Original articles

Influence of rice with different amylose contents on postprandial glycemic response

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Abstract

Objective : Clinical studies suggest that maintaining a lower postprandial glycemic response is important for improvement and prevention of metabolic syndrome and type 2 diabetes mellitus. Amylose, an ingredient in many food grains, is a major factor for the lowering of postprandial glycemic and insulinemic response. The aim of the present study was to determine the influence of rice with different level of amylose on postprandial glycemic and insulinemic response in mice and humans.

Materials and Methods : The two types of rice used in the study contained 29 wt% (high amylose rice) or 17 wt% (low amylose rice) amylose. In mice and humans, postprandial glycemic and insulinemic responses were measured and then the area under the response curves of both rice groups were compared.

Results : In mice, comparisons of postprandial glycemic response showed high amylose rice was lower than that for low amylose rice in all time points. Notably postprandial glycemic responses for high amylose rice at 15, 30, 45 and 60 min

were significantly lower (19%, 31%, 16% and 17% respectively). The area under the glycemic response curve for high amylose rice was a remarkably 16% less than for the low amylose rice. In humans, postprandial glycemic response at 30 min and insulinemic response at 60 min for high amylose rice were significantly lower than for low amylose rice (15% and 40% lower, respectively). Furthermore, general linear measurement multivariate analysis after adjustment for eating time and hemoglobin A1c at baseline showed that postprandial glycemic response at 30 and 60 min and insulinemic response at 60 min, and the area under the glycemic response curve for high amylose rice were significantly lower than for low amylose rice in human.

Conclusion : The higher amylose content of the rice lowered the postprandial glycemic and insulinemic response, demonstrating the potential to prevent or improve metabolic syndrome and type 2 diabetes mellitus.

Key words : amylose , glycemic index , postprandial glycemic response , postprandial insulinemic response

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Introduction

Attention has been focused on dietary carbohydrates, major role-players in postprandial glycemic and insulinemic response. Postprandial hyperglycemia is a leading factor of *de novo* lipogenesis and increased triglycerides accumulation in the liver and the fat tissue (1-2). Additionally, abnormal lipid metabolism occurs between the liver and adipose tissue through circulating nonesterified fatty acids in the chronic stage (3-4). These abnormalities lead to obesity, metabolic syndrome and type 2 diabetes mellitus. The lowering of postprandial glycemic and insulinemic peaks is an essential objective in the prevention of obesity and type 2 diabetes mellitus (5).

There is no doubt that the quality and quantity of carbohydrates considerably affect their rate of absorption. More specifically, the quality of the carbohydrate has a major influence on postprandial glycemic and insulinemic response. The Glycemic Index (GI), pertaining to carbohydrate foods, is based on their effects on blood glucose. In the past two decades, the GI has been the topic of many studies (6). Dietary fiber and amylose content, major factors affecting GI, are very much involved with postprandial glycemic and insulinemic response (7-9). Amylose, linearly polymerized glucose residues linked by alpha 1-4 bonds, is difficult to digest in the small intestine and passes into the large intestine for fermentation (10). With this advantageous characteristic, foods with a higher amylose content may help reduce postprandial glycemic and insulinemic response (9).

We have previously reported that high carbohydrate diets contributed to obesity and metabolic syndrome in Japanese (11-14). Particularly, we indicated that a decrease in carbohydrate intake was a main contributor to weight loss in overweight Japanese (15-16). The staple rice consumed by Japanese contains 85-90 wt% of carbohydrates, has a relatively low amylose content, thus a potential contributor to

postprandial hyperglycemia. Therefore, replacement of low-amylose rice with the higher amylose rice may help to suppress postprandial hyperglycemia and hyperinsulinemia. We performed an animal and human study to examine the effects of the higher and lower amylose rice on postprandial glycemic and insulinemic response.

Materials and Methods

Animal study

Male C57J/BL mice, aged 7 weeks and weighing 20-23 g, were purchased from Oriental Yeast Co., Ltd (Tokyo, Japan). C57J/BL mice are prone to obesity and frequently used in studies on obesity and type 2 diabetic mellitus. All mice were allowed to acclimate one week before use in the present study. The liquefied test meals were prepared using two types of boiled rice. One was produced using high amylose rice known as YUMETOIRO (high amylose : "HA rice") ; the other using normal rice known as KOSHIHIKARI (low amylose rice : "LA rice"). Percentage composition of the two test meals is shown in Table 1.

For the present study, all mice were fasted for 16 hr, and then randomly divided into two groups. 3.1 mg/g body weight of carbohydrate (0.014 kcal/g body weight of test meal) of HA or LA rice was gavaged orally using intragastric tubes. Blood samples were collected from abdominal vein into tubes containing EDTA-2Na at 0, 15, 30, 45, 60, 90 and 120 min following test-meal ingestion, and then measured for glucose and insulin concentrations. At an each time point,

Table 1 Composition of rice.

	LA rice	HA rice
Carbohydrate (% of total energy)	88	88
Fat (% of total energy)	4	4
Protein (% of total energy)	7	7
Amylose (% of total weight)	17	29

HA ; high amylose, LA ; low amylose.

ten mice were sacrificed under ether anesthesia. All procedures in mice experiments were approved by the Animal Care and Use Committee of Shimane University School of Medicine, Japan.

Plasma was separated from collected blood samples by centrifugation (1000 g×15 min). Plasma glucose and insulin concentrations were measured by Glucose C-II test Wako (Wako Pure Chemicals, Osaka, Japan) and Rat Mouse Insulin ELISA kit (Mercodia AB, Uppsala, Sweden), respectively. The increments in plasma glucose and insulin until postprandial 120 min time point were appraised by the AUC using by the trapezoidal rule. The AUCs with rice were calculated by using average values of plasma glucose and insulin concentrations at each at time point.

Human study

A total of 18 volunteers, aged 27-70 years [6 men, 50.3±13.4 years, body mass index (BMI) 24.7 ±2.2, fasting blood glucose 107±10 mg/dl, and hemoglobin A1c (HbA1c) 5.2±0.6 ; 12 women, 53.5 ±13.9 years, BMI 22.4±2.8, fasting blood glucose 96 ±10 mg/dl, and HbA1c 5.1±0.4] participated in a cross-over study. We excluded subjects with type 2 diabetes, who were prescribed medications for diabetes, fasting blood glucose 126 mg/dl or HbA1c 6.5% on the criteria of the Japan Diabetes Society. The ethics committee of Shimane University School of Medicine approved all study protocols, and all subjects gave written informed consent.

Two types of 200 g rice gruel which contained 100 kcal of energy and 19.6 g of carbohydrate were prepared with HA and LA rice. From 8 : 00 to 9 : 30 in the morning after an overnight fast, a half of subjects were randomly selected and given HA rice gruel ; the other half of subjects were given the lower amylose rice gruel. Venous blood was then collected from the antecubital vein at 0, 30, 60, and 120 min after eating rice gruel. In the afternoon of the same day, from 14 : 00 to 15 : 30, six hours after the rice gruel eaten in the morning, the latter half of the cross-over study was done as using the same methods.

Blood glucose and HbA1c were measured by electrode method and high performance liquid chromatography (normal range of HbA1c : 4.1-6.0%), respectively, at the Shimane Institute of Health Science. For measurement of insulin concentration, plasma was separated by same method of mice experiments and Human Insulin ELISA kit (Mercodia AB, Uppsala, Sweden) was used. The AUC's of each subject were independently calculated using the trapezoidal rule.

Statistical analyses

Statistical analyses of animal and human study data were done with SPSS software version 13.0 J (SPSS Inc., Tokyo, Japan). In animal study, results are expressed as means±S.D. Comparisons of two groups in mice were performed by Student's *t*-test, and *P*-value of less than 0.05 was used to assess significance. In human study, comparisons of two groups were performed by paired *t*-test, and *P*-value of less than 0.05 was used to assess significance. In addition, general linear measurement multivariate analysis (GLM) was used to assess the data of human study adjusted for eating time of rice gruel and HbA1c before the ingestion, considering confounding factor of circadian rhythm for test period and prediabetes of subject, respectively.

Results

Animal study

Figure 1A and 1B showed changes in mean postprandial glycemic and insulinemic response curve, respectively. Plasma glucose concentration of LA rice group increased after ingestion, and at 45 min achieved its highest level 410±27 mg/dl. In contrast, plasma glucose concentration of HA rice group increased to its highest level of 343±15 mg/dl that was lower than that of LA rice group. In the time after 45 min, plasma glucose concentrations of both groups decreased, but still, with that of HA rice group was remaining less than LA rice group (Figure 1A). The plasma

Figure 1 Changes in mean postprandial glycemic (A) and insulinemic (B) response in mice. Closed triangles and squares indicate high amylose (HA) rice group and low amylose (LA) rice group, respectively. Vertical bars indicate S.D. Comparisons of two groups were performed by Student's *t*-test, and asterisks indicate significant difference in time point ($P < 0.05$).

glucose concentrations of LA rice group at 15, 30, 45, and 60 min were significantly lower than those of LA rice group (19%, 31%, 16% and 17% respectively, $P < 0.05$). In the comparison of AUCs of postprandial glucose for the two rice groups, HA rice group was 16% less than LA rice group (Figure 3A, 518 mg/dl · hr versus 620 mg/dl · hr).

Figure 1B showed the changes in mean postprandial insulinemic response over a 120 min span. Although there were no significant differences at any of the time points, at 30 min the postprandial insulinemic response of HA rice group was slightly lower than that of LA rice group (9.8 ± 1.0 ng/dl versus 10.5 ± 1.0 ng/dl). A comparison of AUCs showed no significant difference (Figure 3A).

Figure 2 Changes in mean postprandial glycemic (A) and insulinemic (B) response in humans. Closed triangles and squares indicate high amylose (HA) rice group and low amylose (LA) rice group, respectively. Vertical bars indicate S.D. Comparisons of two groups were performed by Paired *t*-test, and asterisks indicate significant difference in time point ($P < 0.05$).

Human study

Figure 2A and 2B showed the results of the cross-over study in humans. In the postprandial glycemic response during the 60 min period, glucose concentrations in HA rice group were lower than for LA rice group. At 30 min, glucose concentrations in both groups peaked, and glucose concentrations for HA rice group were significantly lower than for LA rice group (132 mg/dl versus 152 mg/dl). A comparison of AUCs showed no significant difference, but the AUC for HA rice group was slightly lower than that for LA rice group (Figure 3B, 223 mg/dl · hr versus 238 mg/dl · hr).

Figure 2B showed the changes in mean postprandial insulinemic response over a 120 min span. At 60 min the postprandial insulinemic response of HA rice group was significantly lower

Figure 3 Comparison of AUCs in mice (A) and humans (B). Glycemia and insulinemia were displayed left and right, respectively. Open and gray bars indicate high amylose (HA) rice group and low amylose (LA) rice group, respectively. Verticality bars indicates S.D. Double asterisks indicate significant difference in AUC of human's insulinemia by paired *t*-test ($P < 0.01$).

than that of LA rice group (5.6 ± 3.2 mU/l versus 9.6 ± 6.4 mU/l), and at 30 min that of HA rice group was lower than that of LA rice group (14.6 ± 7.2 mU/l versus 18.6 ± 9.3 mU/l). Furthermore, a comparison of AUCs showed significant difference in Figure 3B (13.9 ± 6.9 mU/l versus 18.1 ± 8.1 mU/l, $P < 0.01$).

GLM multivariate analysis after adjustment for eating time of rice gruel and HbA1c, which have possibilities for affecting postprandial glycemic and insulinemic response, was showed in Table 2. There were significant differences at 30 min and

60 min with glycemic response and at 60 min with insulinemic response. A comparison of AUCs also showed significant difference in glycemia with GLM.

Discussion

The highlight of this study was that HA rice reduced postprandial glycemic response in mice and humans. In the mouse experiment, at all time during the 120 min period after ingestion of rice, the level of the postprandial glycemic response to HA rice was relatively lower than was that of LA rice. In particular, at 15, 30, 45 and 60 min, the response levels were significantly lower : 19%, 31%, 16% and 17%, respectively. Thus, the AUC of the postprandial glycemic response to HA rice was 16% less than that of LA rice. In humans, the difference was remarkably not significant, while for the mice there was clearly a significant difference. The amount of increase in the postprandial glycemic response was less at 60 min after ingestion of HA rice, but still lower than for LA rice. It suggested the magnitude of decrease in postprandial glycemic response by HA rice is due to carbohydrate lead in mice (about 66.7 mg/22 g body weight) and in humans (about 19.6 g/55 kg body weight). This finding indicates that food containing large amounts of amylose may be useful in the prevention of obesity and diabetes mellitus.

Our results for postprandial insulinemic response to HA rice showed significantly lower levels than for that of LA rice in humans, although slightly lower in mice. This finding indicates HA rice has great potential for prevention of insulin over-secretion from the pancreas and for improve insulin tolerance in human. In mice, some possible causes of no significant difference of insulinemic responses were considered. One possible cause may be that the quantity of rice consumed was insufficient to generate a greater difference of insulinemic response. Another possible cause is that, because the present study was single dose study.

Table 2 Adjusted levels of plasma glucose and insulin concentrations in humans.

	LA rice (n=18)	HA rice (n=18)	P
Plasma			
glucose (mg/dl)			
0 min	97±3	95±3	NS
30 min	159±5	135±5	<0.01
60 min	137±6	111±6	<0.01
120 min	99±4	97±4	NS
AUC (mg/dl · hr)	257±9	223±9	0.01
Insulin (mU/l)			
0 min	2.1±0.5	2.6±0.5	NS
30 min	17.7±2.0	14.3±2.0	NS
60 min	9.5±1.2	5.5±1.2	0.02
120 min	2.0±0.6	3.5±0.6	NS
AUC (mU/l · hr)	17.5±1.8	13.7±1.8	NS

GLM analysis in adjustment with eating time of rice gruel and HbA1c before the ingestion was used to assess the differences in plasma glucose and insulin. Data are means±S.E. HA ; high amylose, LA ; low amylose.

Pawlak *et al.* (17) reported on the long-term effects of different GI diets with amylose on normal rats. The body fat mass increases for the low GI group were significantly lower than that of the high GI group after of 17 weeks of feeding. Oral glucose tolerance tests were performed at 5 and 14 weeks with the AUCs for blood glucose and insulin of the low GI group being significantly lower. There were similar reported results by Lerer-Metzer *et al.*, (18). These seem to indicate that the low GI diet using amylose produces favorable effects in the controlling of postprandial glycemic and insulinemic responses. These two studies also reported histochemistry and morphometry data. In the high GI group, a much greater proportion of islets were distinctly abnormal than in the low GI group, showing severely disorganized architecture and extensive fibrosis. The reports concluded that the proportion of fibrotic islets in each individual strongly correlated to AUC of glycemic response for both high and low GI groups.

Although our results for postprandial insulinemic response to HA rice were only slightly lower with no significant difference to the LA rice results, postprandial glycemic response to HA rice

was clearly lower and significantly different than that of LA rice. In light of the conclusions of Pawlak *et al.* (17), and because HA rice resulted in much lower AUC for postprandial blood glucose in our tests, HA rice administered in our study may cause little or no pancreatic islet damage.

Rice, which accounts for a significant part of caloric intake in the Japanese, generally contains 85-90 wt% of carbohydrates, which are absorbed in the small intestine as simple sugar. Amylose, one of the carbohydrates, is classified as an undigested component. LA rice, used as the control in this study, contains amylose as 17 wt%, and is a commonly consumed rice in Japan. HA rice which containing 29 wt% amylose is, in contrast, specially grown and has not yet become popular with the general population, but seems to have significant potential in efforts to reduce postprandial glycemic response.

We speculate that damaging chronic hyperglycemia and hyperinsulinemia, causes of obesity and diabetes mellitus, exhaust and debilitate the pancreas, which leads to further damaging acute responses of blood glucose and insulin concentrations. Thus, we surmise that the lowering of the postprandial glycemic response is

of great importance in the maintaining general good health in individuals, as well as treatments of obesity and type 2 diabetes mellitus patients. Further long and medium term studies with GI foods, focusing on the pancreas, are needed to examine this premise more closely. We anticipate that results will coincide with our conclusion that higher-amylose food has the potential for prevention of metabolic syndrome and type 2 diabetes mellitus.

Conclusion

This study found that the higher amylose content of the rice lowered the postprandial glycemic and insulinemic response and AUC in mice and humans. These results are demonstrating the potential to prevent or improve metabolic syndrome and type 2 diabetes mellitus.

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