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原　著

Effects of the Intake of HYA-containing Food on Postprandial Hyperglycemia : a Randomized, Placebo-controlled, Double-blind Crossover Trial

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Abstract

This study evaluates the effects of the intake of 10-hydroxy-*cis*-12-octadecenoic acid (HYA) in a clinical trial. HYA can inhibit the elevation of postprandial blood glucose levels and may have the effect in preventing diabetes mellitus.

Key words : 10-hydroxy-*cis*-12-octadecenoic acid, postprandial hyperglycemia, randomized placebo-controlled double-blind crossover trial, clinical study

Introduction

Lately, lifestyle-related diseases such as obesity, dyslipidemia, and diabetes mellitus, which result from the Westernization of dietary habits, overeating, and a lack of exercise, are becoming increasing social problems. Metabolic syndrome is a state in which visceral fat obesity has induced at least two of the following conditions : hyperglycemia, hypertension, and dyslipidemia, and the risk of arteriosclerosis has increased. Among Japanese people ages 40 to 74, one in three men and one in five women are thought to have metabolic syndrome or to be at risk of developing it[1)], and the importance of seeking to optimize calorie intake through diet therapy is proposed for preventing and stopping the progression of visceral fat accumulation in metabolic syndrome. Because of such observations, studies of functional food ingredients capable of improving lipid metabolism or alleviating diabetes mellitus have been actively conducted. Much attention has been given to the intake of functional lipids such as conjugated fatty acids (including conjugated linoleic acid), n-3 polyunsaturated fatty acids (including eicosapentaenoic acid and docosahexaenoic acid), and medium-chain fatty acids. In addition, some oxoacids, including 9-oxo-octadecadienoic acid and 13-oxo-octadecadienoic acid from tomatoes, have been reported to alleviate lifestyle-related diseases[2)], e.g., by improving dyslipidemia. Thus, attention has been focused on the physiological functions of rare fatty acids, including oxo fatty acids and hydroxylated fatty acids.

10-hydroxy-*cis*-12-octadecenoic acid (HYA) is a gut microbial metabolite derived from linoleic acid, which is a chief component of vegetable oil. Kishino et al. elucidated the mechanism underlying the formation of HYA by lactic acid bacteria and found that a group of enzymes involved in the generation of HYA acts on the double bond of unsaturated fatty acids such as linoleic acid and α-linolenic acid to catalyze

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the reactions of hydration, oxidation, reduction, and dehydration, thereby producing various new fatty acids[3]. Furthermore, they conducted an analysis of SPF mice and GF mice to verify that metabolites of these unsaturated fatty acids are present in the body and that they are generated by intestinal bacteria. These mice were provided with ordinary feed, and the distribution of free fatty acids in the colon, small intestine, and plasma was analyzed. As a result, hydroxylated fatty acid HYA and 13-hydroxy-*cis*-12-octadecenoic acid, which are the initial metabolites of linoleic acid, and 10-hydroxy-octadecanoic acid, which is the initial metabolite of oleic acid, were identified in various tissues. Moreover, HYA, 13-hydroxy-*cis*-12-octadecenoic acid, and 10-hydroxy-octadecanoic acid were found to be present at significantly higher concentrations in the small intestines of SPF mice than in those of GF mice. These results suggest that fatty acids originating from the consumed feed are converted into hydroxylated fatty acids depending on the presence of intestinal bacteria[3]. Furthermore, the functionality of these fatty acids has been evaluated in various assay systems, and interesting activities have been discovered. Miyamoto et al. reported that HYA can facilitate recovery from intestinal epithelial barrier injury through GPR40. It has been discovered that the addition of IFN-γ and TNF-α to a human intestinal epithelial-like cell line (Caco-2 cells) damages tight junctions ; however, the addition of HYA can promote recovery. Furthermore, it has been demonstrated that when dextran sulfate sodium (DSS)-induced colitis model mice were made to take HYA in advance, recovery from DSS-induced intestinal epithelial inflammation, from shortening of the large intestinal length, from diarrhea and bloody feces associated with inflammation, and from weight loss was observed. These actions are thought to be manifested when HYA inhibits the expression of TNFR2 in intestinal epithelial cells through GPR40[4]. Miyamoto et al. also confirmed that, when HYA is orally administered to mice, GLP-1 and insulin concentrations in the blood increase[5]. These reports suggest that HYA generated by linoleic acid as a result of metabolism by intestinal bacteria, including lactic acid bacteria, acts on long-chain fatty acid receptors expressed in the digestive tract, thereby exerting various physiological activities, and that one of these activities may be the control of blood glucose levels. In recent years, it has been reported that a long-chain fatty acid receptor, GPR40, promotes insulin secretion in a manner dependent on glucose concentration in pancreatic β-cells through stimulation by long-chain fatty acids. These results are consistent with the idea that a long-chain fatty acid that is a ligand of GPR40 may become a candidate drug for the treatment of type 2 diabetes mellitus[6].

Hyperglycemia induces vascular endothelial dysfunction and inflammation. The sudden elevation of blood glucose levels several hours after a meal (a glucose spike) is believed to damage blood vessels, thereby accelerating arteriosclerosis and increasing the risks of myocardial and cerebral infarctions[7]. Because this problem cannot be detected from the fasting blood glucose levels measured during health check-ups, it is sometimes called "hidden diabetes mellitus," and the proportion of people strongly suspected to have diabetes is assumed to be 20.1% for males and 9.3% for females[1]. For these reasons, it is important even for people with prediabetes to take care of sudden changes in blood glucose after meals, and an effective substance that can be consumed routinely is expected to be useful for preventing diabetes mellitus.

Therefore, this study was conducted to evaluate the effects of consuming HYA-containing food on postprandial blood glucose levels in men and women from 20 to 69 years of age.

Methods

1. Test food

Gelatin-coated capsules containing HYA served as a test food. In this study, HYA was given at two dose levels ; 1,000 mg of HYA was given to the low-dose group and 2,000 mg was given to the high-dose group. Capsules containing no HYA were used as the placebo.

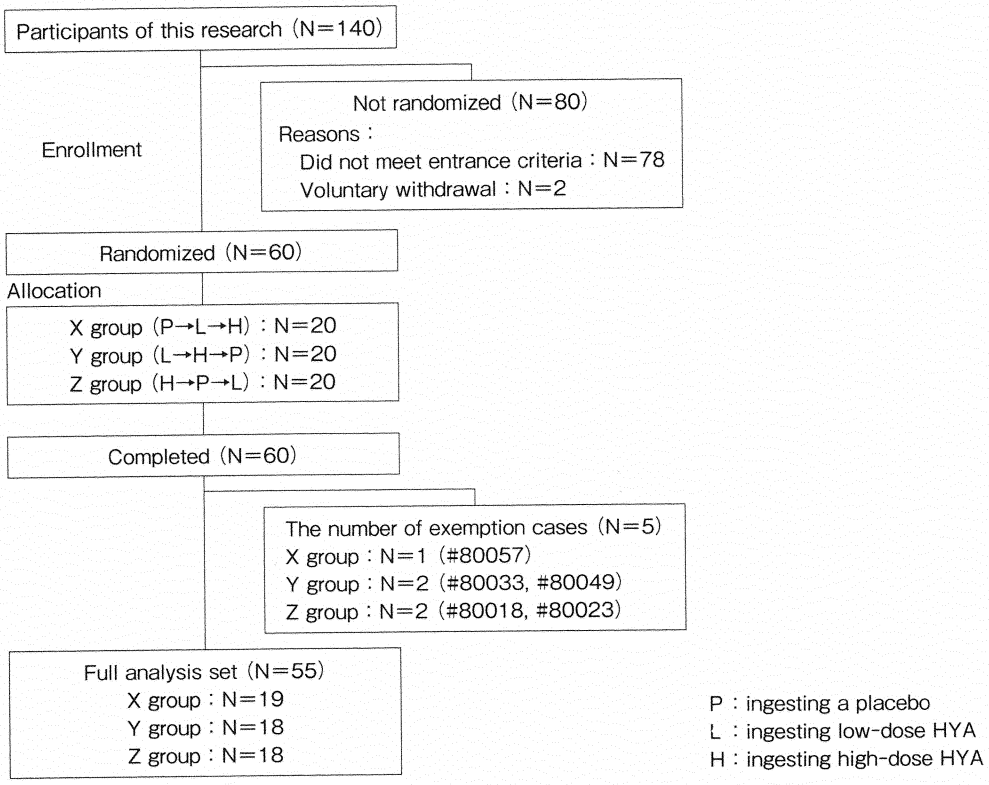

Figure 1　Participant disposition

2. Subjects

In accordance with the Declaration of Helsinki, this study was conducted in compliance with the Ethical Guidelines for Medical and Health Research Involving Human Subjects (Ministry of Education, Culture, Sports, Science and Technology, Japan, and Ministry of Health, Labour and Welfare, Japan), after undergoing a review and receiving approval from the Ethical Review Board of Aisei Hospital, Ueno Clinic Research Ethics Committee (date of approval : July 13, 2016). The principal investigator provided a detailed explanation of the study to the subjects and obtained their voluntary informed written consent. In this study, subjects were recruited by the P-one Clinic, and 60 subjects who satisfied the following inclusion criteria (i) and (ii) and did not match any of the exclusion criteria were enrolled.

(i) Men and women from 20 to 69 years of age

(ii) Persons whose blood glucose level 30 minutes after food ingestion during the preliminary tolerance test was ≧140 and ≦199 mg/dL

3. Study schedule

The test food was consumed in one sitting. The washout period was 1 week. The test food was consumed 3 times in total according to the sequence shown in the assignment table in **Figure 1**.

4. Study method

This study was based on a three-way randomized double-blind crossover design. Subjects were asked to consume.one pack of test food with water without chewing. After that, they were asked to eat load food (300 g of rice) and supplement food (210 g of chicken and egg mixed in a bowl) over the course of 10 minutes. Blood samples were collected before and 30, 60, 90, and 120 minutes after ingestion of the load food.

5. Endpoints

The primary endpoint was the area under the concentration-time curve for the blood glucose level (blood glucose AUC) after ingesting the load food.

The secondary endpoints were the fasting blood glucose level ; blood glucose levels 30, 60, 90, and 120 minutes after ingesting the load food ; the peak blood glucose level after ingesting the load food (blood glucose C_{max}) ; the fasting insulin level ; and insulin levels 30, 60, 90, and 120 minutes after ingesting the load food.

Table 1 Baseline demographic and clinical characteristics

	X	Y	Z	Average	p value
Number of participants	19	18	18		
Male	9	15	8		0.030*
Female	10	3	10		
Age (years)	51.1 ± 10.7	49.2 ± 10.1	50.9 ± 7.3	50.4 ± 9.4	0.797
Height (cm)	165.2 ± 8.5	168.4 ± 9.7	160.7 ± 8.2	164.8 ± 9.2	0.037*
Weight (kg)	61.2 ± 10.7	64.5 ± 10.2	58.1 ± 8.6	61.2 ± 10.0	0.162
BMI (kg/m^2)	22.3 ± 2.4	22.6 ± 2.3	22.5 ± 2.4	22.5 ± 2.4	0.897
Fasting blood glucose (mg/dL)	99.1 ± 8.1	99.8 ± 7.3	98.1 ± 6.5	99.0 ± 7.3	0.783
Blood glucose at 30 minutes after injection (mg/dL)	165.7 ± 12.9	167.9 ± 13.1	170.0 ± 12.0	167.8 ± 12.6	0.588
$AUC_{0\text{-}120\ min}$ (mg · h/dL)	301.0 ± 41.5	306.0 ± 37.1	315.4 ± 29.2	307.3 ± 36.2	0.478
C_{max} (mg/dL)	183.8 ± 25.4	184.1 ± 21.2	188.4 ± 20.7	185.4 ± 22.3	0.792

Data are mean ± SD or number of participants. BMI : body mass index, AUC : the area under the blood concentration-time curve, C_{max} : maximum observed peak blood glucose level. The data were assessed by Chi-square tests (sex) or one-way ANOVA.

The safety endpoint was an adverse event (subjective symptoms and objective signs or findings).

6. Statistical analysis

Values were expressed as the mean ± standard deviation. The level of significance of the tests was a two-sided 5 %.

Results

1. Selection of subjects

The classification and disposition of subjects in this study are shown in **Figure 1**.

A preliminary test was administered to 140 subjects who had given their informed written consent. Sixty subjects, who were selected on the basis of the preliminary test's results, were randomly assigned to one of the groups, and the study was started. For some subjects, the values of some parameters were outside the reference range. Nonetheless, they were enrolled in the study after the principal investigator confirmed the absence of problems from study participation.

2. Disposition of subjects included in the analysis

All 60 subjects who started the study completed the designated schedule and test items. Among these 60 subjects, the following 5 subjects matched the exclusion criteria, and it was deemed appropriate to exclude them from the efficacy analysis before code breaking the randomization table (**Figure 1**). Subjects #80018 (Group Z) and #80023 (Group Z) did not finish the load food in Periods Ⅱ and Ⅲ of the eating schedule. Subject #80033 (Group Y) did not finish the load food at the time of the test in Period Ⅲ of the eating schedule, and ate a sugar-rich food at 21 : 00 on the day before the test in Period Ⅱ of the eating schedule. Subject #80049 (Group Y) was in poor physical condition for a prolonged period after enrollment, and started the oral administration of a prescription drug after the onset of sinusitis. The symptom of diarrhea also appeared after ingesting the load food. Subject #80057 (Group X) ate supper on the day before the tests in Periods Ⅰ and Ⅲ of the eating schedule but did not eat supper on the day before the test in Period Ⅱ of the eating schedule. Therefore, only 55 subjects were included in the efficacy analysis. All 60 subjects who consumed the test food were included in the safety analysis.

3. Characteristics of the subjects included in efficacy analysis

Table 1 shows these characteristics (gender, age, body height, body weight, body mass index (BMI), fasting blood glucose level, blood glucose level 30 minutes after ingesting the load food, blood glucose AUC, and C_{max}). Significant differences were observed among the three groups in gender and height. Significant differences in any other parameters were not detected.

4. Efficacy assessment

The values determined after ingesting low-dose HYA (L), after ingesting high-dose HYA (H), and

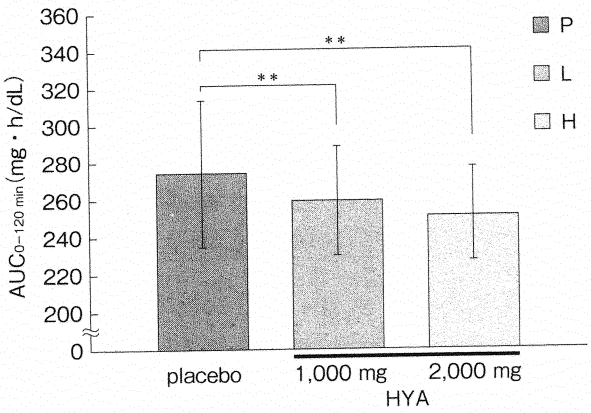

Figure 2　AUC of the blood glucose after ingestion in all subjects

Each value is expressed as the mean ± SD.

The data were assessed by Tukey's multiple comparison tests. * : $p<0.05$, ** : $p<0.01$.

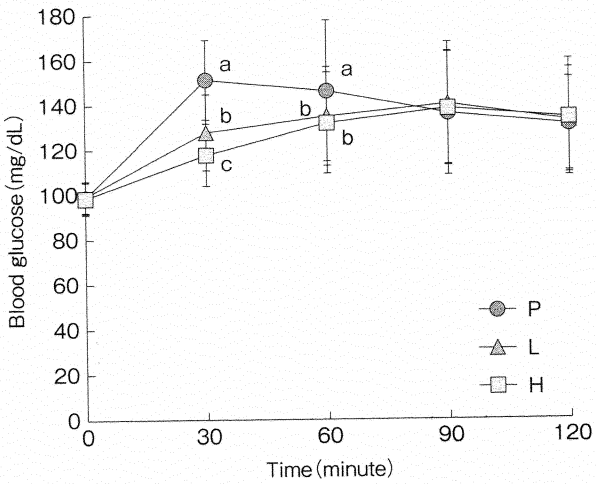

Figure 3　Changes in blood glucose after ingestion in all subjects

Each value is expressed as the mean ± SD. Blood samples were collected before and 30, 60, 90, and 120 minutes after food ingestion.

The data were assessed by Tukey's multiple comparison tests.

a, b, c : Different letters represent significant differences between groups ; $p<0.01$.

after ingesting a placebo (P) and the results of the analysis of variance are shown in **Figures 2—5.**

The blood glucose AUC was 274.2 ± 39.2 mg・h/dL after P ingestion, 259.4 ± 29.0 mg・h/dL after L ingestion, and 252.1 ± 24.8 mg・h/dL after H ingestion. Significant differences were observed between P and L ($p < 0.001$) and between P and H ($p < 0.001$) after the analysis of variance ; however, no significant difference was seen between L and H (**Figure 2**).

The blood glucose level 30 minutes after ingesting the load food was 151.3 ± 17.5 mg/dL after P ingestion, 127.8 ± 16.8 mg/dL after L ingestion, and 117.7 ± 13.8 mg/dL after H ingestion. Significant differences were observed between P and L ($p < 0.001$), P and H ($p < 0.001$), and L and H ($p < 0.001$) after the analysis of variance (**Figure 3**).

The blood glucose level 60 minutes after ingesting the load food was 146.2 ± 31.2 mg/dL after P ingestion, 134.9 ± 21.9 mg/dL after L ingestion, and 131.9 ± 22.4 mg/dL after H ingestion. Significant differences were observed between P and L ($p = 0.002$) and between P and H ($p < 0.001$) after the analysis of variance ; however, no significant difference was seen between L and H (**Figure 3**).

Blood glucose C_{max} was 159.0 ± 23.8 mg/dL after P ingestion, 151.4 ± 21.0 mg/dL after L ingestion, and 150.2 ± 19.7 mg/dL after H ingestion. Significant differences were observed between P and L ($p = 0.012$) and between P and H ($p = 0.003$) after the analysis of variance ; however, no significant difference was seen between L and H (**Figure 4**).

The insulin level 30 minutes after ingesting the load food was 59.99 ± 24.79 μU/mL after P ingestion, 31.30 ± 16.74 μU/mL after L ingestion, and 21.18 ± 14.15 μU/mL after H ingestion. Significant differences were observed between P and L ($p < 0.001$), P and H ($p < 0.001$), and L and H ($p < 0.001$) after the analysis of variance (**Figure 5**).

The insulin level 60 minutes after ingesting the load food was 49.07 ± 19.46 μU/mL after P ingestion, 39.99 ± 21.19 μU/mL after L ingestion, and 29.74 ± 17.30 μU/mL after H ingestion. Significant differences were observed between P and L ($p = 0.004$), P and H ($p < 0.001$), and L and H ($p < 0.001$) after the analysis of variance (**Figure 5**).

The insulin level 90 minutes after ingesting the load food was 41.25 ± 21.29 μU/mL after P ingestion, 45.55 ± 27.98 μU/mL after H ingestion, and 36.15 ± 17.42 μU/mL after H ingestion. Significant differences were observed between L and H ($p = 0.004$) after the analysis of variance ; however, no significant difference was seen between P and L and between P and H (**Figure 5**).

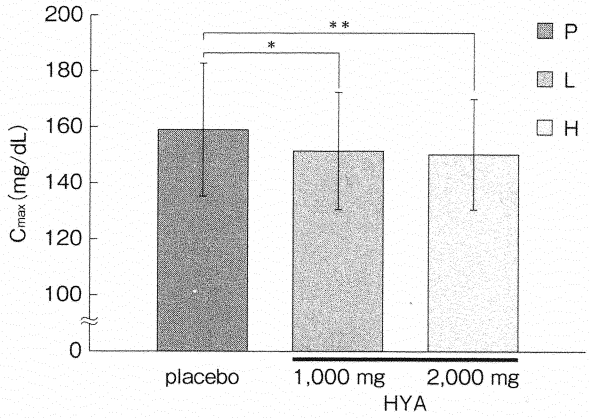

Figure 4 C_{max} of the blood glucose after ingestion in all subjects

Each value is expressed as the mean ± SD.

The data were assessed by Tukey's multiple comparison tests. * : $p<0.05$, ** : $p<0.01$.

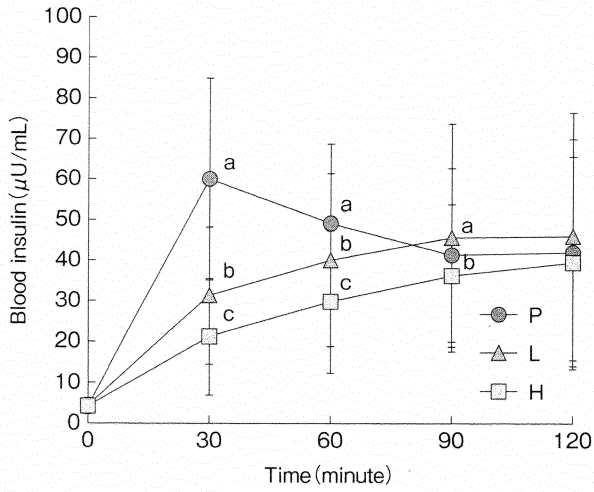

Figure 5 Changes in blood insulin after ingestion in all subjects

Each value is expressed as the mean ± SD. Blood samples were collected before and 30, 60, 90, and 120 minutes after food ingestion.

The data were assessed by Tukey's multiple comparison tests.

a, b, c : Different letters represent significant differences between groups ; $p<0.01$.

5. Safety assessment

A list of adverse events that occurred after ingesting the test food is presented in **Table 2.**

The principal investigator found abdominal symptoms in 5 subjects (loose stool, diarrhea, and abdominal pain) to be "possibly related" to the test food. Patient #80039 (a 66-year-old man) consumed P during the test in Period Ⅰ and developed loose stool on the following day. Patient #80049 (a 51-year-old woman) ate L during the test in Period Ⅰ, and diarrhea presented on the same day. She ate H during the test in Period Ⅱ, and diarrhea appeared on the same day. Patient #80051 (a 50-year-old woman) ate H during the test in Period Ⅰ and developed abdominal pain on the same day. Patient #80057 (a 55-year-old man) consumed L during the test in Period Ⅱ, and diarrhea presented on the following day. Patient #80059 (a 49-year-old woman) ate H during the test in Period Ⅰ, and diarrhea emerged on the same day. All of these clinical signs were mild. The effects of relevant ingredients were suspected to be the cause of the diarrhea because such a sign was not observed after subjects ingested the placebo. As to other adverse events, a total of 16 cases of subjective symptoms were observed in 12 of 60 subjects during the study period. Nevertheless, all symptoms were mild, and there were no serious adverse events. The principal investigator determined all of these events were "not related" to the test food.

Discussion

A total of 60 adults (men and women) susceptible to elevated postprandial blood glucose levels were asked to ingest HYA-containing food once, and the dose at which the increase in postprandial blood glucose levels was inhibited was determined.

The results revealed that blood glucose AUC as the primary endpoint was significantly lower after the ingestion of both low-dose HYA (1,000 mg) and high-dose HYA (2,000 mg) than after the ingestion of a placebo. Moreover, blood glucose levels 30 and 60 minutes after ingesting the load food and blood glucose C_{max} as secondary endpoints were significantly lower after ingesting HYA at low and high doses than after ingesting the placebo. Insulin levels 30 and 60 minutes after ingesting the load food were significantly lower after the ingestion of both low and high doses than after the ingestion of the placebo. Among the parameters that showed significant differences from the placebo group, the blood glucose level 30 minutes after ingesting the load food and insulin levels 30 and 60 minutes after ingesting the load food showed significantly lower values after ingesting the high dose than after ingesting the low-dose. These

Table 2　Summary of treatment-emergent adverse events

Subject No.	Age	Sex	Adverse events
80039	66	Man	Loose stool occurred on the next day after ingestion, and disappeared on that day.
80049	51	Woman	Diarrhea occurred after ingestion, and confirmed disappearance by the last inspection.
80051	50	Woman	Abdominal pain occurred after ingestion, and disappeared on that day.
80057	55	Man	Diarrhea occurred on the next day after ingestion, and disappeared on that day.
80059	49	Woman	Diarrhea occurred after ingestion, and disappeared on that day.

results showed that HYA can inhibit the elevation of postprandial blood glucose levels. Concerning the mechanism behind this beneficial effect of HYA, it has been assumed from the results of nonclinical studies that, because of the biological properties of HYA, it acts on long-chain fatty acid receptors expressed in cells of the gastrointestinal tract, thereby promoting insulin secretion. In this study, however, the promotion of insulin secretion by HYA was not observed. Instead, low insulin levels were observed. This result may be explained by characteristics of the subjects in this study. Although persons with blood glucose levels in the upper border zone were selected as study subjects, they did not necessarily have a lowered insulin-secreting ability. Probably for this reason, the promotion of insulin secretion by HYA was not observed, and insulin secretion was inhibited as the blood glucose level decreased. It is possible that other mechanisms were involved in the inhibition of the elevation of blood glucose levels, and further research is necessary. Because sufficient effects were observed even after ingesting the low-dose, the dose at which HYA exerts effects was assumed to be 1,000 mg, based on the results of this study. Given that the difference from the placebo was sufficient in the low-dose group, it seems possible to obtain beneficial effects at even lower doses of HYA.

With respect to safety, a total of 5 cases of abdominal signs and symptoms (loose stool, diarrhea, and abdominal pain) were reported by 5 subjects as secondary reactions under the conditions of this study. The effects of relevant ingredients were suspected to be the cause of diarrhea because a similar symptom was not observed after the patients ingested the placebo.

These results suggest that HYA can inhibit the elevation of postprandial blood glucose levels and may have the effect in preventing diabetes mellitus.

Acknowledgment

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