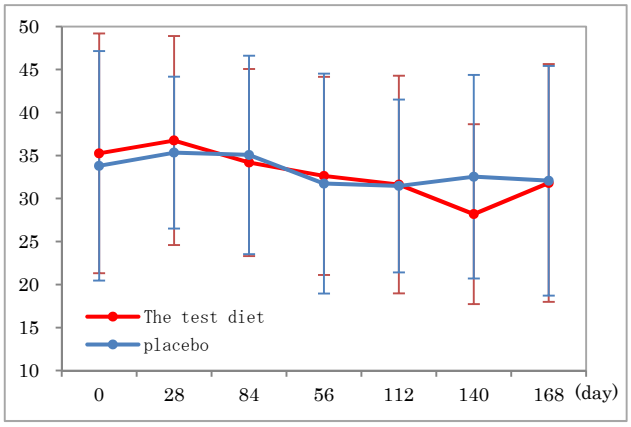

Fig. 15: Time-course change of frontal pigmentation score in 31 subjects for efficacy evaluation (average value ± standard deviation)

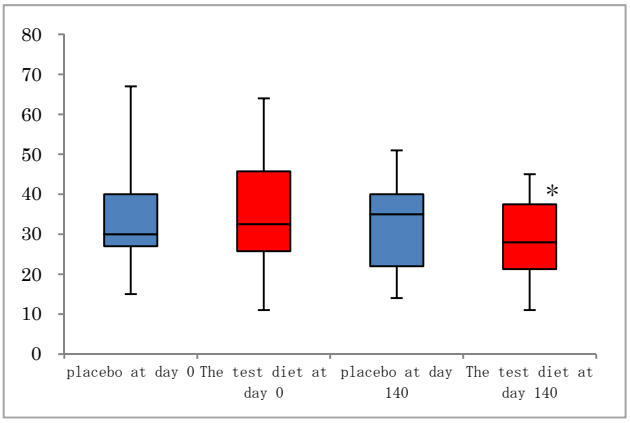

Fig. 16: Time-course change of frontal pigmentation score in 31 subjects for efficacy evaluation from Day 0 to Day 140 (median value and interquartile range) (*:P < 0.05)

Table 24: Time-course change of frontal pigmentation score in 31 subjects for efficacy evaluation (average value ± standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	33.8±13.3	35.3±8.8	35.1±11.5	31.7±12.8	31.5±10.0	32.5±11.8	32.1±13.3
The test diet	16	35.3±13.9	36.8±12.2	34.2±10.9	32.6±11.5	31.6±12.7	28.2±10.5	31.8±13.8

Table 25: Time-course change of frontal pigmentation score in 31 subjects for efficacy evaluation from Day 0 to Day 140 (median value and interquartile range)

Group	SN	0day	140day
placebo	15	30.0(27.0-40.0)	35.0(22.0-40.0)
The test diet	16	32.5(25.8-45.8)	28.0(21.3-37.5)

② Effects on pores

The time course change in frontal prominent pore scores is shown in Table 26 and Fig. 17. The average value at day 168 (2727.2 ± 703.6) decreased by 8.4% compared to the average value at day 0 (2977.5 ± 761.9) in the placebo group. However, the average value at day 168 (2401.7 ± 910.1) decreased by 16.0% compared to the average value at day 0 (2859.4 ± 730.5) in the test diet group, indicating that the frontal prominent pore score decreased more over time in the test diet group than in the placebo group. Also, the median values in the test diet group at day 112 (2307.5 (1973.8-3061.0)) (P = 0.046), day 140 (2337.0 (1696.0-2666.5)) (P = 0.012) and day 168 (2300.5 (1834.5-2731.0)) (P = 0.046) were significantly lower than the median value at day 0 (2670.5 (2466.3-3334.5)) (refer to Table 27 and Fig. 18).

The time course change in frontal pore scores with prominent blackheads is shown in Table 29 and Fig. 19. The average value at day 168 (1642.6 ± 582.8) decreased by 10.2% compared to the average value at day 0 (1830.1 ± 622.8) in the placebo group. In the test diet group, the average value at day 168 (1384.4 ± 739.0)

decreased by 19.1% compared to the average value at day 0 (1712.3 ± 641.8). These results showed that the frontal pore score with prominent blackheads decreased more over time in the test diet group than in the placebo group. Also, the median values in the test diet group at day 112 (1219.5 (978.3-1775.0)) (P = 0.046), day 140 (1229.0 (893.8-1584.5)) (P = 0.012), and day 168 (1223.5 (994.5-1603.5)) (P = 0.012) were significantly lower than the median value at day 0 (1533.0 (1352.8-1939.3)) (refer to Table 30 and Fig. 20).

From the above results, it was suggested that the test diet decreases the appearance of blackheads.

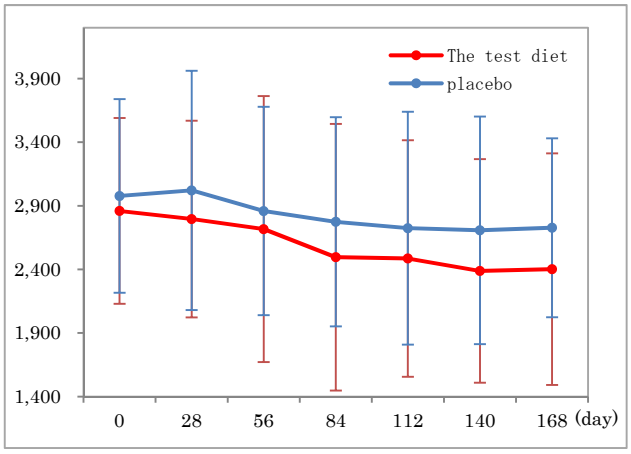

Fig. 17: Time course change in frontal prominent pore scores for the 31 subjects in the efficacy evaluation (average value ±standard deviation)

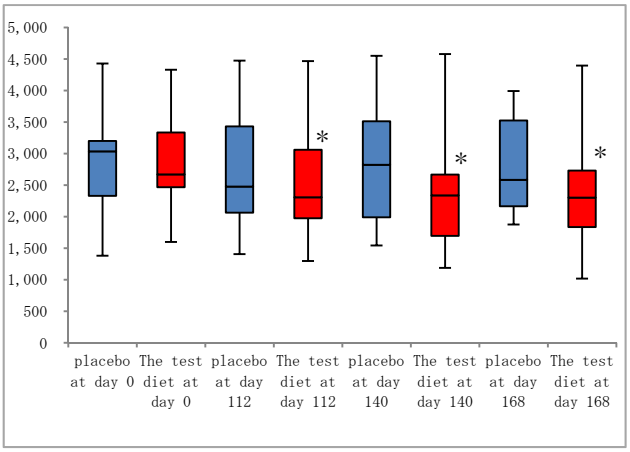

Fig.18: Time course change in frontal prominent pore scores for the 31 subjects in the efficacy evaluation at days 0-112, 140 and 168 (*:P < 0.05)

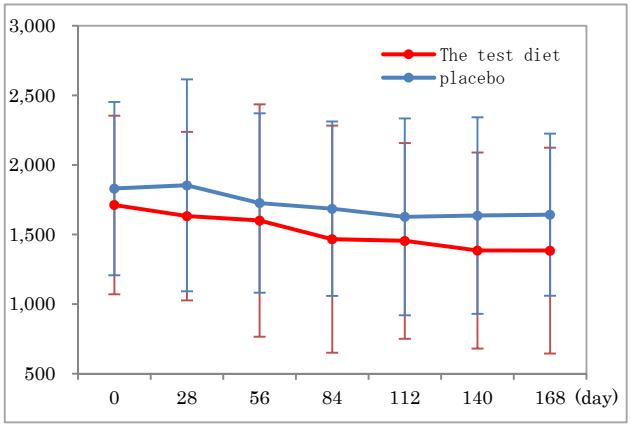

Fig. 19: Time course change in frontal pores with prominent pore scores for the 31 subjects in the efficacy evaluation (average value ± standard deviation)

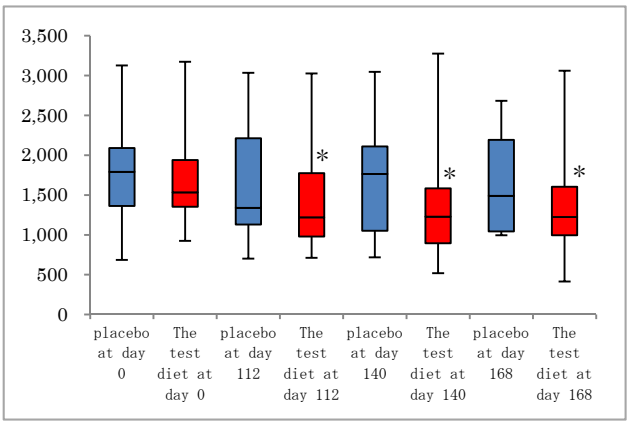

Fig.20: Time course change in frontal pore scores with prominent blackheads for the 31 subjects in the efficacy evaluation at days 0-112, 140 and 168 (*:P < 0.05)

Table 26: Time course change in frontal prominent pore scores for the 31 subjects in the efficacy evaluation (average value ±standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	2977.5±761.9	3021.4±940.8	2859.2±819.6	2773.9±822.4	2724.1±915.5	2707.2±894.5	2727.2±703.6
The test diet	16	2859.4±730.5	2796.1±773.2	2716.9±1045.9	2496.0±1048.1	2485.6±929.9	2387.4±879.0	2401.7±910.1

Table 27: Time course change in frontal prominent pore scores for the 31 subjects in the efficacy evaluation at days 0-112, 140, and 168 (median value and interquartile range)

Group	SN	0day	112day	140day	168day
placebo	15	3036.0(2329.0-3201.0)	2476.0(2062.0-3431.0)	2821.0(1989.0-3511.0)	2583.0(2165.0-3524.0)
The test diet	16	2670.5(2466.3-3334.5)	2307.5(1973.8-3061.0)	2337.0(1696.0-2666.5)	2300.5(1834.5-2731.0)

Table 28: Time course change in frontal prominent pore scores for the 31 subjects in the efficacy evaluation at the end of study compared to baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	11	4	73.3%
The test diet	16	12	4	75.0%

Table 29: Time course change in frontal pores with prominent pore scores for the 31 subjects in the efficacy evaluation (average value ± standard deviation)

Group	SN	0day	28day	56day	84day	112day	140day	168day
placebo	15	1830.1±622.8	1853.3±761.1	1726.3±644.9	1685.3±626.8	1627.3±707.4	1636.1±705.9	1642.6±582.8
The test diet	16	1712.3±641.8	1632.4±605.7	1600.1±834.9	1466.6±816.2	1454.1±703.6	1385.0±704.8	1384.4±739.0

Table 30: Time course change in frontal pore scores with prominent blackheads for the 31 subjects in the efficacy evaluation at days 0-112, 140, and 168 (median value and interquartile range)

Group	SN	0day	112day	140day	168day
placebo	15	1791.0(1362.0-2091.0)	1339.0(1130.0-2212.0)	1764.0(1052.0-2110.0)	1490.0(1043.0-2192.0)
The test diet	16	1533.0(1352.8-1939.3)	1219.5(978.3-1775.0)	1229.0(893.8-1584.5)	1223.5(994.5-1603.5)

Table 31: Changes in frontal pore scores with prominent blackheads for the 31 subjects in the efficacy evaluation at the end of study compared to baseline

Group	SN	Decrease	Increase /unchanged	percentage of decrease
placebo	15	11	4	73.3%
The test diet	16	13	3	81.3%

(5) Others

Regarding other secondary endpoints (general hematology test, biochemical test, urinalysis, and confirmation of changes in skin condition by imaging analysis), there were no significant changes in either the placebo group or the test diet group.

(6) Adverse events

Adverse events occurred in 17 subjects (including one withdrawal) in the test diet group (Table 32) and 17 subjects (including 2 withdrawals) in the placebo group (Table 33). Among a total of 34 subjects, adverse events developed in 13 subjects (a total of 17 cases), among which 5 subjects (a total of 6 cases) belonged to the test diet group. An investigator of the clinical study made medical judgement as to the causal relationships between adverse events and test diet and confirmed that there is no adverse event causally related to the test diet. Based on the above results, there is the very low possibility of occurrence of adverse reaction due to ingestion of the test diet.

Table 32: Causal relationships between adverse events in 17 subjects in the test diet group and the test diet

Adverse events	Causal relationship	No. of adverse events	%(No. /No. of subjects)	Date of confirming adverse events	Drug name	Dose per day	Start of administration	Completion of administration	Outcome	Date of confirming outcome
myalgia of the neck	no causal relationship	1	5.88%	May 12 2013	Loxonin 60mg	180mg (in 3 divided does of 60mg)	May 22 2013	May 29 2013	resolved	May 29 2013
					Mucosta 100mg	300mg (in 3 divided does of 100mg)	May 22 2013	May 29 2013		
					Loxionin tape	appropriate does	May 22 2013	May 29 2013		
urinary frequency	no causal relationship	1	5.88%	April 11 2013	-	-	-	-	not resolved	September 3 2013
a cold	no causal relationship	1	5.88%	April 11 2013	-	-	-	-	resolved	May 17 2013
rash	no causal relationship	1	5.88%	April 15 2013	Rinderon-VG		April 2 2013	April 15 2013	resolved	May 10 2013
worsening of hypertension	no causal relationship	1	5.88%	April 25 2013	Unisia Combination tablets HD	1 tablet (in 1 divided of 1tablet)	April 26 2013	continuing after the end of the study	remission	May 24 2013
insect bite	no causal relationship	1	5.88%	June 27 2013	Allegra tablets 60mg	120mg (in 2 divided does of 60mg)	June 10 2013	June 10 2013	resolved	June 27 2013
					Rinesteron 0.5mg	0.5mg (in 1 divided 0.5 mg)	June 10 2013	June 10 2013		
					Rebamipide tablets 100mg	200mg (in 2 divided does of 100mg)	June 10 2013	June 10 2013		

Table 33: Causal relationships between adverse events in 17 subjects in the placebo group and the test diet

Adverse events	Causal relationship	No. of adverse events	%(No. /No. of subjects)
Cerebral infarction	Causal relationship unknown	1	5.88%
stomach ache	no causal relationship	1	5.88%
upper respiratory tract inflammation	no causal relationship	1	5.88%
a cold	no causal relationship	1	5.88%
left foot pain	no causal relationship	1	5.88%
sensory disturbance of the fingers in both hands	no causal relationship	1	5.88%
periarthritis of the left shoulder	no causal relationship	1	5.88%
atypical genital bleeding	no causal relationship	1	5.88%
edema in both lower limbs	no causal relationship	1	5.88%
upper respiratory tract inflammation	no causal relationship	1	5.88%
urticarial	no causal relationship	1	5.88%

(7) Interaction with combination drugs

We evaluated possible relationships between concomitant drugs the subjects were taking during the study and the test diet. The number of adverse events related to antidiabetic drugs and their causal relationships are shown in Table 34, while the number of adverse events related to concomitant drug use and their causal relationships are shown in Table 35. The clinical study investigator made a medical judgment regarding any possible causal relationship. Adverse events were observed in 4 subjects (3 types) among 6 subjects (5 types) who were taking antidiabetic drugs and were observed in 11 of 26 specific concomitant drugs. However, no causal relationships between adverse events and the test diet were confirmed. From the above results, it was confirmed that there was no interaction between either 5 types of antidiabetic drugs taken by the subjects during the study and the test diet or between 26 other combination drugs and the test diet.

(8) Effects on complications

Among the 17 subjects in the test diet group, 9 subjects (a total of 25 cases) had complications. We examined causal relationships with the test diet when adverse events developed in patients with complications (Table 36). The clinical study investigator determined that there was no relationship between any of the adverse events that occurred in 2 patients with complications (a total of 10 cases) and the test diet.

Based on the above results, there is only a very low possibility of adverse reactions occurring in patients with complications due to ingestion of the test diet.

Table 34: Causal relationships between antidiabetic drugs and the test diet in 17 test diet group subjects

Antidiabetic drugs	medicine taking situation	Causal relationship	No. of adverse events	%(No. /No. of subjects)	Adverse events
Actos Tablet 15	currently taking	no causal relationship	1	5.88%	myalgia of the neck
Amaryl	currently taking	no causal relationship	1	5.88%	urinary frequency/a cold
Amaryl 1 mg Tablet	currently taking	no causal relationship	0	0.00%	
Januvia Tablet	currently taking	no causal relationship	1	5.88%	worsening of hypertension/insect bite
Nesina Tablet	currently taking	no causal relationship	0	0.00%	

Table 35: Causal relationships between concomitant drugs and the test diet in 17 test diet group subjects

Combination drugs	Dose per day	Causal relationship	No. of adverse events	% (No. of subjects with adverse events/No of subjects in the test diet group)	Adverse reactions	Date of onset	Start of administration	Completion of administration	Outcome	Date of confirming outcome
IDpap	Appropriate dose	no causal relationship	3	17.65%	Contusion of the left shoulder/lumbar contusion/contusion of the both thumbs	February 10 2013	February 16 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Avapro Tablet 50 mg	100 mg (in 2 divided doses of 50 mg)	no causal relationship	0	0.00%	hypertension	March 25 2010	March 25 2010	January 22 2011	not resolved	continuing after the end of the study
Avapro Tablet 50 mg	100mg (in 2 divided doses of 50 mg)	no causal relationship	0	0.00%	hypertension	January 24 2013	January 24 2013	April 25 2013	not resolved	continuing after the end of the study
Amlodipine Tablet 2.5 mg	2.5 mg (once daily dosing of 2.5 mg)	no causal relationship	0	0.00%	hypertension	March 25 2010	November 18 2010	December 16 2010	not resolved	continuing after the end of the study
Allegra tablet 60 mg	120 mg (in 2 divided doses of 60 mg)	no causal relationship	1	5.88%	allergic rhinitis	March 8 2013	March 8 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Excelase Combination Tablet	3 tablets (in 3 divided doses of 320 mg)	no causal relationship	0	0.00%	gastroenteritis	February 19 2013	February 19 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Exforge Combination Tablet	1 tablet (once daily dosing of 85 mg)	no causal relationship	1	5.88%	hypertension			continuing after the end of the study	not resolved	continuing after the end of the study
Omeprazole Tablet 10 mg	10 mg (once daily dosing of 10 mg)	no causal relationship	1	5.88%	reflux esophagitis	December 29 2003		continuing after the end of the study	not resolved	continuing after the end of the study
Gaster D Tablet 20 mg	20 mg (once daily dosing of 20 mg)	no causal relationship	0	0.00%	gastroenteritis	February 19 2013	February 19 2013	April 16 2013	not resolved	continuing after the end of the study
Gaster D Tablet 10 mg	10 mg (once daily dosing of 10mg)	no causal relationship	0	0.00%	gastroenteritis	February 19 2013	April 17 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Calblock Tablet 16 mg	16 mg (once daily dosing of 16 mg)	no causal relationship	0	0.00%	hypertension	April 22 2008	April 22 2008	continuing after the end of the study	not resolved	continuing after the end of the study
Cravit Tablet 500 mg	500 mg (once daily dosing of 500mg)	no causal relationship	0	0.00%	vesiculitis	September 18 2010	September 18 2010	September 22 2010	resolved	September 22 2010

Combination drugs	Dose per day	Causal relationship	No. of adverse events	% (No. of subjects with adverse events/No of subjects in the test diet group)	Adverse reactions	Date of onset	Start of administration	Completion of administration	Outcome	Date of confirming outcome
Zyloric Tablet 100 mg	100 mg (once daily dosing of 100mg)	no causal relationship	0	0.00%	hyperuricemia	October 17 2011	October 17 2011	continuing after the end of the study	not resolved	continuing after the end of the study
Zyloric Tablet 50 mg	50mg (once daily dosing of 50mg)	no causal relationship	0	0.00%	hyperuricemia	November 1 2002	November 1 2002	March 20 2010	resolved	March 20 2010
heavy magnesium oxide	0.8 g	no causal relationship	0	0.00%	constipation	February 19 2013	February 19 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Rhubarb powder	0.3 g	no causal relationship	0	0.00%	constipation	February 19 2013	February 19 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Diovan OD Tablet	80mg	no causal relationship	0	0.00%	hypertension	2008		continuing after the end of the study	not resolved	continuing after the end of the study
Nasonex nasal spray	appropriate dose	no causal relationship	1	5.88%	allergic rhinitis	2003	March 8 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Halcion D Tablet 0.25 mg	0.25 mg (once daily dosing of 0.25 mg)	no causal relationship	1	5.88%	insomnia	2010	April 5 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Harnal D Tablet 0.2 mg	0.2 mg (once daily dosing of 0.2 mg)	no causal relationship	0	0.00%	benign prostatic hypertrophy	March 14 2011	March 28 2011	the end of May 2012	remission	August 18 2011
Biofermin Tablet	2.5 mg	no causal relationship	0	0.00%	constipation	February 19 2013	February 19 2013	April 16 2013	not resolved	continuing after the end of the study
Biofermin Tablet	2.5 mg	no causal relationship	0	0.00%	constipation	April 17 2013	April 17 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Fluitran Tablet	1mg	no causal relationship	0	0.00%	hypertension	2008	2008	continuing after the end of the study	not resolved	continuing after the end of the study
Preminent Tablet	1 tablet (once daily dosing of 62.5 mg)	no causal relationship	0	0.00%	hypertension	April 22 2008	June 9 2009	continuing after the end of the study	not resolved	continuing after the end of the study
Mucosta Tablet 100 mg	300 mg (in 3 divided doses of 100 mg)	no causal relationship	3	17.65%	contusion of the left shoulder/lumbar contusion/contusion of the both thumbs	February 10 2013	February 16 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Unisia Combination Tablet HD	1 tablet (once daily dosing of 13 mg)	no causal relationship	1	5.88%	hypertension	March 25 2010	January 23 2011	February 19 2011	not resolved	continuing after the end of the study
Unisia Combination Tablet HD	1 tablet (once daily dosing of 13 mg)	no causal relationship	1	5.88%	hypertension	2010	January 31 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Lac-B fine granule N	2.5g	no causal relationship	0	0.00%	constipation	February 19 2013	February 19 2013	April 16 2013	not resolved	continuing after the end of the study
Lac-B fine granule N	3.0g	no causal relationship	0	0.00%	constipation	February 19 2013	April 17 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Livalo Tablet 1 mg	1 mg (once daily dosing of 1 mg)	no causal relationship	0	0.00%	hyperlipidemia	2005	March 17 2012	continuing after the end of the study	not resolved	continuing after the end of the study

Combination drugs	Dose per day	Causal relationship	No. of adverse events	% (No. of subjects with adverse events/No of subjects in the test diet group)	Adverse reactions	Date of onset	Start of administration	Completion of administration	Outcome	Date of confirming outcome
Lipitor Tablet 10 mg	10 mg (once daily dosing of 10 mg)	no causal relationship	0	0.00%	hypercholesterolemia	2003	March 4 2012	continuing after the end of the study	not resolved	continuing after the end of the study
Ribomine ophthalmic solution	appropriate dose	no causal relationship	1	5.88%	allergic rhinitis	2003	March 8 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Loxonin Tape	appropriate dose	no causal relationship	3	17.65%	contusion of the left shoulder/lumbar contusion/contusion of the both thumbs	February 10 2013	February 16 2013	continuing after the end of the study	not resolved	continuing after the end of the study
Loxonin Tablet	180 mg	no causal relationship	3	17.65%	contusion of the left shoulder/lumbar contusion/contusion of the both thumbs	February 10 2013	February 16 2013	continuing after the end of the study	not resolved	continuing after the end of the study

Table 36 Causal relationships between complications and the test diet in 17 subjects in the test diet group

Complications	Causal relationship	No. of adverse events	%(No. /No. of subjects)
Allergic rhinitis	no causal relationship	1	5.88%
variant angina	no causal relationship	1	5.88%
enterogastritis	no causal relationship	0	0.00%
reflux esophagitis	no causal relationship	1	5.88%
hypertension	no causal relationship	2	11.76%
hypercholesterolemia	no causal relationship	0	0.00%
hyperuricemia	no causal relationship	0	0.00%
hyperlipidemia	no causal relationship	0	0.00%
lumbar contusion	no causal relationship	1	5.88%
ascending colon diverticulitis	no causal relationship	0	0.00%
benign prostatic hypertrophy	no causal relationship	0	0.00%
gallstone	no causal relationship	0	0.00%
contusion of the left shoulder	no causal relationship	1	5.88%
insomnia	no causal relationship	1	5.88%
constipation	no causal relationship	0	0.00%
asymptomatic cerebral infarction	no causal relationship	1	5.88%
contusion of the both thumbs	no causal relationship	1	5.88%

5. Consideration

HbA1c, a primary endpoint of this study, is currently the most important method used for diabetes diagnosis. If we look at the time course change in HbA1c levels for the test diet group, HbA1c gradually decreased at days 28, 56, 84, 112, and 140, showing a significantly lower level at day 168 compared to day 0. These data suggested that ingestion of the test diet is effective at lowering HbA1c levels and improving diabetes.

In addition, regarding other endpoints for diabetes, a significant difference was confirmed between day 168 and day 0 in IRI and HOMA-β levels, and improvement trend was observed in GLU and IRI antibody levels. As to IRI in patients whose IRI level was more than the standard value (12.4 μU/mL) at the starting date of ingestion (day 0) (6 patients in the placebo group/4 patients in the test diet group), the median value in the test diet group decreased to within a range of standard value. From the above results, it was suggested that the test diet exerted a prominent effect especially in patients with excessive secretion of insulin. Based on these multiple factors, the possibility of improving diabetes through the improvement of insulin action was considered, and effectiveness of this test diet in patients with diabetes became evident as demonstrated by the significant difference in HbA1c levels at the end of the study.

As to other endpoints, a significant decrease was shown in γ-GTP level at days 56, 84 and 112, and in GPT level at days 28, 56, 84 112, 140, and 168, while the average value of GOT level showed a decreasing trend. These findings suggest an improvement in hepatic functions. Regarding the skin analysis using Robo Skin Analyzer RSA 50 and Clinical Suite 2.1, significant differences compared to day 0 were observed in pigmentation score at day 140; and in prominent pore score and blackhead scores at days 112, 140, and 168. These results suggest that the test diet may also have an unintended effect on skin appearance. The potential multiple beneficial effects of the test diet and its effectiveness against diabetes will become more evident through close examination of these and future results on this topic.

No causal relationships between the test diet and the adverse events that developed during the 168 day study period were confirmed and no adverse events were confirmed to have been caused by interactions between the test diet and concomitant drugs. Also, no adverse events that developed in patients with complications were considered to be related to the test diet. Both S. crispa and E. grauca have long been used as food ingredients and the high degree of safety that both exhibit have been confirmed in past studies. Therefore, there is very little possibility of occurrence of adverse reaction due to ingestion of the test diet containing these natural ingredients.

Based on the results described above, it is clear that the test diet combining LB-Scr and E. grauca B2 is safe and potentially effective at providing protective and palliative effects against Type-2 diabetes without causing any

adverse reactions. Diabetes is one of the most prominent diseases worldwide, with a rapid and widespread increase in its prominence. It was suggested that the test diet can provide evidence towards improving this disease and all advances will constitute a great contribution to society as they are realized in the future.

6. Reference

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