# Original article Effect of heat-moisture-treated high-amylose corn starch-containing food on postprandial blood glucose.

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# Abstract

*Purpose*: Heat-Moisture-Treated high-amylose corn starch (HMT-HAS) including a large amount of amylose is a recalcitrant ingredient. The purpose of this study was to verify its effect on postprandial blood glucose level.

**Methods:** The subjects were 19 males and 13 females  $(30.9 \pm 10.7 \text{ years})$  of healthy students and company workers. The control food was a bread roll made with wheat starch blended with 50% wheat flour replacement and the test food was bread blended with 50% HMT-HAS (Amylogel <sup>TM</sup> HB-450). The postprandial blood glucose levels after ingesting these breads were tested on different days. The blood glucose level tests were conducted with a self-blood glucose meter, 15, 30, 45, 60, 90 and 120 minutes after the subjects started ingesting the test food. A hematological test, blood blochemistry general test and background test for subjects were conducted in advance. This study was conducted with the approval of the Doshisha University ethical committee.

**Results:** The postprandial blood glucose levels of all subjects were higher after they ingested the control food (bread including wheat starch) than the test food (bread including HMT-HAS). The incremental area under the curve (iAUC) of blood glucose level changes were compared between the control food and test food, and as a result, it was significantly lower after ingesting the test food than the control food and the maximum value of glucose concentration change ( $\Delta$ Cmax) was significantly lower after ingesting the test food than the control food. As a subclass analysis, the result of the comparison between company workers' group (36.9 ± 9.3 years) and students' group (22.6 ± 1.3 years) showed that the homeostasis model assessment of insulin resistance (HORM-IR) was higher in the company workers' group and the maximum value of glucose concentration (Cmax) was significant higher in the same group; however, there was no significant difference in the postprandial blood glucose levels after the ingestion of the food containing HMT-HAS.

**Conclusion:** The ingestion of the bread including HMT-HAS inhibited postprandial hyperglycemia more than the one blended with wheat starch. Its inhibiting action on postprandial hyperglycemia was more remarkable in the groups of higher age and higher insulin resistance. It was suggested that postprandial blood hyperglycemia can be alleviated by ingesting bread blended with HMT-HAS and it can contribute to health maintenance.

**KEY WORDS:** heat-moisture-treated high-amylose corn starch (HMT-HAS), post-prandial hyperglycemia, wheat starch, amylose, insulin

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# Introduction

The phenomenon that reducing sugars, such as glucose, reacts with protein non-enzymatically and forms glycated protein, and as a result, generates advanced glycation end products (AGEs), is called "glycation" 1, 2). The stress on a living body caused by reducing sugars and aldehyde is called "glycative stress," and it is one of the risk factors for the acceleration of physical aging. For the alleviation of glycative stress, the inhibition of postprandial hyperglycemia, inhibition of AGE generation and decomposition and elimination of AGEs are cited. For the purpose of inhibiting postprandial hyperglycemia, there are methods of selecting low glycemic index (GI) foods as staple foods by which the rise of postprandial blood glucose (GLU) becomes gradual, such as ingesting dietary fibers such as vegetables before carbohydrates and limiting the ingestion of carbohydrates<sup>3</sup>. A notable postprandial hyperglycemia (the maximum GLU value [Cmax] more than 140 mg/dL) has been called a "glucose spike" (Fig. 1), and it has been known to cause a variety of tissue damage, i.e. vascular endothelial disorder. Recently, it has been clarified as the result of an in vitro experiment that it is possible that the open-linear form of glucose molecules exposes the aldehyde group and it causes a chain reaction and multiple various intermediate aldehydes are produced in a simultaneous manner<sup>4</sup>). We called this phenomenon an "aldehyde spark." From the viewpoint of glycative stress, the prevention of postprandial hyperglycemia leads to the prevention of an aldehyde spark, so that it is more important than ever thought before.

This time, a clinical ingestion test was conducted for the purpose of evaluating the effect of the food blended with processed starch (test food) on the GLU changes after the food ingestion. The subjects were healthy males and females. The control food was a table roll (bread) made with wheat starch blended with 50% replacement of wheat flour. The test food was bread blended with heat-moisture-treated highamylose corn starch (HMT-HAS)<sup>5</sup>). The inhibition effect of the test food in the rise of GLU was verified by an open-label single ingestion test.

# Method

# **Subjects**

The subjects of this study were healthy males and females 20 years old or older and 60 years old or younger, and they had submitted letters of consent for participation in this study. The test food was the table roll, bread blended with HMT-HAS (Amylogel HB-450; Sanwa Starch Co., Ltd., Kashihara-city, Nara). The inhibition effect of the test food on postprandial hyperglycemia was verified by a single ingestion.

A briefing for the explanation of the test was conducted after obtaining the approval of the ethical committee concerning "studies involving human subjects" of Doshisha University, and 32 subjects whose consents had been obtained and who did not conflict with the following exclusion criteria were incorporated in this study:



#### Fig. 1. "Glucose spike" evokes "Aldehyde spark."

The glucose spike evokes an "aldehyde spark." Various types of aldehyde are simultaneously formed with a chain-reaction, *i.e.* GO, MGO, 3DG, GA. Glucose is normally saccharides with a cyclic form, however, in 0.002% of glucose, a portion of the molecule structure is decyclized to present an open-linear form, exposing the aldehyde group (-CHO) which is highly reactive. AGEs, advanced glycation end products; RAGE, receptor for AGEs; ER, endoplasmic reticulum; GO, glyoxal; MGO, methyglyoxal; 3DG, 3-deoxyglucosone; GA, glyceraldehyde.

- 1. Individuals who are using medical products for any disease
- 2. Individuals who are under treatment or have a history of impaired glucose tolerance, mental disabilities, sleep disorder, hypertension, diabetes, dyslipidemia or other serious dysfunctions
- 3. Individuals who used a drug for treatment in the past 1 month (one-shot medicine for headache, menstrual pain and colds are excluded)
- 4. Individuals who are under treatment of or have a history of serious liver dysfunction, kidney damage, heart disease or blood disease
- Individuals who are under treatment of or have a history of digestive organ disease (a history of appendicitis is excluded)
- Individuals whose body mass index (BMI) is over 30kg/m<sup>2</sup>
- Individuals who donated blood over 200 mL in the past 1 month or over 400 mL in the past 3 months
- 8. Individuals with serious anemia
- 9. Individuals who are sensitive to test products or individuals who have the fear of severe allergy in other foods and medical supplies
- 10. Individuals who are pregnant or breastfeeding or individuals who could possibly become pregnant
- 11. Individuals who had a habit to continuously ingest healthpromoting foods advocating glycometabolism or healthy foods in the past 3 month or will ingest those foods during the test period (ingest for the purpose of health maintenance is acceptable)
- 12. Individuals who were judged inappropriate as the subjects for this study by a responsible doctor

#### Test Design

This test was an open-label test with a control.

Table roll made with HMT-HAS blended with 50% replacement of wheat flour (bread containing HMT-HAS) was used as the test food. Table roll made with wheat starch blended 50% replacement (bread containing wheat starch) was used as the control food (*Table 1-1, 1-2*).

The subjects were instructed to avoid excessive exercise, eat a specified dinner menu and sleep longer than 6 hours on the day before the test. The menus of the dinner on the day before the test are shown in *Table 2*<sup>6,7)</sup>. The subjects selected their dinner from (1) and (2) with their free will and decided to take the same menu both times. Alcohol intake was prohibited from the previous day of the test to the completion of the day of the test.

On the day of the test, they were waiting quietly in sitting position, while DVDs of animation and travels were televised and they were prohibited from telephoning, sleeping, excessive brain activities and physical activities with a possibility of sweating until the end of the test. After the ingestion of the test food, they were only allowed to take water.

On the day of the first test, after waiting quietly, the subjects underwent physical measurements and, by collecting blood samples, hematologic test and blood biochemical test. At the same time, they conducted self-monitoring of GLU (the first time). After that, they ingested three pieces of control food (the dough was divided and baked so that the carbohydrate amount was 75 g). A piece of control food was cut into six pieces for the subjects to chew a piece 10 times and swallow. Food ingest time was from 9 to 10 minutes.

	Control food	Test food
Flour	25.9	24.1
Starch	26.7	24.9
Vital gluten	0.8	0.7
Yeast	1.1	1.0
Yeast food	0.1	0.0
Refined sugar	6.4	6.0
Salt	1.0	0.9
Powdered skim milk	1.6	1.5
Margarine	6.4	6.0
Water	30.2	34.9

#### Table 1-1. Composition of test meal (%).

## Table 1-2. Nutrition facts of test meal.

test food	unit (g)	energy (kcal)	protein (g)	fat (g)	carbohydrate (g)	sugar (g)	fiber (g)	sodium chloride equivalent (g)
Control food	100	298.4	4.8	6.6	53.5	52.6	0.9	1.2
Test food	100	244.6	4.6	6.1	49.6	32.5	17.1	1.1

#### Table 2. Dinner menu on the day before the test.

	Selection ①	Selection ②
Cooked white rice	200 g	300 g
Ingredients of beef bow	1 pack	1 pack
Ingredients of beef bow (mini size)	—	1 pack
Freeze-dried miso soup	1 pack	1 pack
Energy intake (kcal)	668	1,014
PFC ratio	12:39:48	12:41:46

Cooked rice: "Papatto Rice" Koshihikari (Hagoromo Foods Corporation, Suruga-ku, Shizuoka).

Side dishes: Ingredients of beef bowl, Ingredients of beef bowl (mini size) (Yoshinoya Holdings Co., Ltd., Chio-ku,

Tokyo, Japan) 5, 6), Freeze-dried miso soup: Yoshinoya Holdings Co., Ltd

The subjects conducted self-monitoring of GLU 15 minutes (the 2nd time), 30 minutes (the 3rd time), 45 minutes (the 4th time), 60 minutes (the 5th time), 90 minutes (the 6th time) and 120 minutes (the 7th time) after starting ingestion. On the day of the second test, the subjects conducted self-monitoring of GLU after waiting quietly (the 1st time). Then they ingested three pieces of test food together with 200 mL of water. The ingestion method, the way of waiting quietly during self-monitoring of GLU and the test implementation time were the same as those of the first test.

The test period was from August 2017 to December 2017.

## Self-monitoring of GLU

The subjects conducted self-monitoring of GLU using a self-monitoring blood glucose meter (Glucocard G Black: GT-1830, Arkray Co., Kamigyo-ku, Kyoto, Japan). The measurement was conducted twice and the average value was used. When the difference between the two values was more than 10%, a 3rd value was measured and the average value of the two values with the least difference was used.

## Physical measurements

Body height, body weight, body fat percentage, BMI, systolic and diastolic blood pressures and pulse rate were measured.

## Blood tests

Peripheral blood tests and biochemical tests were conducted using blood samples. The test items for this analysis were white blood count (WBC), red blood count (RBC), hemoglobin content (Hb), hematocrit value (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), blood urea nitrogen (BUN), creatinine (CRE), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transpeptidase ( $\gamma$ -GTP), total cholesterol (TC), triglyceride (TG), low-density-lipoprotein cholesterol (LDL-C), highdensity lipoprotein cholesterol (HDL-C), HbA1c, fasting plasma glucose (FPG), insulin (imunoreactive insulin: IRI) and glucagon. The measurements using blood samples were conducted at LSI Medience Corporation (Chiyoda-ku, Tokyo, Japan).

## Statistical analysis

Analytical items were GLU measured value, incremental area under the curve (iAUC) of GLU level changes, amount of change from initial value ( $\Delta$  blood glucose value), Cmax and change value of Cmax ( $\Delta$  Cmax), and fundamental statistics (average value, standard deviation and 95% confidence range) were calculated.

For the test of significant difference, the comparisons between groups at the time of ingesting the control food and the test food were evaluated using unpaired t test or Bonferroni's multiple test (paired). For the test of significant difference, an appropriate statistical analytical software (Excel Statistics, Social Survey Research Information Co., Ltd.) was used. The level of significance was assumed to tend to be less than 5% and 10%. This system was prepared so that various statistical analyses can be done from the viewpoint of exploratory research such as a stratified analysis in responding to subject characteristics and research of correlation from the viewpoint of affectivity.

## Ethical review

This study was conducted in compliance with the declaration of Helsinki (revised at 2013 WMA General Assembly, Fortaleza, Brazil) and the ethical guideline regarding the "medical and health research involving human subjects (announced by the Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare). For this test, the "Committee for Medical Test involving human subjects" was held at the Ethical Review Committee concerning the "study involving human subjects" of Doshisha University (Kamigyo-ku, Kyoto, Japan), where the morality and validity of this test was discussed, and approval was granted (Application No. 17054). This test was pre-registered as a clinical trial (UMIN No.000029026).

# Result

# General background

All 32 subjects of this study did not fall under the Judgment Classification D, requiring medical treatment, of the Japan Society of Ningen Dock and they were all in a healthy condition (*Table 3*). The breakdown of subjects was 19 males and 13 females, and their average age was  $30.9 \pm 10.7$ .

The subjects can be classified in the two groups of 13 college students (Doshisha University students and graduate students, 6 males and 7 females) and 19 company workers (Sanwa Starch Co., Ltd., 13 males and 6 females). The company workers were higher in age, body weight, BMI, TC, LDL-C and homeostasis model assessment of insulin resistance (HORMA-IR) than the college students (p < 0.01). On the other hand, their pulse rates and IRI values were significantly lower than those of students (p < 0.01). The difference between the average ages of the two groups was 14.3 and it is presumed that aging affected the difference in the test values. Regarding the test items concerning carbohydrate metabolism, company workers' IRI values were 2.7 µunit/mL (1.8 times) lower than those of the students' group and their HORMA-IR values were 0.5 points higher (1.7 times) than those of the students' group. The difference in carbohydrate metabolism between the groups was within the reference range. The insulin resistance associated with aging tends to increase in the corporate workers' group (*Table 4*).

#### Table 3. Subject background profile.

		Average	SD
Age	year	30.9 ±	10.7
Height	cm	166.7 ±	7.7
Weight	kg	60.0 ±	10.8
BMI		21.5 ±	2.7
Blood pressure (systolic)	mmHg	119.7 ±	13.1
(diastolic)	mmHg	70.7 ±	10.4
Pulse	/min	66.8 ±	14.1

n = 32. BMI, body mass index; SD, standard deviation.

#### Table 4. Comparison between College students and Company workers.

		College student (n=13)		Compar (n:	Company worker (n=19)		p value
Gender	Male Female	6 7			13 6		0.419
Age		22.6 ±	1.3	36.9	±	9.3	< 0.001
Height	cm	164.1 ±	7.1	168.1	±	8.0	0.118
Weight	kg	52.3 ±	6.4	64.1	±	9.1	< 0.001
BMI	-	19.4 ±	1.6	22.6	±	2.1	< 0.001
Blood pressure (systolic)	mmHg	124.2 ±	13.1	119.1	±	16.4	0.095
(diastolic)	mmHg	70.4 ±	9.5	73.9	±	13.8	0.899
Pulse	/min	74.7 ±	15.4	61.9	±	11.5	0.005
ТС	mg/dL	165.9 ±	22.5	196.9	±	39.3	0.004
LDL-C	mg/dL	88.5 ±	18.5	107.4	±	34.5	0.004
HDL-C	mg/dL	63.5 ±	10.3	75.6	±	17.5	0.500
TG	mg/dL	76.5 ±	32.9	72.4	±	39.3	0.782
FPG	mg/dL	82.9 ±	6.6	89.9	±	9.4	0.454
HbA1c	%	5.1 ±	0.2	5.2	±	0.2	0.138
IRI	μU/mL	5.9 ±	2.3	3.2	±	1.1	0.002
Glucagon	pg/mL	107.5 ±	10.4	112.8	±	14.2	0.793
HORMA-IR		0.7 ±	0.2	1.2	±	0.6	0.004

Data are expressed as mean ± SD, paired t test. BMI, body mass index; TC, total cholesterol; LDL-C, low-density-lipoprotein-cholesterol; HDL-C, high-density-lipoprotein-cholesterol; TG, triglyceride; FPG, fasting plasma glucose; IRI, immunoreactive insulin; HORMA-IR, homeostasis model assessment of insulin resistance; SD, standard deviation.

## Changes pf postprandial GLU (Whole analysis)

The GLU change curves of all subjects show that they were higher after ingesting control food (bread containing wheat starch) than after ingesting test food (bread containing HMT-HAS). The postprandial GLU levels showed significant decreases 30, 45, 60, 90 and 120 minutes after starting test food intake (*Table 5, Fig. 2*). iAUC showed a significantly lower level in test food compared with control food (p < 0.01, *Table 6, Fig. 3-a*). Cmax showed a significantly lower level in test food compared with control food (p < 0.01, *Table 7, Fig. 3-b*).  $\Delta$ Cmax showed a significantly lower level in test food compared with control food (p < 0.05, *Table 8, Fig. 3-c*).

# Changes of postprandial GLU (Subclass analysis)

All subjects were classified into two groups of 13 college students and 19 company workers and analyzed. In the group of college students, the GLU change curve after ingesting the control food was higher than after ingesting the test food, which was the same as the analysis of GLU change curves of all subject groups. Postprandial GLU levels significantly lowered 30 minutes after starting test food intake comparing with control food (p < 0.05) and tended to lower 45 minutes after starting test food intake (p < 0.1, *Table 9*, *Fig. 4*). There was no significant difference in iAUC and Cmax between the two groups. A significant reduction was shown in  $\Delta$ Cmax by ingesting test food compared with control food (p < 0.05, *Table 10*, *Fig. 5*).

In the company workers' group, a higher GLU change curve was shown by ingesting test food compared with control food, which was the same as the analysis of all subjects' groups after ingesting each food. The postprandial GLU showed a significant reduction 45 minutes, 60 minutes, 90 minutes and 120 minutes after starting ingestion of the test food compared with control food (p < 0.01, *Table 11, Fig. 6*). iAUC was significantly reduced by ingesting test food compared with control food (p < 0.05, *Table 11, Fig. 7-a*). Cmax and  $\Delta$ Cmas were significantly reduced by ingesting test food compared with control food (p < 0.05, *Table 11, Fig. 7-a, b*).

#### Table 5. Changes in GLU (mg/dL) after meals: Total analysis.

	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control food	$0.0\pm0.0$ -	$16.3 \pm 16.6 5.7$	58.8 ± 17.1 5.9	$61.9 \pm 20.5$ 7.1	48.1 ± 18.0 6.3	33.5 ± 18.3 6.4	29.5 ± 17.7 6.1
Test food	$0.0 \pm 0.0$ -	17.6 ± 13.1 4.5	48.0 ± 17.8 6.2	49.5 ± 22.4 7.8	35.8 ± 20.6 7.1	25.8 ± 16.1 5.6	$15.0 \pm 12.7 \ 4.4$

Data are expressed as mean ± SD, 95% CI, n = 32. GLU, blood glucose; SD, standard deviation; CI, confidence interval.



## Fig. 2. Changes in GLU level after meals.

Data are expressed as mean  $\pm$  95% CI, n = 32, \* p < 0.05, \*\* p < 0.01 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose; CI, confidence interval.

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	iAUC	Cmax (mg/dL)	ΔCmax (mg/dL)	
Control food	4583.9 ± 1422.1 492.7	$162.5 \pm 22.2$ 7.7	$68.5 \pm 17.6$ 6.1	
Test food	3530.5 ± 1202.9 416.8	$151.3 \pm 20.8$ 7.2	$57.4 \pm 19.4  6.7$	

#### Table 6. iAUC, Cmax and $\triangle$ Cmax: Total analysis.

Data are expressed as mean  $\pm$  SD, 95% CI, n = 32. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta$ Cmax, maximum value of GLU concentration change; SD, standard deviation; CI, confidence interval.

#### Table 7. Multiple comparison analysis by Bonferroni: Total analysis.

Level 2	Level 2	Changes in GLU level (min)							iAUC	Cmax	ACmax
		0	15	30	45	60	90	120	IAUC	Cillax	
Control	Test	-	1.000	0.007	0.001	0.001	0.033	< 0.001	< 0.001	0.001	0.001

n = 32. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta$ Cmax, maximum value of GLU concentration change.

## Table 8. Changes in GLU (mg/dL) after meals: Subclass analysis in College students.

	0 min	15 min	30 min	45 min	60 min	90 min	120 min
Control food	$0.0 \pm 0.0$ -	17.3 ± 15.7 5.4	62.0 ± 17.0 5.9	64.2 ± 19.1 6.6	46.7 ± 16.7 5.8	31.5 ± 13.3 4.6	30.8 ± 20.2 7.0
Test food	$0.0 \pm 0.0$ -	22.1 ± 14.0 4.8	49.5 ± 14.4 5.0	49.0 ± 22.2 7.7	36.2 ± 23.3 8.1	29.1 ± 18.1 6.3	$19.0 \pm 14.5 5.0$

Data are expressed as mean ± SD, 95% CI, n = 13. GLU, blood glucose; SD, standard deviation; CI, confidence interval.



#### Fig. 3. Comparison between test group and control.

#### a: iAUC, b: Cmax, c: $\Delta$ Cmax.

Data are expressed as mean  $\pm$  95% CI, n = 32, \* p < 0.05, \*\* p < 0.01 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta$ Cmax, maximum value of GLU concentration change; CI, confidence interval.

Table 9. M	Iultiple co	mparison a	nalysis b	v Bon	ferroni:	<b>Subclass</b>	analysis in	College students.
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Level 2	Level 2	Changes in GLU level (min)							iAUC	Cmax	ACmax
		0	15	30	45	60	90	120	TAUC CIIIdx		
Control	Test	-	0.837	0.036	0.051	0.225	1.000	0.178	0.144	0.264	0.048

n = 13. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta$ Cmax, maximum value of GLU concentration change.



Fig. 4. Changes in GLU level after meals in College students.

Data are expressed as mean  $\pm$  95% CI, n = 13, \* p < 0.05, † p < 0.1 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose; CI, confidence interval.

#### Table 10. Changes in GLU level after meals: Subclass analysis in Company workers.

	0 min	15 min	30 min	45 min	60 min	90 min	120 min	
Control food	$0.0 \pm 0.0$ -	15.7 ± 17.5 6.1	$56.5 \pm 17.2 \ 6.0$	$60.3 \pm 21.8$ 7.6	49.0 ± 19.3 6.7	34.9 ± 21.4 7.4	28.6 ± 16.3 5.7	
Test food	$0.0\pm0.0$ -	14.5 ± 11.8 4.1	46.9 ± 20.0 6.9	49.9 ± 23.1 8.0	35.5 ± 19.2 6.6	23.6 ± 14.6 5.1	$12.3 \pm 10.8 \ 3.8$	

Data are expressed as mean ± SD, 95% CI, n = 19. GLU, blood glucose; SD, standard deviation; CI, confidence interval.



## *Fig. 5.* $\Delta$ Cmax in College students.

Data are expressed as mean  $\pm$  95% CI, n = 13, \* p < 0.05 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose;  $\Delta$ Cmax, maximum value of GLU concentration change; CI, confidence interval.

Table 11. Multiple comparison analysis by Bonjerroni: Subclass analysis in Company workers.											
Level 2	Level 2	Changes in GLU level							iAUC	Cmax	ACmax
		0	15	30	45	60	90	120	mee	Cinax	
Control	Test	-	1.000	0.168	0.031	0.003	0.005	< 0.001	< 0.001	0.003	0.017

n = 19. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta$ Cmax, maximum value of GLU concentration change.





Data are expressed as mean ± 95% CI, n = 19, \* p < 0.05, \*\* p < 0.01 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose; CI, confidence interval.



#### Fig. 7. Comparison between test group and control in Company workers.

#### a: iAUC, b: Cmax, c: $\Delta$ Cmax.

Data are expressed as mean ± 95% CI, n = 19, \* p < 0.05, \*\* p < 0.01 vs control by Bonferroni multiple comparison analysis with related data. GLU, blood glucose; iAUC, incremental area under the curve; Cmax, maximum value of GLU concentration;  $\Delta C$ max, maximum value of GLU concentration change; CI, confidence interval.

# Discussion

#### Summary of results

The postprandial GLU showed a higher level for all subjects by ingesting control food (bread containing wheat starch) than test food (bread containing HMT-HAS). iAUC was significantly reduced by ingesting test food compared with control food, and  $\Delta$ Cmas was significantly reduced by ingesting test food compared with control food. From these results, it is predicted that HMT-HAS blended in test food inhibits postprandial hyperglycemia.

From the subclass analysis, it is presumed that HMT-HAS blended in the test food more effectively inhibited the postprandial hyperglycemia in the company workers' group than in the college students' group. The comparisons of iAUC, Cmax and  $\Delta$ Cmax between college students' group and company workers' group showed that only the Cmax in company workers' group after ingesting control food was high (p < 0.05). It showed the possibility that the hyperglycemic state of the company workers' group whose insulin resistance tends to increase can be alleviated by replacing their food with a food that contains HMT-HAS, and it can also alleviate glycative stress.

Regarding the background factors of the 32 subjects of this study, they were classified into company workers' group and college students' group. The average age of the company workers was 14.3 years older than that of the college students, and their body weight, BMI, TC, and LDL-C were higher while their pulse rate was significantly lower than those of college students. Regarding the items relating to carbohydrate metabolism, the corporate workers were 2.7 µunit/mL (1.8 times) lower in IRI value than college students and their HORMA-IR was high, so that it was presumed that they had the tendency for higher insulin resistance. Looking at the efficacy of the test food, even though no clear efficacy was observed in college students, the effect to alleviate postprandial hyperglycemia was observed in corporate workers. It was presumed that the efficacy of this food can be exhibited in the middle aged older than 30, those whose insulin resistance increases and those who are prone to have postprandial hyperglycemia more than in young people around 20 years old.

There are many opportunities to take bread as a staple food at breakfast and lunch. HMT-HAS is a dietary fiber material, so it is possible to ingest this material on a daily basis by blending it into bread and it contributes to the health maintenance of those who ingest it.

## Starch

What represents staple foods eaten in the world are rice and wheat, and the main component of both foods is starch. Rice (rice plant) is cultivated everywhere in the world. Asian rice is broadly classified into Japonica rice and Indica rice. Cooked Japonica rice is soft and sticky and cooked Indica rice is hard and less sticky. The main component of rice is starch which becomes the main energy source. The digestibility of rice starch is affected by different shapes, such as powder or particle, or cooked rice, noodle or rice cake<sup>8,9</sup>.

In Japan, there are various functional rice processed foods including rice powder bread, rice powder noodles and sprouted brown rice centering on the new rice characteristic. Sticky rice and low-amylose rice are fast in digestion and absorption. On the other hand, the inhibiting effect of high-amylose rice and super-hard rice on postprandial hyperglycemia is recognized. It is also expected to have an effect on the prevention and inhibition of the progression of life-style diseases with strong glycative stress such as visceral obesity, metabolic syndrome and type 2 diabetes<sup>8,9</sup>.

Starch is a natural polymer of polymerized glucose and it consists of amylose, molecules of glucose arranged in a straight chain, and amylopectin, a molecule with many branches. The crystal structure of starch is maintained because of amylose and amylopectin regularly arranged by hydrogen bonding. Starch is a white powdery substance that does not dissolve in water and leaves deposits if it is left alone in water. The origin of the Japanese word for starch, *"denpun,"* comes from the Japanese words of sediment and powder.

In order to dissolve it, starch must be added to water and heated. The starch granules under water start absorbing water above a certain temperature and swell. When the swelling reaches the maximum point, starch is broken and becomes pasty. This phenomenon is called the gelatinization of starch.

Gelatinization properties of starch differ depending on the ratio of amylose and amylopectin. In the case of rice, the lower amylose ratio is, the more cooked rice becomes sticky.

HAS is a functional starch containing many dietary fibers. It also has the names of indigestible starch and resistant starch. HAS expresses stickiness only at high concentrations, and its gelatinization temperature is very high <sup>10,11</sup>.

HMT-HAS was used in this test food. The test food (HMT-HAS) containing dietary fibers increased approximately three times compared to normal bread and was developed by being heat-treated (heat-moisture treated) in a water amount in which starch is not gelatinized<sup>5</sup>.

#### Animal study

An animal test using HAS, or feed containing HAS, has been conducted. As the results of measuring fat metabolism and the cumulative amount of body fat of the rats fed with a diet containing 10%-40% of HAS, the weight of epididymal adipose tissue and the ratio of body fat and body protein were significantly lower or showed the tendency for a low level in the groups ingesting HAS (10%, 20% and 40%). In the concentrations of serum lipids (TG, TC and phospholipid) and the content of neutral fat in the liver, decreases depending upon the dose of HAS in the animal feed were recognized <sup>12</sup>.

The results of verification concerning the difference of the content of amylose in the feed for mice showed that the postprandial GLU response of the high-amylose group (29% amylose) was low at all times compared with the low-amylose group (17% amylose), and it was significantly lower in particular 15, 30, 45 and 60 minutes after ingesting it, and AUC of the high-amylose group was 16% lower than that of the low-amylose group <sup>13</sup>.

# GLU test

The responses of GLU and insulin of the high-amylose group of human subjects (29% amylose) 60 minutes after eating were significantly lower than those of the low-amylose group (17% amylose), and the GLU response 30 and 60 minutes after eating and the insulin response and AUC 60 minutes after eating in the high-amylose group were significantly lower than those of the low-amylose group<sup>13</sup>.

A GLU test comparing low GI rice blended with barley

and high-amylose rice and the control rice for 10 patients with type 2 diabetes showed that the degrees of elevation of GLU and insulin 60 minutes after eating the rice blended with high-amylose rice were significantly inhibited <sup>14</sup>. No inhibiting effect on GLU and insulin by glucagon was observed, and on the contrary, they were slightly increased.

A meal tolerance test (total energy 616 kcal; energy ratio: carbohydrate 56%, lipids 27%, protein 14% and dietary fibers 3%) was conducted on the patients with diabetes (n = 21) using sulfonylurea drug only or together with other drugs, and as a result of the comparison between high-amylose rice (containing amylose 25%) and Koshihikari (containing amylose 17%), the postprandial GLU by high-amylose rice remained at a low level, and Cmax was significantly lower (7% in average, p < 0.001)<sup>15</sup>.

There is a report that in the test where postprandial GLU changes following the intake of foods containing different amounts of amylose targeting seven adult males with normal glucose tolerance, there was no significant difference in GLU and insulin levels among three foods of 75 g of glucose, cooked rice and rice cakes containing the same amount of glucose<sup>16</sup>.

#### Long-term ingestion study

There is a report of clinical tests of the comparison between high-amylose rice (containing amylose 25%) and Koshihikari (containing amylose 17%).

A test was conducted where the subjects with untreated diabetes and glucose intolerance (52 adult males) ingested one package each of the cooked rice (containing carbohydrate 50 g) of high-amylose rice (containing amylose 25%) or Koshihikari (containing amylose 17%) twice a day for 12 weeks consecutively. As a result, no significant difference was observed in fasting GLU levels, but the HbA1c and 1.5-AG (anhydro-glucitol) levels of those who ingested high-amylose rice were significantly improved <sup>17</sup>.

#### Sensory evaluation

High-amylose rice, which is highly expected to improve food self-sufficiency, does not taste good in the form of bread, in particular, so improvement is required. Its taste has been evaluated by the sensory evaluations of rice containing various amyloses and the hardness of bread.

The taste of rice bread containing different amounts of amylose was evaluated by sensory test by the semantic differential (SD) method and its physical property was tested by a tensipresser, and the results are shown below <sup>18</sup>.

As for its physical property, the more amylose it contained, the harder it became. However, the high-amylose rice bread which is said to be not delicious showed room for improvement and was evaluated as "it rather does not taste good." As the result of the sensory evaluations of different varieties of rice containing different rates of amylose such as sticky rice (containing amylose 0%), low-amylose rice (containing amylose 11.9%), middle-amylose rice (containing amylose 16.2%) and high-amylose rice (containing amylose 27.9%), high-amylose rice was evaluated to be the hardest and not sticky in the mouth <sup>19</sup>.

The results of the sensory test of GI rice blended with barley and high-amylose rice and the control rice (ordinary rice) are shown below<sup>14</sup>). There were three answers of "good" and seven answers of "ordinary" in texture of ordinary rice, and there were two answers of "not so good" and eight answers of "medium" in the texture of low GI rice. As for the condition of cooked rice, there were six answers of "perfect" in ordinary rice and one answer of "perfect" in GI rice, and there was one answer of "soft" in ordinary rice and three answers of "soft" in low GI rice. There were five answers of "tasty but not great" in ordinary rice and four answers of "tasty but not great" and one answer of "not so tasty" in low GI rice." There were seven answers of "even though there is a slight uncomfortable feeling, if it is good for levels of GLU and insulin, I will eat it," and three answers of "I will eat it taking the cost into consideration."

It is considerable that the food materials for further "delicious" and "soft" bread can be developed by further studies in the future.

#### Safety

Amylogel HB-450 is an insoluble dietary fiber material with the content of dietary fibers heightened by heat-moisture treatment. Dietary fibers have the effect of excreting waste and harmful materials out of the body (fiber detox). Among them, resistant starch is superior in process ability, so that it has been applied to various food products.

As an edible product for humans, Amylogel HB-450 started being sold in 1997 and it has been successfully used for many food products. It is described in the certificate of Quality Assurance Department of Sanwa Starch Co., Ltd. that there has been no report about health damage by this product.

#### Limitation of this study

The tests conducted for this study was to show that the postprandial hyperglycemia after ingesting bread including HMT-HAS was inhibited more than after ingesting control food (bread containing wheat starch). Postprandial hyperglycemia triggers aldehyde spark. However, in this study, intermediate aldehydes (glyoxal (GO), methyglyoxal (MGO), 3-deoxyglucosone (3DG) and others) were not measured. Therefore, it was only to evaluate the possibility by estimation that an aldehyde spark can be alleviated.

It was not possible to clarify to what extent the alleviation of postprandial hyperglycemia can be achieved by a single intake and contribute to the alleviation of glycative stress from a long-term point of view based on the test in this study.

# Conclusion

HMT-HAS has the effect of alleviating postprandial hyperglycemia and glucose spikes as a material containing dietary fibers, so that it is expected that it contributes to the health maintenance of those who ingest it by alleviating glycative stress.

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# **Conflict of Interest Statement**

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