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Improvement of glucose metabolism via mung bean protein consumption: A clinical trial of GLUCODIA™ isolated mung bean protein in Japan

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ABSTRACT

Background: The main component of mung bean protein, accounting for more than 80%, is 8Sα globulin. Its structure closely resembles that of soybean β-conglycinin. Thereby, the mung bean protein is expected to have similar physiological effects to those of β-conglycinin, but there is no clinical evidence for these effects.

Purpose of this study: The aim of this study was to confirm the positive effects of mung bean protein (GLUCODIA™) on glucose metabolism in clinical trials.

Method: This clinical study was conducted using a double-blind placebo-controlled design with 45 prediabetes patients.

Results: Many of the subjects were pre-diabetes with blood glucose levels exceeding 140 mg/dl by 2-hour plasma glucose level. However, the initial mean fasting plasma glucose level was less than 100 mg/dl. Therefore, mung bean protein did not lower fasting plasma glucose levels. The test period extended from summer to autumn, and increased fasting plasma glucose levels in the placebo group were observed due to seasonal factors. However, this increase was suppressed in the test group. Similarly, the mean insulin level increased in the placebo group, but the increase was also suppressed in the test group. Among obese subjects with a high body mass index, significant increases in fasting plasma glucose and insulin levels in the placebo group were observed. In the comparison between the test and the placebo groups with the average elevation value, there was a significant difference in fasting blood glucose level and significant tendencies in insulin level and homeostatic model assessment for insulin resistance value between the two groups.

Conclusion: Mung bean protein suppresses fasting plasma glucose and insulin levels. Consequently, it may have an inhibitory effect on insulin resistance, a trigger of metabolic syndrome.

Key words: mung bean protein, insulin, obesity, body mass index, randomized clinical trial, seasonal variation.

INTRODUCTION

Mung bean is widely eaten as a porridge and/or vermicelli in China, Southeast Asia, and India. It is also a Chinese medical agent, with antifebrile effects [1, 2]. The polyphenol component of the mung bean seed coat has been shown improvement of plasma glucose levels and antioxidant property [3, 4], while the starch of mung bean increases suppression of plasma glucose levels [5]. However, there are a few reports on the physiological effects of mung bean protein.

The main component of mung bean protein is 8Sα globulin, with a structure very similar to that of soybean β-conglycinin [6], which has been reported to have positive effects on lipid and glucose metabolism [7, 8]. Therefore, mung bean protein is expected to have similar effects owing to its structural similarity to β-conglycinin. We have reported the plasma triglyceride-lowering effect of mung bean protein due to improved insulin sensitivity in rats [9].

Type 2 diabetes is a lifestyle-related disease that has spread worldwide, especially in developed countries. With the progression of type 2 diabetes symptoms, microscopic plasma vessels in the body are gradually destroyed, causing serious damage to various organs of the body [10]. The complications of type 2 diabetes progression include severe conditions such as diabetic neuropathy, diabetic retinopathy, and diabetic nephropathy [11-13].

This study examined the effects of mung bean protein of improving glucose metabolism by assessing insulin sensitivity in prediabetes subjects.

MATERIALS AND METHODS

Experimental food

Isolated mung bean protein (GLUCODIA™) was manufactured using a method identical to that used for the production of isolated soy protein and administered in the form of candy. GLUCODIA™, consisting of 92% mung bean protein, 3% minerals, and 5% water, was sterilized for food use and spray dried. The composition of a test candy is shown in Table 1. The test candy contained 0.625 g of mung bean protein per piece. In the control experiment, placebo candy with the same taste as the test candy was produced by replacing mung bean protein with the milk protein casein (Table 1).

Table 1) Contents of chable tablets.

Materials	Test chable tablet Content (%)	Placebo chable tablet Content (%)
GLUCODIA™ (mung bean protein)	47.0	-
Milk protein (casein Na)	-	47.0
Maltose	34.5	34.5
Citrate acid	2.5	2.5
Flavor	1.5	1.5
Lubricant	2.5	2.5
Cellose	12.0	12.0
Total	100	100

Subjects

All of the test subjects were Japanese men and women 20 to 59 years of age. Individuals undergoing treatment for hyperlipidemia, diabetes, or liver dysfunction or with food allergies were excluded from the study. According to the guidelines of the Japan Diabetes Society, prediabetes is defined as a fasting plasma glucose level of 110-125 mg/dl or 2-hour plasma glucose level (2-h PG) using the 75 g glucose tolerance test (OGTT) of 140-200 mg/dl [14]. This study screened patients for prediabetes patients based on health diagnostic results. Furthermore, some subjects were also screened for hypertriglyceridemia (fasting plasma triglyceride (TG); 150-400 mg/dl). The subjects were screened at two medical examinations (Pre-trial 1 and 2) before trial enrolment.

A total of 189 potential subjects visited the facility for testing at Pre-trial 1. A specific history was obtained from these potential subjects, and they underwent a physical examination and laboratory tests. Based on the results of testing at Pre-trial 1, 98 individuals were identified as suitable for this trial. The 98 individuals visited the facility for testing at Pre-trial 2 and underwent laboratory testing. The subjects consumed 75 g of glucose (Trelan G solution 75 g (AY Pharmaceuticals Co.) for the plasma test. Based on the results of this test, 45 individuals were selected as trial subjects. These subjects visited the medical facility for testing on the date that diet consumption started. A specific history was obtained from these subjects; then they underwent a physical examination and laboratory tests.

Test design

This study was a double-blind placebo-controlled design that lasted for 12 weeks. The subjects were assigned at random to one of two groups: the test group consumed only the test candy containing GLUCODIA^{TM} (n = 23), while the placebo group consumed only the casein-containing placebo candy (n = 22). During the consumption period, subjects visited the medical facility twice during their consumption of that diet (6 weeks and 12 weeks from the start of the test), and plasma samples were collected. The subjects in the test group were instructed

to consume two pieces of candy twice daily, for a total of 2.5 g mung bean protein; i.e., before breakfast and dinner (0.68 g GLUCODIA^{TM} per piece × 92% mung bean protein per GLUCODIA^{TM} × two pieces × twice a day = 2.5 g). Subjects in the placebo group were administered the casein candy in the same manner. A specific history was taken, and subjects underwent a physical examination and laboratory tests. OGTT was performed at the start and the end of the test, and plasma was collected before and 30, 60 and 120 min after consumption of glucose. The subjects recorded information such as their consumption of the test diet, whether or not they took medication, alcohol consumption, and their physical state in a diary from the start until the conclusion of diet consumption. Additionally, the subjects recorded their diet and dietary content for duration of the test period.

The test design was registered in the University Hospital Medical Information Network (UMIN) Clinical Trials Registry (UMIN000014317).

Data collection

Plasma samples were collected from each participant periodically from the start of the consumption period. Plasma samples were collected at 09:00 in the morning after the subjects had fasted from 22:00 the previous night. The following hematological and serum biochemistry measurements were performed by LSI Medience Corporation (formerly Mitsubishi Chemical Medience Corporation, Tokyo, Japan): white plasma cell count, red plasma cell count, hemoglobin, hematocrit, and platelet count; TG, total cholesterol and low-density lipoprotein (LDL)-cholesterol for lipid metabolism; aspartate transaminase, alanine transaminase, gamma-glutamyl transpeptidase, alkaline phosphatase (ALP), and lactate dehydrogenase; total protein, albumin, uric acid, creatine, and total bilirubin for nitrogen metabolism; plasma glucose and HbAlc for glucose metabolism; and the electrolytes Na, K, Ca, Fe, and Cl.

Statistical analysis

A Bonferroni-corrected paired t-test was used to compare changes within each group based on measurements at the start of the test. Figures are presented as the

mean ± standard errors and a significance level (two-tailed) of < 0.05 was considered to indicate a significant difference. A significance level (two-tailed) between 0.05 and 0.10 was considered to indicate a potentially significant difference.

Ethical considerations

The protocol for this trial closely followed the ethical principles set out in the Declaration of Helsinki (2008 revision) and the Ethical Guidelines for Epidemiological Research (2007, Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, and Labor and Welfare [Notice No. 1]). The potential subjects were selected based on inclusion and exclusion criteria in the trial protocol. In order to protect the human rights of the subjects, aspects such as the subject's health, age, sex, ability to provide consent, his or her dependence on the principal investigators, and whether subjects were participating in other trials were considered. Whether or not subjects would be asked to participate in this trial was carefully considered. Careful attention was given to personal information on the subjects involved in this trial with regard to consent forms, case reports, handling of raw data, and publication of data. When case reports were drafted, manuscripts were published, or data were presented, measures were taken to prevent individual trial subjects from being identified (such as designating subjects with a subject identification number), and subjects' personal information was protected.

RESULTS

Subjects

During the evaluation of efficacy, data from three subjects would markedly reduce the accuracy of that evaluation. Therefore, these three subjects were excluded from analysis. The rationales for exclusion were: (1) Fasting plasma glucose (FPG) and insulin levels markedly higher than the mean ± 2SD prior to consumption of the test diet. (2) Subjects with insulin levels equal to reference levels due to hemolysis of laboratory specimens.

Therefore, data from 42 subjects were analyzed (Fig. 1).

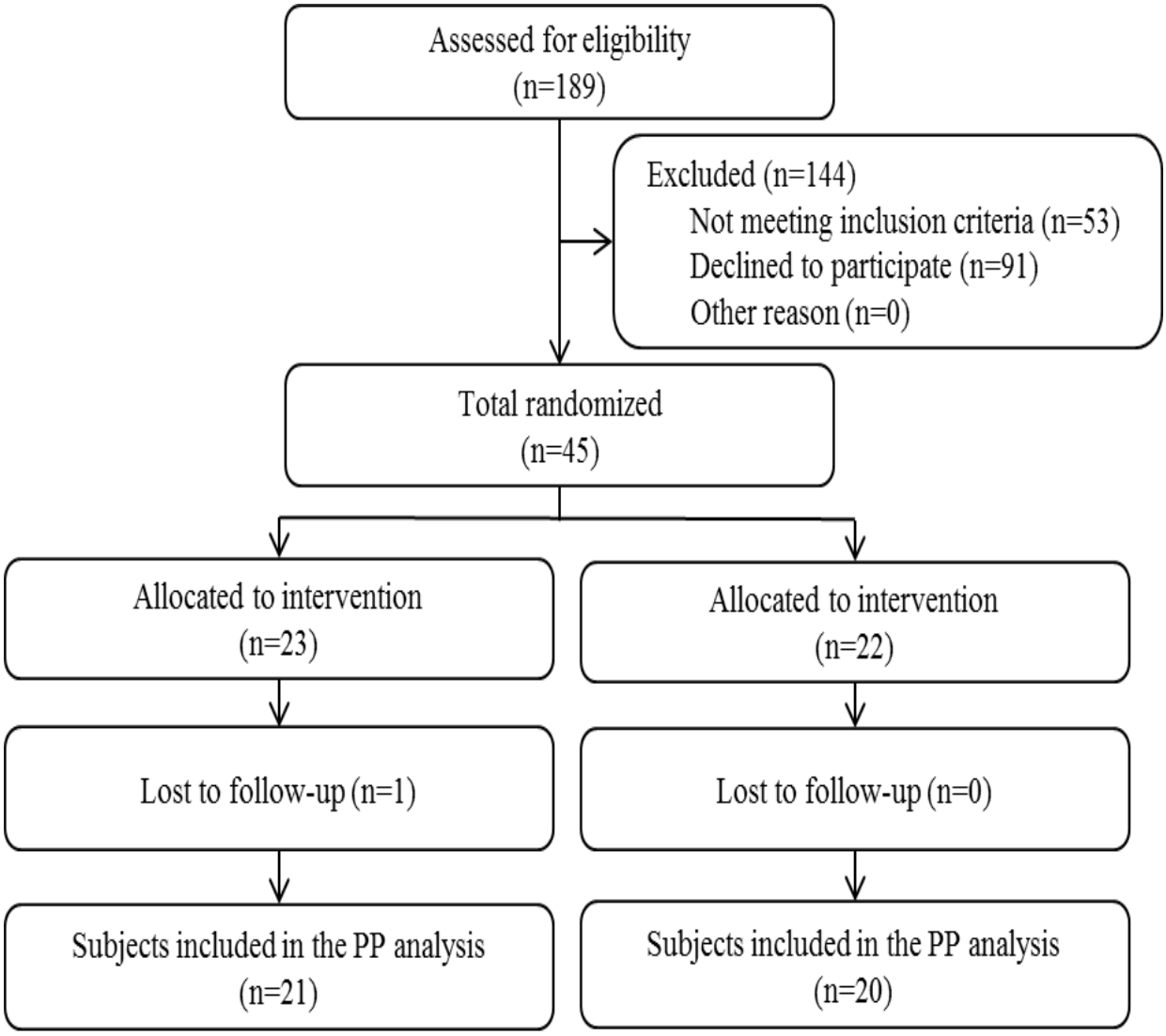

Figure 1: Disposition of study participants. A total of 189 potential participants were screened, and 45 eligible participants were enrolled in the study. Forty-one participants completed the study. One participant was removed from the Per Protocol (PP) analysis.

The background information of the 42 subjects in both groups is summarized in Table 2. The FPG values in the test and placebo groups were 97 ± 2 and 95 ± 2 mg/dl; the TG values were 130 ± 13 and 114 ± 12 mg/dl respectively. There were no significant differences in the initial FPG and TG between the test and placebo groups. The body mass indexes (BMIs) in the test and placebo groups were 29.2 ± 1.0 and 26.2 ± 1.3 kg/m^2 respectively. There was a significant tendency in the difference between the two groups ($p < 0.1$).

Table 2) Initial and final data of clinical characteristics and plasma levels of total study subjects.

			Test	Placebo	p value[1)] (Test vs Placebo)
N(male/female)			21 (13/8)	20 (11/9)	
age			50.7 ± 1.3	51.5 ± 1.1	
BMI	(kg/m^2)	initial	29.2 ± 1.0	26.2 ± 1.3	**0.095**
		final	29.2 ± 1.0	26.2 ± 1.3	**0.072**
Fastig Plasma Glucose	(mg/dl)	initial	97 ± 2	95 ± 2	0.418
		final	97 ± 3	100 ± 3 *	0.725
Insulin	(μU/ml)	initial	8.5 ± 1.0	6.6 ± 0.9	0.315
		final	8.9 ± 1.1	9.6 ± 1.6 *	0.958
HOMA-IR		initial	2.1 ± 0.3	1.6 ± 0.2	0.297
		final	2.1 ± 0.3	2.5 ± 0.5 **	0.969
HbA1c	(%)	initial	6.0 ± 0.1	6.0 ± 0.1	0.431
		final	6.0 ± 0.1	6.0 ± 0.1	0.927
Triglyceride	(mg/dl)	initial	130 ± 13	114 ± 12	0.382
		final	131 ± 14	123 ± 20	0.375
Total Cholesterol	(mg/dl)	initial	206 ± 7	205 ± 7	0.876
		final	209 ± 6	216 ± 6 *	0.506
LDL-Cholesterol	(mg/dl)	initial	131 ± 7	129 ± 7	0.794
		final	127 ± 7	140 ± 10 #	0.389

Data represent the mean ± SE.

*, ** show significant differenes between initial and final data (* $p<0.05$, ** $p<0.01$), and # show a significant tendency between initial and final data ($p<0.1$).

1) The p values show comaprisons between test group and placebo group.

Evaluation of safety

The results of the physical examination reflected physiological variations in the subjects, so the principal investigators deemed that the test food posed no problems in terms of safety. Additionally, some laboratory results fell outside the standard value ranges depending on the individual subject, but these results were specific to the subject or they reflected physiological variations. The principal investigators deemed that the test food posed no clinical problems.

The subjects reported several subjective symptoms during the trial, but these symptoms were due to lifestyle, seasonal factors, or physical condition or were pre-existing symptoms. Since the severity of these symptoms was minimal, the

principal investigators deemed that they were not associated with the test food.

FPG

The mean FPG results are shown in Table 2 and Fig. 2a. The FPG level in the test group did not change during the consumption period. In contrast, the FPG level in the placebo group significantly increased from 95 ± 2 to 100 ± 3 mg/dl. Additionally, the net changes in FPG level in the test and placebo groups were −0.4 ± 2.0 and 5.4 ± 2.5 mg/dl respectively, but the difference between the two groups was not significant (p = 0.114, Fig. 2a).

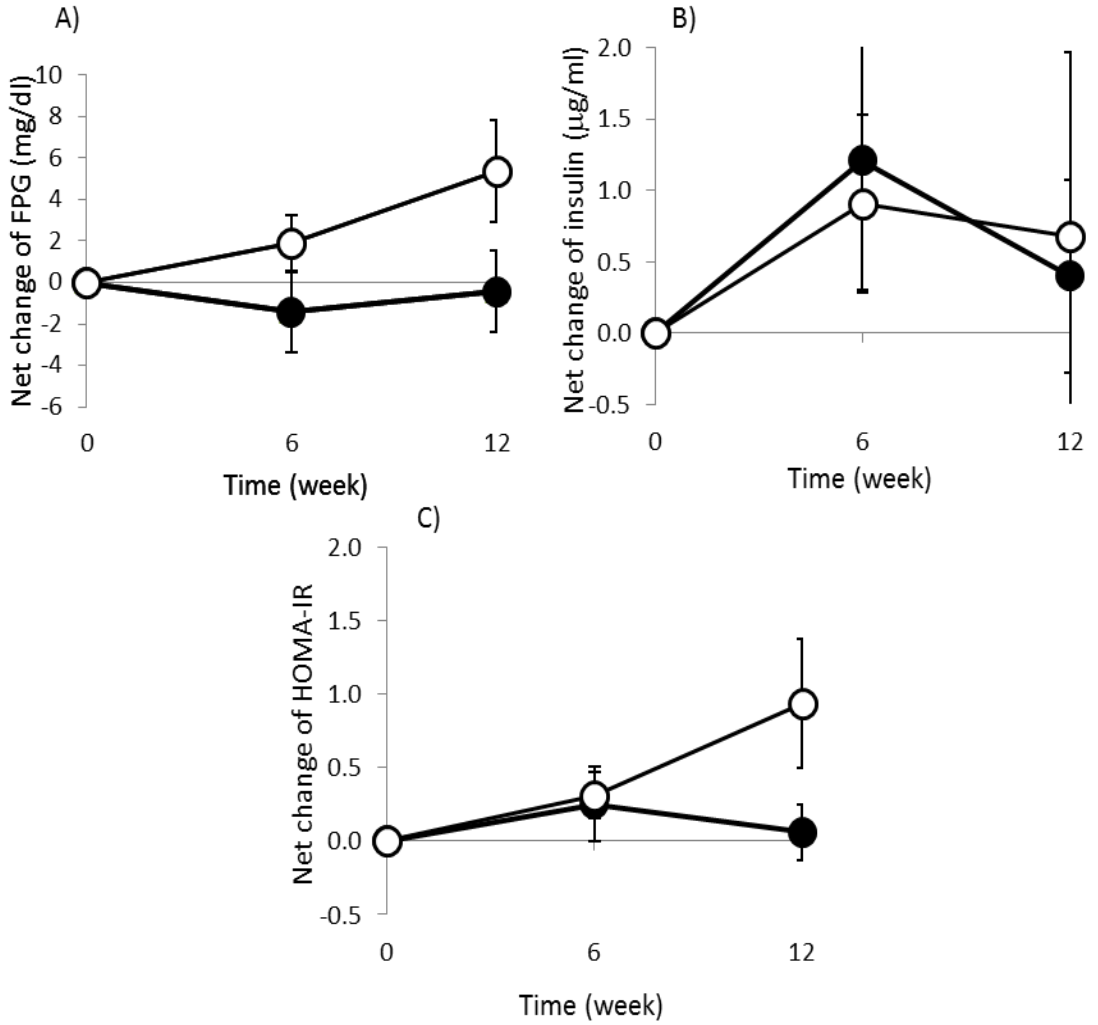

Figure 2: Net changes of total subjects in the parameters of sugar metabolism. A), fasting plasma glucose; B), insulin; C), homeostasis model assessment-Insulin Resistance (HOMA-IR). Test group (solid) and placebo group (open). Bar represent SEMs.

Insulin

The results of insulin testing are shown in Table 2 and Fig. 2b. The fasting insulin level in the test group showed almost no change (8.5 ± 1.0 to 8.9 ± 1.1 μU/ml). In the placebo group, the fasting insulin level significantly increased from 6.6 ± 1.0 to 9.6 ± 1.6 μU/ml ($p < 0.05$, Table 2). However, the net change did not differ significantly between the test and placebo groups ($p = 0.111$, Fig 2b).

Homeostatic Model Assessment for Insulin Resistance

Homeostatic model assessment for insulin resistance values (HOMA-IR) were calculated as follows based on each subject's plasma glucose and insulin levels.

HOMA-IR = fasting insulin levels (μU/ml) × FPG levels (mg/dl) /405.

Similar to the insulin level, HOMA-IR values in the test group showed almost no change. In the placebo group, HOMA-IR values significantly increased from 1.59 ± 0.23 to 2.52 ± 0.51 ($p < 0.01$, Table 2). However, the net change in HOMA-IR values showed no significant difference between the two groups ($p = 0.118$, Fig. 2c).

Glycated hemoglobin (HbA1c)

There were no significant differences in HbA1c levels between or within groups (Table 2).

TG and cholesterol

The TG level in the test group changed little (130 ± 13 to 131 ± 14 mg/dl). In the placebo group, the TG level increased from 114 ± 12 to 123 ± 20 mg/dl. Significant differences in TG levels were not noted between or within groups (Table 2).

Because there was a difference in the initial values between the two groups, TG levels were analyzed, focusing on subjects with high triglyceride levels (>150 mg/dl). The TG level in the test group was reduced. In contrast, the TG level increased in the placebo group (Table 3).

Table 3) Triglyceride of hyperlipidemia sujbects.

			Test	Placebo	p value[1)] (Test vs Placebo)
Numbers of subjects			9	5	
Triglyceride	(mg/dl)	initial	302 ± 112	250 ± 55	0.683
		final	164 ± 26	299 ± 70	0.127
Net chang of TG	(mg/dl)		-138 ± 135	50 ± 49	0.122

Data represent the mean ± SE.

1) The p values show comaprisons between test group and placebo group.

The total cholesterol level in the placebo group increased significantly ($p < 0.05$), and LDL cholesterol level increased with a significant tendency ($p < 0.1$). In the test group, total cholesterol and LDL cholesterol level showed almost no change (Table 2).

OGTT

Plasma glucose levels 120 min after administration of the glucose load were lower in the test group than those in the placebo group at the start and end of the diet consumption (Table 4a and b). In contrast, insulin levels showed the opposite trend. There were no significant differences between groups for either glucose or insulin levels.

Table 4a) Plasma glucose and insulin for OGTT at initial time of this study.

Time (min)	0	30	60	120
Glucose (mg/dl)				
Test	97 ± 2	165 ± 4	193 ± 7	159 ± 8
Placebo	95 ± 2	160 ± 5	203 ± 8	169 ± 9
Insulin (μU/ml)				
Test	8.5 ± 1.0	40.4 ± 4.8	56.7 ± 7.7	67.2 ± 10.7
Placebo	6.6 ± 0.9	30.3 ± 4.2	47.5 ± 5.7	54.4 ± 7.5

Data represent the mean ± SE.

Table 4b) Plasma glucose and insulin for OGTT at final time of this study.

Time (min)	0	30	60	120
Glucose (mg/dl)				
Test	97 ± 3	174 ± 6	197 ± 7	153 ± 8
Placebo	100 ± 3	173 ± 8	204 ± 10	169 ± 12
Insulin (μU/ml)				
Test	8.9 ± 1.1	46.7 ± 6.9	65.9 ± 7.9	77.5 ± 12.1
Placebo	9.6 ± 1.6	43.5 ± 5.7	51.9 ± 6.5	66.1 ± 10.7

Data represent the mean ± SE.

Background information on obese subjects

In this study, the BMI in the placebo group was lower than that of the test group ($p<0.1$, Table 2). Among the total subjects, those with a BMI above 25 kg/m^2 were selected and analyzed as obese subjects. The background information on these subjects is shown in Table 5. The initial BMIs in the test and placebo groups were 30.2 ± 0.9 and 30.0 ± 1.1 kg/m^2 respectively. No significant differences were observed between the groups.

FPG levels in obese subjects

There was little change in FPG level in the test group (96 ± 2 mg/dl prior to diet consumption vs. 95 ± 3 mg/dl after 12 weeks of diet consumption). In contrast, the FPG level in the placebo group increased from 96 ± 2 to 103 ± 4 mg/dl, a difference with a significant tendency ($p < 0.1$, Table 5). The net changes in FPG level in the test and placebo groups were −1.3 ± 2.2 and 7.0 ± 3.5 mg/dl respectively, which was a significant difference ($p < 0.05$, Fig. 3a).

Table 5) Initial and final data of clinical characteristics and plasma levels of obese study subjects.

			Test	Placebo	p value[1)] (Test vs Placebo)
N(male/female)			18 (12/6)	12 (7/5)	
age			50.3 ± 1.6	51.2 ± 1.6	
BMI	(kg/m^2)	initial	30.2 ± 0.9	30.0 ± 1.1	0.950
		final	30.2 ± 0.9	30.0 ± 1.2	0.692
Fastig Plasma Glucose	(mg/dl)	initial	96 ± 2	96 ± 2	0.884
		final	95 ± 3	103 ± 4 #	0.113
Insulin	(μU/ml)	initial	9.4 ± 1.0	9.2 ± 0.8	0.692
		final	9.7 ± 1.2	13.6 ± 2.0 *	***0.031***
HOMA-IR		initial	2.3 ± 0.3	2.2 ± 0.2	0.662
		final	2.3 ± 0.3	3.6 ± 0.7 *	***0.043***
HbA1c	(%)	initial	6.0 ± 0.1	6.0 ± 0.1	0.884
		final	6.0 ± 0.1	6.1 ± 0.1 *	0.439
Triglyceride	(mg/dl)	initial	140 ± 14	129 ± 17	0.632
		final	137 ± 15	138 ± 31	0.439
Total Cholesterol	(mg/dl)	initial	206 ± 8	208 ± 6	0.723
		final	210 ± 7	213 ± 5 #	0.723
LDL-Cholesterol	(mg/dl)	initial	131 ± 8	135 ± 7	0.662
		final	128 ± 8	137 ± 6	0.465

Data represent the mean ± SE.

* shows a significant differenes between initial and final data ($p<0.05$), and # show a significant tendency between initial and final data ($p<0.1$).

1) The p values show comaprisons between test group and placebo group.

Insulin levels of obese subjects

The fasting insulin level in the test group showed almost no change (9.4 ± 1.0 to 9.7 ± 1.2 μU/ml). The level in the placebo group significantly increased from 9.2 ± 0.8 to 13.6 ± 2.0 μU/ml ($p < 0.05$), and a significant difference between the two groups was observed ($p < 0.05$, Table 5). Additionally, the net changes in insulin levels in the test and placebo groups were 0.4 ± 0.8 and 4.4 ± 2.2 μU/ml respectively, and there was significant tendency between the two groups ($p < 0.1$, Fig. 3b).

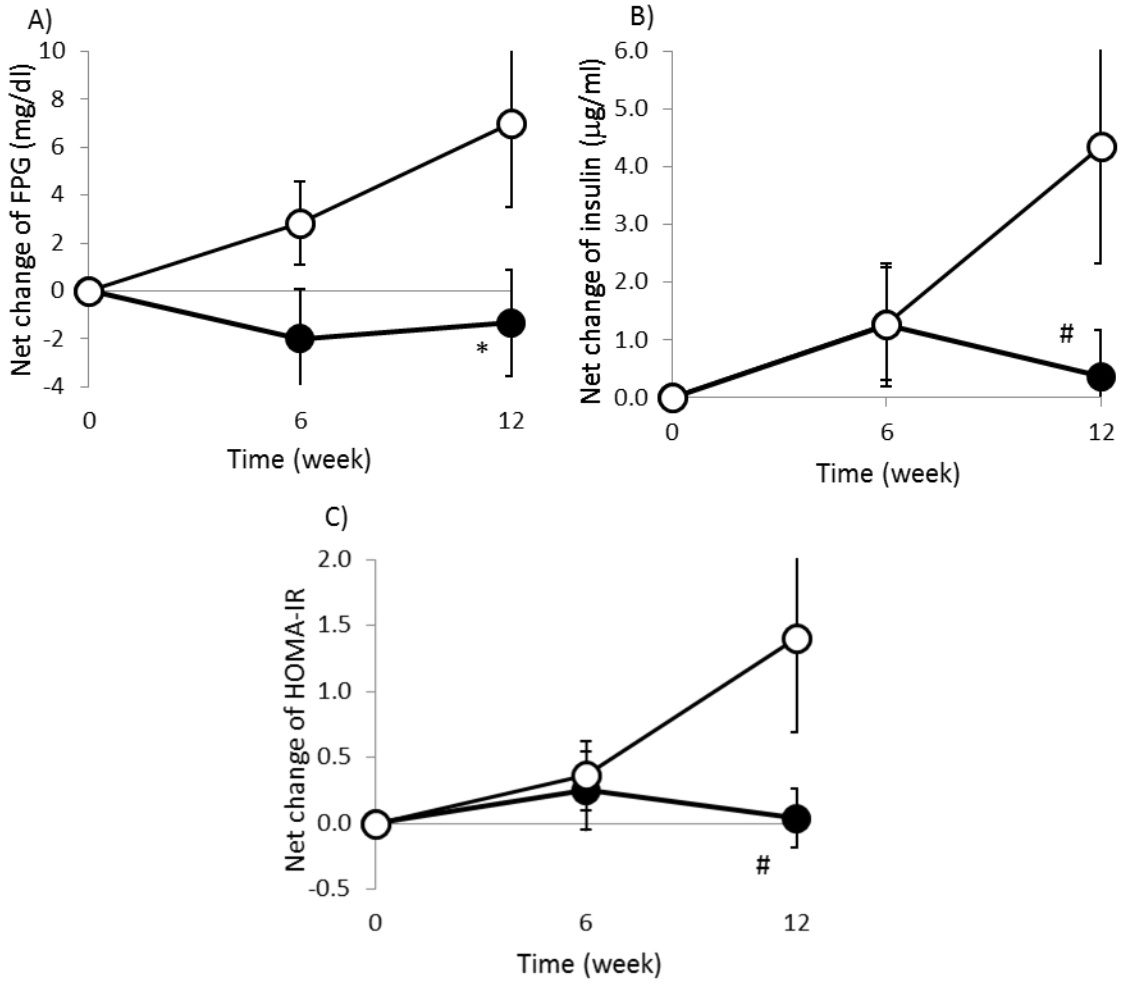

Figure 3: Net changes of obese subjects (initial BMI > 25kg/m^2) in the parameters of sugar metabolism. A), fasting plasma glucose; B), insulin; C), homeostasis model assessment-Insulin Resistance (HOMA-IR). Test group (solid) and placebo group (open). Bar represent SEMs. * shows a significant difference between test and placebo groups ($p<0.05$), and # shows a significant tendency between test and placebo groups ($p<0.1$).

HOMA-IR values in obese subjects

HOMA-IR in the test group showed almost no change, similar to the findings of the total subjects (Table 5). In the placebo group, HOMA-IR increased

significantly, from 2.2 ± 0.2 to 3.6 ± 0.7 ($p < 0.05$). The net change of HOMA-IR showed a significant tendency between the test and placebo groups ($p < 0.1$, Fig 3c).

TG and cholesterol levels in obese subjects

Triglyceride levels slightly decreased in the test group, while those in the placebo group increased, but the difference was not significant. Total cholesterol levels rose in the control group with a significant tendency ($p < 0.1$), but the LDL cholesterol levels did not change (Table 5).

DISCUSSION

This study included prediabetes subjects, based on the diabetes diagnostic guidelines of the Japan Diabetes Society [14]. The subjects in this study were considered to be positive for prediabetes based on 2-h PG values after an OGTT, which is one of the diagnostic methods. The FPG levels in the subjects were within the normal range.

In this study, the 2-h PG value after OGTT in the test group decreased to 153 ± 8 mg/dl from 159 ± 8 mg/dl. The values in the placebo group did not change (Table 4a and b). The insulin value in the OGTT was high in the test group, which suggests that insulin was sufficiently secreted to quickly lower plasma glucose levels to the steady-state value. FPG levels increased significantly after 12 weeks of diet consumption in the placebo group but changed little in the test group (Table 2 and Fig 2a). Additionally, fasting insulin levels in the placebo group increased significantly after 12 weeks of diet consumption. However, in the test group, no changes in the fasting insulin levels were observed (Table 2 and Fig 2b).

In this study, the initial mean BMI differed significantly between the two groups (Table 2). Therefore, data from obese subjects (BMI > 25 kg/m^2) were analyzed. In these subjects, the effects of improved FPG and insulin levels were greater than those for the total subjects (Table 5 and Fig 3a-c). A principal component of mung bean protein is 8Sα globulin, which is structurally similar to that of soybean β-conglycinin [5]. Soybean β-conglycinin reportedly improves

insulin sensitivity in rats and decreases fasting insulin levels or reduces visceral fat and body fat in clinical studies [7]. In previous study [7], the reducing effect of visceral fat by β-conglycinin was remarkably observed in obese subjects with visceral fat areas greater than 100 cm^2. Thus, the same trend as the effect of β-conglycinin was observed in mung bean protein.

This clinical study was conducted from the summer to the autumn. Seasonal variations are frequently observed in the metabolism of mammals [15]. Some human physiological parameters (such as blood pressure, heart rate, lipid profile, insulin, and serum cortisol) are influenced by seasonal cycles [16-20]. In particular, a seasonal variation of HbA1c as glucose homeostasis has been reported in various geographic regions, including the United States, the UK, Poland, Sweden, Spanish, Portugal, and Korea. These investigations revealed the highest and lowest HbA1c values in winter and summer respectively [16, 21-26]. In Japan, Sakura et al. reported the highest HbA1c values in March and lowest values in August among diabetic patients with type 1 or type 2 diabetes [27]. Ishii et al. reported that meal HbA1c levels in type 2 diabetic patients were elevated by ~0.5% in winter compared with those during the period between spring and autumn [28]. Sohmiya et al. reported a relationship between seasonal changes in body composition and HbA1c level in type 2 diabetic patients [29]. They reported increased and decreased body fat and HbA1c levels in winter and summer respectively, without any appreciable change in body weight.

In the current study, FPG and insulin levels increased in the placebo group, but there was little change in HbA1c levels in either group. HbA1c, which is the product of non-enzymatic glycation of the hemoglobin molecule, reflects plasma glucose levels throughout the previous 2-3 months [30]. Significant increases in FPG and insulin levels in the placebo group were considered the result of these naturally occurring factors preceding the increase of HbA1c levels. Therefore, if this study were continued through the winter months, it is presumed that HbA1c levels would have increased. Mung bean protein may control these spontaneous variations and act to stabilize FPG and insulin levels.

Insulin resistance indicates a state where insulin action fails to keep up with insulin levels in the plasma owing to decreased signal transducers for the insulin

receptor. Accumulation of visceral fat is a key factor for the induction of insulin resistance [31].

In the current study, mung bean protein was shown to control spontaneous seasonal variations and to stabilize FPG and insulin levels. These effects were greater in obese subjects (Table 5 and Fig 3a-c). These findings indicate that mung bean protein may improve insulin sensitivity through the accumulation of visceral fat. TG levels largely decreased as a result of analysis limited to the subject of hyperlipidemia (Table 3). Soybean β-conglycinin reportedly improves high TG levels by induction of β-oxidation, downregulation of fatty acid synthase, and inhibition of TG absorption in mice and rats [32, 33]. Consumption of mung bean protein may act in a similar manner. Indeed, mung bean protein has been reported to reduce plasma TG levels by the active suppression of fatty acid synthase by inhibition of sterol regulatory element-binding protein 1 (SREBP-1) expression in the rat liver [9]. In this study, triglyceride, total-cholesterol and LDL-cholesterol levels increased in the placebo group (Table 2, 3 and 5). Some literature reported that casein increased the cholesterol level as casein-induced hypercholesterolemia in swine [34]. But in our previous clinical study [7], these levels in the placebo, casein group almost no change. Thus, this phenomenon was suggested to be induced as one of the seasonal variations observed in the metabolism of mammals [15].

Mung bean is a traditional food in Asia. It is generally eaten as a boiled porridge, and mung bean starch is used in noodle form, but the protein itself is not typically consumed as a food. Mung bean protein is safer than soy protein in terms of being a non-genetically modified organism and not typically an allergic source. Future studies should confirm the effects of mung bean protein in subjects with higher levels of insulin resistance.

CONCLUSIONS

Mung bean protein acted to maintain fasting blood glucose and insulin levels constant by controlling these seasonal variations. These effects were particularly observed in obese subjects. It is useful for the maintenance of the insulin sensitivity to keep insulin secretion constant. Mung bean protein may improve

plasma triglyceride levels. As a result, this finding suggests that mung bean protein may improve insulin sensitivity. In future studies, the investigators would like to evaluate the effect of mung bean protein on visceral and body fat or adiponectin.

Competing interests: This study was made by CRO, with the funds of the Fuji Oil Holding Inc. affiliated with the author. This study received no specific grant from any other funding agency, commercial or not-for-profit sectors.

Author's contributions: The authors' responsibilities were follows: Takayasu Motoyama contributed to the idea of the study. Mitsutaka Kohno and Sakamoto Mai participated in the design of the study. Yuhko Shigihara and Hideo Sugano performed the production of experimental foods.

The authors declare that there are no financial and personal relationships with other people and organisations that could inappropriately influence the present study.

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