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Original article

# Inhibitory effect of *Kaempferia parviflora* Wall. Ex. Baker (Zingiberaceae) rhizome on postprandial hyperglycemia.

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# Abstract

**Objectives:** Persistent postprandial hyperglycemia is a risk factor for glycative stress. The purpose of the present study was to examine how dry powder of *Kaempferia parviflora* Wall. Ex. Baker rhizome powder (KPP) affected postprandial blood glucose level. The present study verified its inhibitory effects on activities of glycolytic enzymes, and the inhibitory effects on postprandial hyperglycemia, where KPP was ingested prior to cooked rice intake.

Methods: For the examination of the inhibitory effects of KPP extract on carbohydrate-degrading enzymes, the inhibitory effects on  $\alpha$ -amylase and  $\alpha$ -glucosidase were examined using hot-water extract of KPP.  $\alpha$ -Amylase derived from pig pancreas was used.  $\alpha$ -Glucosidase was prepared from intestinal acetone powder from rats. Acarbose was employed as the positive control for both  $\alpha$ -amylase and  $\alpha$ -glucosidase models. Inhibitory effects on glycolytic enzymes were assessed using 50% inhibitory concentration (IC<sub>50</sub>). Research participants for the investigation of inhibitory effects on postprandial hyperglycemia were eleven healthy men and women, twenty years of age or older. The investigation was performed after obtaining written consent of the participants. The standard meal was only cooked rice, and the test meal was black galangal processed food combined with KPP, which was ingested prior to the cooked rice. In the test, blood glucose changes after ingestions of the standard food (ingestion of cooked rice alone) and the test food (ingestion of KPP prior to cooked rice intake) were examined. Before the commencement of ingestion, blood glucose levels were measured. Cooked rice was then ingested for ten minutes. Measurements for alternation of blood glucose levels were performed at 15 minutes, 30 minutes, 45 minutes, 60 minutes, 90 minutes, and 120 minutes after the commencement of the cooked rice intake. Using self-monitoring blood glucose devices at finger sites, participants punctured a finger and measured blood glucose levels by themselves. The assessment of glycative stress employed values of increased blood glucose level, the maximum value of glucose concentration change ( $\Delta C$ max) and the incremental area under curve (iAUC) of blood glucose levels. Statistical analysis of test results employed a paired t-test for a comparison between the two groups and a Bonferroni test for multiple comparisons among three groups.

**Results:** Extraction liquid of KPP additive-concentration-dependently inhibited the degradation of substrates by  $\alpha$ -amylase and  $\alpha$ -glucosidase. Fifty percent inhibitory concentration (IC<sub>50</sub>) of KPP extract for  $\alpha$ -amylase was 1/85 or less effective than acarbose. For  $\alpha$ -glucosidase, IC<sub>50</sub> of KPP extract was 1/620 or less of acarbose. When ingesting KPP prior to cooked rice intake, blood glucose level, the maximum blood glucose level changes ( $\Delta$ Cmax) and the incremental area under curve (iAUC) of blood glucose level were smaller in comparison with the intake of only cooked rice. The differences were, however, not statistically significant. For subclass analysis, subjects were classified into groups according to iAUC in ingesting only cooked rice. Values of iAUC were listed in descending order, and were classified into three groups, high-value group (H group), middle-value group (M group) and low-value group (L group). Values of iAUC were higher in H group than L group (p < 0.01), were higher in H group in comparison with M group, and were higher in M group in comparison with L group (p < 0.05). Subclass analysis on  $\Delta$ Cmax for the ingestion of cooked rice alone suggested a tendency that values were higher in H group than in the L group (p < 0.05). Values of  $\Delta$ Cmax in H group showed a tendency for lower values in the prior ingestion of KPP before cooked rice intake than in the intake of only cooked rice (p < 0.1).

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**Conclusion:** Inhibitory effects of KPP extract on glycolytic enzyme activities were marginal in comparison with acarbose. In the case of H group, which was recognized to have highly increased blood glucose levels in ingesting only cooked rice, the rise in postprandial blood glucose levels was reduced when they had the prior ingestion of KPP, compared with the intake of only cooked rice. Consequently, it was recognized that the ingestion of KPP prior to meals was an optional countermeasure to lower glycative stress for individuals who tend to have elevated postprandial blood glucose levels.

**KEY WORDS:** α-amylase inhibition, α-glucosidase inhibition, postprandial hyperglycemia inhibition, *Kaempferia parviflora* 

# Introduction

Advanced glycation end products (AGEs) are formed in the body, where aldehydes, metabolites of alcohol, lipids, and reducing sugar such as glucose, react with protein. Protein modifications to AGEs accompany browning, cross-linkage and inflammation in large part, and thus, cause physiological and physical damage to cells and tissues. The negative impacts on the body due to the formation and accumulation of AGEs are designated as glycative stress<sup>1,2</sup>.

Persistent hyperglycemia and glucose spikes, which elevate hyperglycemia for a short time after eating, promote significant glycative stress. Glycative stress is recognized to be a significant risk factor for aging. Glycative stress is a risk factor for skin aging, diabetic complication, osteoporosis and cognitive impairment. Countermeasures for the reduction of glycative stress are the inhibition of postprandial blood glucose, the inhibition of glycation and the degradation and excretion of AGEs<sup>3</sup>.

It has been previously reported that when eating carbohydrates, the combined ingestion of materials which have inhibitory effects on glycolytic enzymes such as  $\alpha$ -glucosidase<sup>4</sup>), and water-soluble dietary fiber<sup>5</sup>) can reduce the elevation of blood glucose after eating a meal. Foods that include these materials have been commercially advertised as functional food. It is also reported that some dietary methods, such as diets where vegetable salads 6,7) or vinegar 8 are ingested before eating cooked rice, can reduce the rise in postprandial blood glucose levels. We have been examining types of carbohydrates as staple foods, and how foods for combined ingestion can affect postprandial blood glucose alternations. In conclusion, we have reported that effective dietary methods to reduce postprandial hyperglycemia are to eat grapefruit<sup>9)</sup> or plain yogurt<sup>10)</sup> before carbohydrates, to add dietary fiber<sup>11)</sup> to udon noodles, and not to eat udon or cooked rice alone but to eat them with a side dish such as an egg, vegetable salad, mapo eggplant<sup>12</sup>, or gyudon (a bowl of rice topped with beef)<sup>13)</sup>.

*Kaempferiaparviflora* Wall. Ex. Baker (KP) is one of the plants of the *Zingiberaceae* family, and its rhizome are provided as an ingredient of functional food. KP is characterized by the blackish purple of the inner rhizome, and thus, in Japan, KP has the appellations of black galangal, black ginger, and black curcuma. KP is distributed in the Kingdom of Thailand, Laos, India, and Myanmar<sup>14</sup>). KP roots contain anthocyanin and polymethoxy flavonoid (PMF) as feature components. It has been reported that PMF, which is contained in KP, has effects on the improvement of metabolic syndrome, metabolic function of muscles <sup>15</sup>),

and obesity  $^{16}$ , as well as to enhance physical fitness  $^{17, 18)}$ . KP rhizome also have anti-glycation effects, and PMF  $^{19)}$  and hydrophilic components are involved in the effects as the active components  $^{20)}$ .

With a purpose of KP examination as a newly found inhibitory activity on glycative stress, the present study verified inhibitory effects on glycolytic enzymes by KP extraction liquid and examined the inhibitory effects on blood glucose rise by the ingestion of KP rhizome powder prior to eating cooked rice.

# **Methods**

# Dried powder sample of Kaempferiaparviflora (KP) rhizome

Two types of samples were used: One was a rhizome powder which was produced by vacuum dehydration at a low temperature (KPP). The other was a black galangal functional food, a hard gelatin capsule in which KPP was contained. These samples were provided by Rene Co. Ltd. (Osaka, Japan).

# KPP extraction liquid

KPP extraction liquid was obtained from a mixture of 2 g of KPP as a sample, and 40 mL of purified water. The mixture liquid was incubated in a water bath at 80°C for 60 minutes. This incubated liquid was centrifuged at 2,500 rpm ( $800 \times g$ ) for ten minutes, and later, the supernatant was filtered. Subsequently, 5 mL of extraction liquid was put in an aluminum tray, and then dried and vaporized at 120°C for two hour. Consequently, the concentration of the solid KPP extract was calculated by weighing the volume of evaporation residues.

# Inhibitory effects on activities of glycolytic enzymes

#### 1) Inhibitory effects on α-amylase

Inhibitory effects on  $\alpha$ -amylase were measured employing  $\alpha$ -Amylase Measuring Kit (for analysis of fermented food production) (Kikkoman Biochemifa Company, Minato-ku, Tokyo, Japan) and pancreatic amylase from pigs (Sigma-Aldrich, St. Louis, MO). Activities of  $\alpha$ -amylase were measured as follows: Into a microtube, 200 µL of substrate solution and 200 µL of enzyme liquid sample were dispensed, and this was preliminarily heated at 37°C for five minutes. In another microtube, 30 µL of  $\alpha$ -amylase solution 60 U/mL or aqueous sodium solution at 0.5% (enzyme blank), and

30 µL of KPP extraction liquid or positive control liquid were dispensed. Liquid mixture of amylase was prepared. Acarbose (Wako Pure Chemical, Osaka, Japan) was used as the positive control for the inhibitory action. After completing preliminary heat, 40 µL of the liquid mixture of  $\alpha$ -amylase was added into the microtube. Thus, amylase reaction started. After the reaction at 37°C for ten minutes, 800 µL of stop solution was added. Subsequently, 200 µL of this mixture was poured in a microplate and absorbance was measured with a wavelength of 400 nm. The blank value was an absorbance of the following: substrate-enzyme sample solution was heated at 37°C for ten minutes and 800 µL of stop solution was added. Subsequently, 30 µL of KPP extract solution was added. Absorbance of this mixture was measured. The inhibitory ratio of  $\alpha$ -amylase was obtained by the following calculation formula.

- The inhibitory ratio of  $\alpha$ -amylase (%) = (1 A / B) × 100
  - A: Absorbance value of KPP extract addition model B: Absorbance value of extractant addition model
  - (control: all coloring)

#### 2) Inhibitory effects on α-glucosidase

Inhibitory effects on a-glucosidase were measured employing QuantiChrom α-Glucosidase Assay Kit DAGD-100 (BioAssay System, Hayward, CA). α-Glucosidase was prepared as follows: Mixture, which consisted of 0.2 g of small intestinal acetone powder from rats (Sigma-Aldrich) and 3 mL of potassium phosphate buffer solution 50 mmol/L (pH 7.0), was treated homogeneously (30-second treatments with twelve intervals of 20 seconds). Subsequently, this mixture was centrifuged at 10,000 rpm at 4°C for 30 minutes and the supernatant liquid was obtained. After setting the temperature of the microplate reader measurement at 30°C, 200 µL of either KPP extraction liquid, acarbose solution, or potassium phosphate buffer solution 50 mol/L (pH 7.0) (control) was dispensed into a microtube. Subsequently, 8 µL of substrate solution,  $\alpha$ -nitrophenyl- $\alpha$ -D-glucopyranoside (PNP-G), was added to prepare the substrate mixture. Afterwards, 20 µL of α-glucosidase solution or potassium phosphate buffer solution 50 mmol/L at pH 7.0 (blank reaction) was dispensed into each well of the microplate. Finally, 200 µL of substrate mixed solution was added and the reaction was initiated. Absorbance changes with a wavelength of 405 nm were measured for 30 minutes. Acarbose was used as the positive control for inhibitory action. The inhibitory ratio of  $\alpha$ -glucosidase was obtained by the following calculation formula.

The inhibitory ratio of  $\alpha$ -glucosidase (%) =  $(1 - A/B) \times 100$ A: Absorbance value of KPP extract addition model

B: Absorbance value of extractant addition model (control: all coloring)

#### Inhibitory effects on postprandial blood glucose

#### 1) Research participants

Research participants of the investigation regarding inhibitory effects on postprandial hyperglycemia were recruited from individuals who were related to Life and Medical Sciences, Doshisha University. Participants were healthy men and women 20 years old or older who did not meet the following exclusion criteria: individuals who were allergic to food or medicine, who were pregnant or lactating, who had medical treatment with administration of medication, who had a disease under medical observation, who were diagnosed with diabetes, who showed notable disorders of cardiopulmonary functions, who were prescribed medicine for the treatment of hypertension, who had undergone surgery for their gastrointestinal tract, and who were suspected to have an infection. Participants were fully informed of the research and the research was conducted with written consents of the participants.

#### 2) Test items and their contents

Research participants filled out a self-administered questionnaire regarding subject profile such as age, medical history, and absence or presence of food allergy. In addition, they had a biochemical examination of their blood prior to the ingestion test. Blood glucose measurements of the participants were employed by a medical lancet device (Naturalet EZ device, Arkray, Kyoto, Japan), puncture needle for blood sampling (Naturalet EZ, Arkray) and blood glucose self-monitoring equipment (Glucocard G Black, GT-1830, Arkray). Participants punctured their finger, collected a blood sample, and measured their blood glucose level by themselves.

#### 3) Protocol of testing

The present study was conducted referring to the unified protocol of Japanese Association for the Study of Glycemic Index<sup>21</sup>, as was the same of previous report <sup>9-13</sup>. Participants were instructed to behave as usual during the day before the test and to act in conformity of the following restrictions: to refrain from strenuous exercise, to finish eating supper before 22:00, not to ingest anything but water from 22:00 to the commencement of the test, and to avoid overeating and overdrinking, intake of excessive alcohol, and staying up late. When participants were in poor state of health on the day before the test or before and during the test, the test was cancelled or postponed.

Measurements of blood glucose level were performed twice to gain the mean value for measurement data. When there was a separation of 10% or larger, a third measurement was added. Measured data was decided as the average value of two values that had the smallest separation. The ingestion of the test food started after the commencement of the test and its duration was ten minutes. The manner of ingestion was 30 cycles of chewing per bite.

The measurements of blood glucose level were as follows: The first measurement was before the commencement of ingestion. Subsequently, measurement was performed at 15 minutes after the commencement of the test (the second measurement), 30 minutes (the third), 45 minutes (the fourth), 60 minutes (the fifth), 90 minutes (the sixth), and 120 minutes (the seventh).

#### 4) Test foods

Nutrition facts of test meals that were examined in the present study are shown in *Table 1*. Nutrient components were calculated based on the values shown for each food. The standard food employed in the present study was a commercially available cooked rice pack (*Sato no Gohan* Niigata Prefecture, Koshihikari 200 g, Sato Foods Industries, Niigata, Japan). When ingesting cooked rice, *furikake* rice seasoning was ingested (2.5 g) (*Noritama*, Marumiya Foods,

Tokyo, Japan). For the test food, KPP, three capsules (370 mg x 3) of black galangal processed food (Rene, Osaka, Japan) were ingested. The standard food was ingested with 150 mL water for ten minutes from the commencement of the test. KPP was ingested five minutes before the commencement of the test (after the first measurement of blood glucose level).

#### Table 1. Nutrition facts of test meal

Test food (unit)	Cooked rice	KPP
Weight (g)	200	1.11
Energy (kcal)	294	3.7
Protein (g)	4.2	0.1
Fat (g)	0	0.02
Carbohydrate (g)	67.8	0.9

Cooked rice, 200 g; KPP, 370 mg x 3 capsules; KPP, *Kaempferia parviflora* Wall. Ex. Baker rhizome powder.

#### 5) Selection of analysis subjects

The targets for the safety analysis were subjects who ingested the test food at least once. The targets for the validity analysis were subjects who completed the prescribed test schedule and test contents. However, subjects were excluded from analysis data when they met the following exclusion criteria for validity analysis: individuals who were recognized to notably show behaviors that deteriorated the reliability of data results, and when it was discovered, after the commencement of the test, individuals who previously met the exclusion criteria but were not able to abide by restriction rules.

#### 6) Ethical standard

The present study regarding the test for the inhibitory effects on postprandial hyperglycemia was conducted in compliance with the Declaration of Helsinki (Note of Clarification added at the 2004 World Medical Association General Assembly in Tokyo) with the ethical principles and protection of personal information. The present study abided by the Ministerial Ordinance on Good Clinical Practice (GCP) for Drug (Ordinance of Ministry of Health and Welfare No.28 of March 27, 1997) and the Ethical Guidelines for Epidemiological Research established by Japan's Ministry of Health, Labour and Welfare, and the Ministry of Education, Culture, Sports, Science and Technology. Further, the Doshisha University Ethics Review Committee on Research Involving Human Subjects held a meeting to deliberate on the ethics and validity and approved this study (Application number: #16020). Moreover, this study was conducted with the prior registration in the open database that is held by the University Hospital Medical Information Network (UMIN) of the National University Hospital Council of Japan (Registry number: UMIN000023814).

# Statistical Analysis

As for data results of the inhibitory effects on glycolytic enzyme activities, 50% inhibitory concentration (IC<sub>50</sub>) was

obtained by the calculation with the inhibitory ratio values of KPP extracts with three concentration types.

Based on data results of the inhibitory effects on postprandial hyperglycemia, the differences between fasting blood glucose and postprandial blood glucose levels were the volume of changes ( $\Delta$ ), where the values of blood glucose before the commencement of the test (fasting state) were subtracted from the values that were measured over time after the ingestion of the test food. The maximum value of glucose concentration change was the  $\Delta Cmax$ measurement from the test commencement time until 120 minutes after commencement. Incremental area under curve (iAUC) was calculated with the unified protocol of the Japanese Association for the Study of Glycemic Index (GI). Measured values were expressed with the mean values  $\pm$  the standard error mean (SEM). Statistical analysis of test results employed paired t-tests for comparison between two groups, and Bonferroni tests for multiple comparisons among three groups (BellCurve for Excel, Social Survey Research Information, Tokyo, Japan). A two-sided test was employed, and a hazard rate less than 5% indicated significant difference.

# Results

#### 1) Inhibitory effects on glycolytic enzymes

The results of inhibitory effects on glycolytic enzymes are shown in *Fig. 1*. KPP extraction liquid concentrationdependently inhibited the degradation of substrates by  $\alpha$ -amylase. At a fifty percent inhibitory concentration (IC<sub>50</sub>) for  $\alpha$ -amylase, KPP extract showed 433.3 µg/mL or more, and acarbose showed 5.10 µg/mL. KPP extract was 1/85 or less of acarbose in terms of effectiveness for  $\alpha$ -amylase. Extraction liquid of KPP additive-concentration-dependently inhibited the degradation of substrates by  $\alpha$ -glucosidase. As IC<sub>50</sub> for  $\alpha$ -glucosidase, 3.722 µg/mL or more KPP extract was present, and acarbose contained 0.006 µg/mL. For  $\alpha$ -glucosidase, KPP extract contained 1/620 or less acarbose.

# 2) Inhibitory effects on postprandial hyperglycemia

# Assessment of safety

There was no report of adverse events in subjects that were related to the test of inhibitory effects on postprandial hyperglycemia in this study (data not included).

#### **Target for effectiveness evaluation**

There was no subject who was excluded from the list of analysis targets for the inhibitory effects on postprandial hyperglycemia. Thus, all the research participants were the valid subjects for analysis (eleven subjects). Subjects of the present study were five men and six women, age  $22.0 \pm 0.4$ years old (the mean value  $\pm$  standard deviation) (men;  $22.2 \pm 0.4$  years old, women;  $21.8 \pm 0.4$  years old), height 162.3  $\pm 4.6$  cm (men;  $164.9 \pm 3.3$  cm, women;  $159.2 \pm 4.1$  cm), weight  $53.3 \pm 6.1$  kg (men;  $52.4 \pm 8.5$  kg, women;  $52.4 \pm 2.7$ kg), BMI  $20.2 \pm 1.7$  (men;  $19.8 \pm 0.6$ , women;  $20.6 \pm 2.4$ ). Further, there were no subjects who showed abnormal values in the results of the biochemical blood examination (*Tables* 2 and 3).

# Evaluation of effectiveness

#### 1) Total analysis

Alternation in blood glucose levels after the ingestion of test meals, the maximum value of glucose concentration change ( $\Delta$ Cmax) and incremental area under the curve (iAUC) are shown in *Fig. 2* and *Table 4*. Blood glucose levels in the time range of 30–60 minutes,  $\Delta$ Cmax and iAUC were lower in the prior ingestion of KPP to cooked rice than in the ingestion of only cooked rice. The measurement differences were, however, not statistically significant.

#### 2) Subclass analysis

Subclass analysis on iAUC was performed as follows: Subjects who ingested only cooked rice were classified into three groups according to the descending order list: highvalue group (H group, n = 3), middle-value group (M group, n = 4) and low-value group (L group, n = 3, *Table 5*). Values of iAUC when ingesting only rice were larger in the H group (6879 ± 696 mg/dL\*min) than in the L group (3764 ± 492 mg/dL\*min, p < 0.01) and were larger in the H group than in the M group (5323 ± 242 mg/dL\*min). Values of iAUC were smaller in the M group than in the L group (p < 0.05). Similarly, there was a tendency for value of  $\Delta$ Cmax to be larger in the H group than in the L group (p = 0.1). However, differences in iAUC and  $\Delta$ Cmax were not recognized among H group, M group and L group, when KPP was ingested prior to cooked rice.

The iAUC values of the H group were lower when KPP was ingested prior to cooked rice than in the ingestion of only cooked rice (p < 0.05). Furthermore, the values of  $\Delta$ Cmax in the H group showed a tendency to be smaller when KPP was ingested prior to cooked rice than in the ingestion of only cooked rice (p < 0.1). No differences in iAUC and  $\Delta$ Cmax were recognized between the M group and the L group when comparing between the prior ingestion of KPP and the ingestion of only cooked rice.

# Discussion

Acarbose and voglibose are drugs that inhibit the activities of glycolytic enzymes such as  $\alpha$ -amylase and  $\alpha$ -glucosidase in the body. They have the effect of inhibiting the elevation of blood glucose levels after meals by ingesting 50–100 mg of the above-mentioned drugs prior to a meal<sup>22)</sup>. Similarly, plant-derived materials, such as the extract of guava leaves <sup>4)</sup>, mulberry leaves <sup>23)</sup>, and *Salacia oblonga* roots <sup>24)</sup> have inhibitory effects and are used as functional foods to reduce postprandial blood glucose rise. It was recognized that KPP extract has an inhibitory effect on the activities of  $\alpha$ -amylase and  $\alpha$ -glucosidase, which are glycolytic enzymes. However, its effectiveness was extremely low in comparison with acarbose.

The total analysis showed that the mean value of blood glucose rise was lower with ingestion of KPP prior to cooked rise than in the ingestion of only cooked rice. The measurement value, however, did not recognize a statistically significant difference. The subclass analysis indicated that the reductions of iAUC and  $\Delta Cmax$  were recognized in the H group when KPP extract was ingested prior to cooked rice; the group where the blood glucose level increased significantly with the ingestion of only cooked rice was referred to as the H group. There are multiple mechanisms for the reduction of postprandial hyperglycemia, other than the inhibitory effects on the activities of glycolytic enzymes: carbohydrate absorption by dietary fiber, discharge delay from the stomach to the small intestine due to acid such as acetic acid, lactic acid, citric acid, and the promotion of incretin secretion due to digestion of proteins. It is reported that an effective dose of water-soluble dietary fiber is 4-10g for the reduction of postprandial hyperglycemia<sup>5, 25, 26)</sup>. Although the volume of dietary fiber which was contained in KPP test food was not measured, 0.9 g was measured as the volume of carbohydrates. Therefore, influences due to the dietary fiber were marginal, as was recognized. It is





Data are expressed as mean  $\pm$  SD; triplicate measurements. Acarbose,  $\alpha$ -amylase and  $\alpha$ -glucosidase inhibitor; KPP, hot water extract of *Kaempferia parviflora* Wall. Ex. Baker; IC<sub>50</sub> ( $\alpha$ -amylase), KPP: > 433 µg/mL, acarbose: 5.10 µg/mL; IC<sub>50</sub> ( $\alpha$ -glucosidase), KPP: 3.722 mg/mL, acarbose: 0.006 mg/mL.

# Table 2. Participant Profiles

	Total	Male	Female
Number of subjects	11	6	5
Age	$22.0 \pm 0.4$	$22.2 \pm 0.4$	$21.8 \pm 0.4$
Body height	$162.3 \pm 4.6$	$164.9 \pm 3.2$	$159.2 \pm 4.1$
Body weight	53.3 ± 6.1	52.4 ± 8.5	52.4 ± 2.7
BMI	$20.2 \pm 1.7$	$19.8 \pm 0.6$	$20.6 \pm 2.4$

Data are expressed as mean  $\pm$  SD. BMI, body mass index; SD, standard deviation.

# Table 3. Blood chemistry

Test item	Reference range	Unit	Measured value
Total cholesterol	150 - 219	mg/dL, serum	$183.3 \pm 28.1$
TG	30 - 149	mg/dL, serum	58.0 ± 15.0
HDL-C	40 - 85 (male) 40 - 95 (female)	mg/dL, serum	71.5 ± 20.2
LDL-C	65 - 139	mg/dL, serum	95.4 ± 23.8
DHEA-s	-	μg/dL, serum	359.4 ± 171.5
IGF-I (somatomedin C)	-	ng/mL, serum	$218.6 \pm 30.5$
Cortisol	3.7 - 19.4	μg/dL, plasma	$15.9 \pm 4.2$
IRI	1.7 - 10.4	U/mL, serum	4.4 ± 1.6
FBG	70 - 109	mg/dL, whole blood	80.2 ± 5.3
HbA1c [NGSP]	4.6 - 6.2	%, whole blood	$5.3 \pm 0.2$
Glucagon	70 - 174	pg/mL, plasma	141.0 ± 38.1
Pentosidine	15.6 - 43.0	pmol/L, plasma	$20.6 \pm 6.0$

Data are expressed as mean  $\pm$  SD, n = 11. TG, triglyceride; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol; DHEA-s, dehydroepiandrosterone-sulfate; IGF-I, insulin-like growth factor-I; IRI, immune reactive insulin; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; NGSP, National Glycohemoglobin Standardization Program; SD, standard deviation.





Data are expressed as mean  $\pm$  SEM, n = 11;  $\Delta$ Blood glucose, changed values of blood glucose from the time 0 min; SEM, standard error mean.

Table 4. Comparison of iAUC and $\Delta C$ max after meals: Total analysis				
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Evaluation item	Cooked rice	KPP + cooked rice	p-value
iAUC	5322.0 ± 444.2	4815.7 ± 406.4	0.347
ΔCmax	81.7 ± 5.8	70.3 ± 5.1	0.166

Data are expressed as mean  $\pm$  SEM, n = 11, statistical analysis by paired t-test. iAUC, incremental area under the curve (unit: mg/dL\*min);  $\Delta$ Cmax, maximamu value of glucose concentration change (unit: mg/dL); SEM, standard error mean.

# Table 5. Comparison of iAUC and $\Delta Cmax$ after meals: Subclass analysis

Evaluation item	Subgroup	Cooked rice	KPP + cooked rice
	Н	$6879.4 \pm 347.8^{a}$	$5091.6 \pm 671.1$ *
iAUC	М	$5322.5 \pm 139.7^{a}$	4720.1 ± 1170.4
	L	$3764.3 \pm 245.8^{a}$	4611.6 ± 580.9
	Н	99.4 $\pm$ 6.3 <sup>b</sup>	71.0 ± 6.6 †
$\Delta C$ max	М	$74.5 \pm 5.2$	$72.0 \pm 11.3$
	L	$69.4 \pm 9.5^{b}$	$68.3 \pm 11.4$

Data are expressed as mean  $\pm$  SEM, n = 11; L, lower tertile (n =4); M, middle tertile (n = 3); H, higher tertile (n =4). Statistical analysis between two group; \*p < 0.05, †p < 0.1 vs cooked rice by paired t-test. Statistical analysis between three groups, Bonferroni correction; a, p < 0.05; b, p < 0.1 iAUC, incremental area under the curve (unit: mg/dL\*min);  $\Delta$ Cmax, maximum value of glucose concentration change (unit: mg/dL); SEM, standard error mean.

reported that an effective dose of vinegar, which contains approximately 4% acetic acid, is  $10-20 \text{ g}^{27}$ . The volume of carboxylic acid which was contained in KPP, however, was marginal. Therefore, influences due to the vinegar were marginal, as was recognized. It was recognized that influences from protein were marginal, as the volume of protein in the KPP test food was 0.1 g, while ingestion of food which contains approximately 40-50 g of protein inhibits postprandial hyperglycemia<sup>28-30</sup>. Judging from the above, there was a possibility that the effectiveness of KPP ingestion in the inhibition of postprandial hyperglycemia had a different mechanism from the above mechanism that had been previously reported.

The subjects of this study regarding the inhibitory effects on postprandial hyperglycemia test were healthy men and women at a mean age of  $22.0 \pm 0.4$  years, who were recognized to have no abnormal carbohydrate metabolism in examination items of the blood test. However, there existed the subjects of H group. The subjects of the H group showed high blood glucose rise when they ingested only cooked rice. This could be a glucose spike; which is a precipitous postprandial increase in blood glucose levels. This glucose spike is a significant risk factor for overload on pancreatic beta cells due to the excessive secretion of insulin, and could result in insulin resistance <sup>31</sup>). Furthermore, it is reported that along with the precipitous rise in postprandial blood glucose levels, concentrations of aldehyde such as 3-deoxyglucosone, glyoxal and methyglyoxal are elevated <sup>32, 33)</sup>. These are referred to as an "aldehyde spark" <sup>34</sup>). It is known that methyglyoxal induces apoptosis in the nerves and the vascular endothelial cells<sup>35, 36)</sup>. Thus, increases of blood aldehyde due to postprandial hyperglycemia could lead to vascular aging and the progression of diabetic complication. This aldehyde spark must be avoided by even young and healthy people in their twenties who do not have abnormal examination items

in blood test. It was recognized that the prior ingestion of KPP before meals was an optional countermeasure to lower glycative stress for individuals whose postprandial blood glucose levels tend to increase.

# Conclusion

Inhibitory effects on glycolytic enzyme activities of KPP extract were marginal in comparison with acarbose. However, for the research participants who showed high postprandial blood glucose increases when ingesting only cooked rice, the values of postprandial blood glucose levels were reduced with the ingestion of KPP before cooked rice intake. Consequently, it was recognized that the prior ingestion of KPP before meals was an optional countermeasure to lower glycative stress for individuals who tend to have increased postprandial blood glucose levels.

# Statement of conflict of interest

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