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Original

Effects of Intake of Puffed *wx ae* Double Mutant Brown Rice on Human Blood Glucose and Lipid Levels

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The amylopectin in *waxy amylose-extender* (*wx ae*) double mutant rice characteristically has a small number of branch chains and long side chains. In this study, we conducted single intake trials and middle term intervention trial to puffed non-glutinous *Uruchi* and *wx ae* brown rice. Postprandial blood glucose response to a single intake of puffed *Uruchi* and *wx ae* brown rice were measured with 3 men and 4 women (47.1 ± 12.7 years of age (mean ± standard deviation [SD])). Subjects who were fasted for 10 to 12 h, has ingested 20 g of the puffed sample. Incremental blood glucose levels of *wx ae* at 45 and 60 min ($p < 0.01$), and its peak (30 min) were much lower than for *Uruchi*. Middle term intervention trial had assigned 14 healthy men and women to eat either puffed brown rice of *wx ae* or *Uruchi* for 15 days to compare possible effects on blood glucose and serum lipid levels. Each group consumed 60 g of puffed brown rice a day, at any time of day, in addition to their ordinary meals. Those in the experimental diet (ED) group (4 men and 3 women; 45.1 ± 16.8 years of age) ate *wx ae* brown rice, while those in the control diet (CD) group (5 men and 2 women; 45.1 ± 15.1 years of age) ate *Uruchi* rice. Fasting blood samples were obtained before and after the experiment to examine and compare clinical chemical test values. The results showed that 1,5-anhydroglucitol (1,5-AG) declined significantly in the CD group and total cholesterol levels declined in the ED group, while triglyceride values rose in the CD group. Thus we expect that *wx ae* brown rice can be used as a functional food superior to *Uruchi* brown rice.

Key words: *wx ae* double mutant rice; resistant starch; blood glucose; serum lipid

1 Introduction

In recent years, lifestyle diseases have accounted for an increasing percentage of deaths. It is therefore important to improve the content of meals to prevent lifestyle-related diseases such as type 2 diabetes or dyslipidemia. Consequently, many attempts have been made to use the functionality of various foods, including functional rice.[1−3]

A *Japonica* rice (*Oryza sativa*) mutant, the *waxy amylose-extender* (*wx ae*) double mutant, produces a unique starch of pure amylopectin, with branched chains longer than those of normal rice amylopectin, the result of defective starch branching enzyme IIb and granule-bound starch synthase I.[4] The *wx ae* starch shares a feature of resistant starch (RS) and represse increase of the post-prandial glucose level in mice.[5] RS cannot be digested in the small intestine,[6] and is effective against hypoglycaemia,[7, 8] hypocholesterolaemia,[9, 10] and fat accumulation.[11]

To make functional food part of the regular diet, we must think about how to cook it. We used puffed *wx ae* brown rice in this study because an elevated intake of milled rice seems to be associated with an increased risk of lifestyle-related diseases,[12, 13] while brown rice seems to decrease the risk of lifestyle-related diseases.[14−16] We were able to give a roasted flavor to the *wx ae* rice and eliminate a smell of bran by puffing it.

Based on preliminary animal testing results, we planned a 15-day study using human subjects with the aim of verifying whether puffed *wx ae* brown rice inhibits postprandial blood glucose elevation and improves serum lipid levels.

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2 Subjects and Methods

2.1 Materials

We used a *waxy amylase-extender* mutant line AMF18 which is a double-recessive mutant for *waxy* (*wx*) and *amylase-extender* (*ae*). It was derived from a cross between the EM21 and EM16 lines. The *wx* mutant line EM21 and the *ae* mutant line EM16 are genetically defective in granule bound starch synthase I and starch branching enzyme IIb, respectively. Those mutants were generated by treating fertilized egg cells of japonica rice (*Oryza sativa*) cv *Kinmaze* with *N*-methyl-*N*-nitrosourea.[17] We used *Uruchi* rice cv *Kinmaze* as a control to measure postprandial blood glucose response to a single intake, and *Uruchi* rice cv *Hinohikari* as a control in a middle term intervention trial. *Hinohikari* and *wx ae* rice plants were grown in the summer of 2009 in Nara, Japan and *Kinmaze* rice plants were grown in the summer of 2007 in an experimental field at Osaka Prefecture University.

We puffed the rice using a puffing machine SL (Tachibana Kiko, Fukuoka, Japan). After puffing, the rice was kept dry until the intervention trial.

2.2 Puffed Uruchi and wx ae brown rice for trials

We measured the postprandial blood glucose response to a single intake of puffed *Uruchi* and *wx ae* brown rice. Seven healthy men and women (3 men and 4 women; 47.1 ± 12.7 years of age) who work at Osaka Prefecture University were recruited for single intake trials for puffed *Uruchi* and *wx ae*. Subjects fasted for 10-12 h before the trials, and then ingested 20 g of the puffed sample with 80 mL water. Blood samples were taken from the tip of the finger after 0, 15, 30, 45, 60 and 120 min. Blood glucose levels were determined by the glucose oxidase method using a NIPRO Freestyle Freedom blood glucose monitoring system (NIPRO Co., Osaka, Japan). The single intake trials of puffed *Uruchi* and *wx ae* were repeated with the same subjects a week later.

2.3 Middle term intervention trial with puffed rice

We recruited 14 healthy men and women (aged 20-68 years) comprising teachers, staff and students of Osaka Prefecture University.

The ED consisted of 60 g of puffed *wx a*e brown rice, while the CD comprised 60 g of puffed *Uruchi*. It is the maximum volume that we can intake without unreasonableness consecutively. The experiment was performed for 15 consecutive days in March 2010. The subjects were randomly assigned to either the CD (5 men and 2 women; 45.1 ± 15.1 years of age [mean ± SD]) or the ED (4 men and 3 women; 45.1 ± 16.8 years) group. They were asked to consume their quota of puffed rice at any time of the day in addition to their usual meals. The study was designed as a parallel group comparison.

Before the experiment, we investigated the normal dietary habits of the participants. During the experiment, we used diet records to examine the influence of the new diet on their meals. Calculations were made with Excel "Eiyokun" software Ver. 5.0 and Food Frequency Questionnaire (FFQg Ver. 2.0, Kenpakusha). We also recorded the amount and hardness of their stools, presence or absence of abdominal wind and status of other digestive organs. The amount of energy consumed was determined with a portable pedometer (PW-900, Yamasa Tokei Co., Ltd). To determine physical status, we measured the participants' weight and body fat percentage using the Body Planner DF-800 (Yamato-Scale Co., Ltd).

Fasting blood samples were obtained in the early morning before and after the experiment to determine levels of blood glucose, glycoalbumin and 1,5-AG. In addition, we measured levels of serum triglyceride, total cholesterol, high density lipoprotein (HDL) cholesterol and low density lipoprotein (LDL) cholesterol. LDL cholesterol concentrations were calculated with the Friedewald formula.

Informed consent was obtained from each of these subjects. The study was approved by the Research Ethics Review Committee of Osaka Prefecture University of Comprehensive Rehabilitation (approval number: 08-307, 2009-05) and conducted observing the terms set by the committee. Blood tests were performed in cooperation with the Houtokukai Medical Association Houwa Hospital.

Data were expressed as mean value ± SD. Statistical processing was performed with the Wilcoxon t-test within groups, while group comparisons were made with the Mann-Whitney U-test. We used Dr. SPSS II software (IBM) for the analyses. Data were considered significant when $p < 0.05$.

3 Results

Fig. 1 shows the subjects' postprandial incremental blood glucose response. Incremental blood glucose levels were lower after ingesting puffed *wx ae* brown rice ($p < 0.01$; 30, 45 and 60 min) than after ingesting puffed *Uruchi*. Blood glucose concentration at its peak (30 min) was much lower for *wx ae* than for *Uruchi*. Based on these results and given that puffed rice is an easy-to-eat food with a roasted flavor, we decided to conduct a middle term intervention trial using puffed *Uruchi* and *wx ae* brown rice.

Throughout the middle term intervention trial, both CD and ED groups showed no significant change in physical status, including weight, body fat percentage, and the amount of energy intake and amount of energy consumed (Table 1). Also, no adverse events, including diarrhea, were reported during the period.

Table 2 indicates changes in clinical blood test values. In both groups, fasting blood glucose levels tended to decline slightly. Glycoalbumin did not fluctuate in either group before or after the experiment. A significant decline in 1,5-AG was observed in the CD group ($p = 0.040$).

Triglyceride levels were significantly elevated in the CD group ($p = 0.022$), while total cholesterol values declined significantly in the ED group ($p = 0.042$). HDL and LDL cholesterol concentrations tended to decrease in both CD and ED groups.

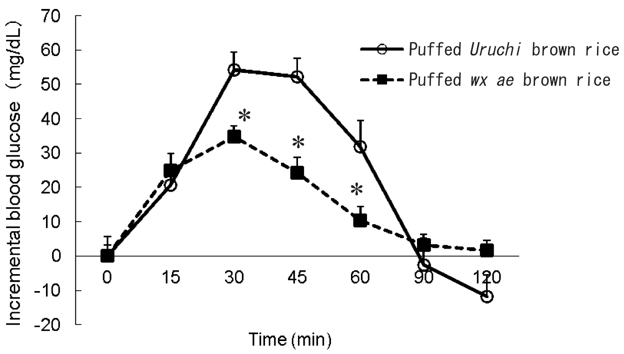

Fig. 1 Postprandial blood glucose response to puffed *Uruchi* and *wx ae* brown rice.
Puffed *Uruchi* brown rice group (n = 8), Puffed *wx ae* brown rice group (n = 7).
*Significant difference groups within the time were analyzed by the Wilcoxon t-test at $p < 0.01$. Data are mean ± SD.

4 Discussion

Uruchi brown rice inhibits the postprandial incremental blood glucose response better than white rice does.[16, 18] Our results suggest that *wx ae* brown rice inhibits this response better still. We recruited subjects for middle term intervention trials for *wx ae* and puffed *Uruchi*. We measured the participants' body weight, body fat percentage, amount of energy intake, amount of energy consumed, blood glucose and serum lipid level.

No significant change was observed in the amount of

Table 1 Changes in physical status of subjects

	CD group						ED group					
Subjects	Male : 5 Female : 2						Male : 4 Female : 3					
Age (year)	45.1 ± 15.1						45.1 ± 16.8					
Height (cm)	163.0 ± 7.8						164.3 ± 6.1					
	day0			day16			day0			day16		
Weight (kg)	61.9	±	10.8	61.4	±	10.2	65.2	±	16.8	64.9	±	16.4
BMI (kg/m^2)	23.3	±	2.5	21.9	±	3.2	24.2	±	2.0	23.3	±	4.4
Body fat (%)	22.9	±	4.3	21.9	±	6.2	27.8	±	5.7	27.4	±	5.7
Energy intake (kcal)	2009	±	362	2054	±	277	2022	±	236	2062	±	23
Energy consumed (kcal)	1891	±	374	1929	±	318	1935	±	508	1912	±	455

CD; control diet, ED; experimental diet, BMI; body mass index. Data are mean ± SD.

Table 2 Changes in clinical chemical test values

	CD group						ED group					
	day 0			day 16			day 0			day 16		
Blood glucose (mg/dL)	96	±	8	92	±	8	89	±	7	87	±	5
Glycoalbumin (%)	12.7	±	0.7	12.7	±	0.7	12.9	±	0.6	12.9	±	0.6
1,5-AG (μg/mL)	26.5	±	9.1	24.8	±	8.7*	24.4	±	8.3	23.9	±	8.5
TG (mg/dL)	77	±	41	111	±	63.5*	83	±	39	91	±	46
TC (mg/dL)	207	±	31	197	±	31	204	±	46	191	±	49*
HDL-C (mg/dL)	62	±	13	58	±	11	65	±	20	62	±	17
LDL-C (mg/dL)	127	±	38	115	±	28	115	±	29	104	±	30

CD group (n = 7), ED group (n = 7). CD; control diet, ED; experimental diet.
1,5-AG; 1,5-anhydroglucitol, TG; triglyceride, TC; total cholesterol,
HDL-C; high density lipoprotein cholesterol, LDL-C; low density lipoprotein cholesterol.
*Significant difference between the day 0 and day 16 within the same group was analyzed by Wilcoxon t-test at $p < 0.05$. Data are mean ± SD.

energy intake or amount of energy consumed in either group. We concluded that these factors did not influence blood test results.

1,5-AG is the most abundant sugar in healthy human blood after glucose. It is contained in a wide variety of foods, but only in minute amounts. Normally, it is reabsorbed into the kidney after excretion. If its blood concentration exceeds the threshold value of renal excretion, renal reabsorption is inhibited, 1,5-AG is discharged into the urine and its blood concentration declines,[19] which serves as an indicator of hyperglycemia after meals. The 1,5-AG concentration value is used mainly for improving high blood sugar after meals or for determining the beneficial effects of diet or exercise therapy.

Recently, 1,5-AG has been introduced into the guidelines on the management of postprandial hyperglycemia implemented by the International Diabetes Federation (IDF).[20, 21] In our study, the 1,5-AG levels of the subjects were all 14 μg/mL or above, falling within the normal range. A significant decrease was observed in the CD group from Day 0 through Day 16. This can probably be explained by leakage of sugars into urine due to transient hyperglycemia after meals. This phenomenon was not observed in the ED group.

Diagnostic criteria for metabolic syndrome include triglycerides at 150 mg/dL or above and HDL cholesterol at less than 40 mg/dL. It is essential to maintain normal serum lipid levels to stay healthy. In our experiment, the CD subjects showed negative effects, including decreased HDL cholesterol and elevated triglyceride levels. Ueno et al. reported that total cholesterol and LDL cholesterol dropped in subjects who consumed unpolished brown rice for a month,[22] and dietary fiber improves serum cholesterol levels.[23−25] In our study, total cholesterol levels decreased significantly in the ED group, which is consistent with these results. Lowered total cholesterol in the ED group may be explained by the fact that *wx ae* brown rice contains as much as four times the dietary fiber of *Uruchi* brown rice.

Triglyceride synthesis increases after carbohydrate-rich meals, because such foods provide sufficient levels of acetyl-CoA, a component of fatty acid synthesis, and nicotinamide adenine dinucleotide phosphate (NADP), a coenzyme necessary for the synthetic reaction. High-carbohydrate consumption is associated with hypertriglyceridemia.[26] Suzuki et al. divided subjects into two groups and asked them to eat either *Uruchi* white rice or brown rice. Both groups consumed a large amount (50 g/day) of sugar. As a result, the white rice group showed higher elevation of triglycerides and cholesterol than the brown rice group.[27] Similarly, triglyceride levels increased significantly in the CD group in our study. This was presumably because *Uruchi* rice starch was quickly digested and absorbed into sugars, resulting in synthesis of triglycerides in the liver. In contrast, probably because *wx ae* rice starch, rich in RS, is digested slowly and less is absorbed, triglyceride levels did not increase in the ED group in our study.

5 Conclusion

We reconfirm that puffed *wx ae* brown rice improves both carbohydrate and lipid metabolism more effectively than puffed *Uruchi* brown rice in middle term intervention trial. Our results suggest that puffed *wx ae* brown rice has a different functionality from that of *Uruchi*, and *wx ae* may be useful as a new dietary ingredient for preventing lifestyle-related diseases. Now we plan to do a long-term crossover study.

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