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The effect of low and high glycemic index based rice varieties in test meals on postprandial blood glucose, insulin and incretin hormones response in prediabetic subjects

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Abstract

Evidences have shown that low glycemic index (LGI) and glycemic load (LGL) of rice exert significant benefits to the population who are at risk to type 2 diabetes. LGI rice could minimize the fluctuation of blood glucose and insulin level. This study was aimed to determine the GI values of four new breeding rice varieties developed by using pseudo-backcrossing techniques. Furthermore, the comparison of GI values with that of commercial varieties (Jasmine and Basmati rice) was also described. Twenty two healthy subjects were fed with a reference food and cooked rice varieties containing 50 g available carbohydrate. All the new breeding rice varieties had GI values ranged between 48.1%-66.1%. Moreover, the effect of low GI (PK+4#20A09) and high GI (Jasmine rice) rice in test meals were studied in 12 prediabetic subjects to determine the changes of postprandial blood glucose, insulin, glucagon-like peptide-1 (GLP-1) and glucagon-dependent insulinotropic polypeptide (GIP). Results showed that LGI-LGL test meal (GI=41.7%, GL=21.3) could significantly lower blood glucose ($P<0.05$) at time point 30, 45, 60 and 90 minute and insulin concentration ($P<0.05$) at time point 60 minute when compared to that of HGI-LGL (GI=70.3%, GL=35.8). There was increase of GLP-1 level in subjects fed with LGI-LGL test meal whereas the mean GIP was significantly ($P<0.05$) decreased at time point 30 and 60 minutes. Our result suggested that new breeding rice, PK+4#20A09, could have beneficial effect on lowering the glycemic response in prediabetic subjects.

Introduction

Over the past two decades, the prevalence of diabetes mellitus has been increasing globally and is currently a major public health concern (Shaw *et al*., 2010). Carbohydrate is the only nutrient that directly raises postprandial blood glucose and insulin response (Ma *et al.*, 2008). Blood glucose after digesting foods could be revealed by using glycemic index (GI) and glycemic load (GL) (Jenkins *et al*., 1981). The GI value and the amount of carbohydrate in food are very important factors for controlling blood glucose and insulin response (Pinhero *et al*., 2016). Therefore, the use of carbohydrates with low GI and GL may produce beneficial effect by minimizing the fluctuation of blood glucose and insulin level (Ludwig, 2002). Many investigators have also shown that glycemia and insulinemia attributable to low GI and GL diets mediated the changes in the incretin hormones (Lemmens *et al*., 2011; Runchey *et al.*, 2013). The incretin hormones; glucagon-like peptide-1 (GLP-1) and glucagon-dependent insulinotropic polypeptide (GIP) are gut derived insulinotropic hormones synthesized and released from intestinal L and K cells, in response to nutrient ingestion that enhance the glucose-stimulated insulin secretion, respectively.

Rice (*Oryza sativa* L.) is the most important staple food for over half of the world's population (Fairhurst and Dobermann; 2002). Rice is generally considered as high GI food, but has a large variation in GI values ranging as low as 54 to as high as 121 (Atkinson *et al*., 2008). Moreover, it is known that consumption of white rice is linked with an increased risk for type 2 diabetes (Hu *et al.*, 2012). However, rice varieties with high amylose content have shown to produce a lower blood glucose and insulin response (Jain *et al*., 2012; Syahariza *et al*., 2013). This is because amylose is harder to break down than simple sugars like glucose and ensures a sustained release of sugar into blood without spiking immediately after a meal (Trinidad *et al*., 2013). Thus, efforts to produce this

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variety of ''new breeding lines'' with low-medium GI were important to promote public health and compete in the world market.

The new rice varieties were developed by using pseudo-backcrossing techniques in Rice Science Center, Kasetsart University, Nakhon Pathom, Thailand (Ruengphayak *et al*., 2015). However, no available data of GI study on these new breeding rice varieties was found in literature review. Therefore, this study was aimed to determine the proximate composition and amylose content of new rice breeding varieties. In addition the GI values of these new rice breeding varieties were determined in healthy subjects. The test meals prepared from low and high GI rice varieties were also investigated on postprandial blood glucose, insulin responses, GLP-1 and GIP in prediabetic subjects.

Materials and Methods

Phase I

Study subjects

Twenty two healthy subjects, aged between 20-45 years, were recruited in the study. Inclusion criteria included: men or non-pregnant women, who had a fasting blood sugar (FBS) ≤ 5.6 mmol/L, HbA1c ≤ 5.9%, had body mass index (BMI) ranged between 18.5-22.9 kg/m^2. Exclusion criteria were subjects who were smoking and taking medications that affect glucose metabolism. All the subjects had given their informed consent before entering into the study and the protocol was approved by the Human Ethics Committee of Mahidol University Institutional Review Board (MU-IRB 2013/001.0401), which conformed to the Helsinki Declaration.

Test rice and reference food

The study involved six polished rice varieties (*Oryza Sativa* L.), which included four new improved breeding lines as Pinkaset+4; PK+4#1_E06, PK+4#20A09, PK+4#B09 and PK+4#117A08 that were obtained from Rice Science Center, Kasetsart university, Kamphaeng Saen, Nakhon Pathom, Thailand (Ruengphayak *et al*., 2015). Two commercial rice varieties i.e, Thai jasmine rice was grown in Thailand, whilst basmati rice was imported from India. All rice varieties were prepared using the same procedure by an automatic rice cooker (1.8L, Sharp KS-19ET) including the amount of water and rice ratio (2:1), except for jasmine rice (1.5:1), according to the cooking process used in Thai household setting.

Glucose powder (glucolin®, Thailand) dissolved in 250 ml water was used as the reference food. The reference food was consumed at the beginning, middle and at the end of the test foods. The three varieties of rice were consumed in random order between the reference food sessions with at least a week gap between measurements to minimize carry-over effects. All the test samples contained 50 g of available carbohydrate. To avoid the effect of starch retrogradation, all the tested rice varieties were cooked freshly and immediately served to the subjects (Karim *et al.*, 2000) and then fed to each subject along with 100 ml of clear soup and 150 ml. of water.

Chemical analysis in test rice

All the cooked rice samples were analyzed for proximate composition using AOAC (2005) method. Dietary fiber was measured by enzymatic-gravimetric method using the Megazyme kit (K-TDFR, Megazyme International Ireland, Bray Business Park, Wicklow, Ireland). The carbohydrate content of each sample was calculated by subtracting the sum of moisture, total fat, protein and ash from 100. Available carbohydrate was estimated from the difference between the total carbohydrate and dietary fiber values.

Amylose content was analyzed using the method described by Juliano et al. (1981) and calculated based on the method described by McGrance *et al*. (1998) using a standard curve plotted from absorbance of amylose standards.

Experimental design

The day prior to study, subjects consumed standard meal with similar serving size, composition and water for dinner. They were prohibited for performing any vigorous exercise, drinking alcohol, caffeine and smoking. This was verified by taking a brief behavioral questionnaire and a 24 hours dietary recall.

Each subject was given the study protocol on six different occasions in the morning after a 10-12 overnight fasting. FBS were taken at -10 and 0 minute before the consumption of food (the baseline value taken as mean of these two values). Subjects consumed cooked rice within 15 minute (the first bite was considered as 0 minute) and blood samples were obtained at 15, 30, 45, 60, 90 and 120 minute. Subjects remained sedentary during each session. Blood sample was measured by glucose oxidase method using an automatic analyzer (Hitachi P800 Modular Chemistry analyzer, Roche diagnostics Ltd, Thailand).

Calculation of the glycemic index, glycemic response and maximum increase in plasma glucose

The GI value of the rice was calculated based on the method described by FAO/WHO (1998) as the incremental area under the curve (IAUC) of a 50 g carbohydrate portion of the test food expressed as a percent of the response to the same amount of carbohydrate from a reference food taken by the same subject. The IAUC for each food ignoring the area below the fasting level was calculated using the GraphPad Prism 5.0. (GraphPad Software Inc., San Diego, CA, USA).

The glycemic response (GR) was calculated geometrically as the mean IAUC after the test food alone, ignoring the area below the fasting blood level (Olausson *et al*., 2014). The maximum increase in plasma glucose (MIPG) was an increase in the postprandial blood glucose subtracted by FBS (Olausson *et al.*, 2014).

Phase II

Prediabetic subjects

Twelve prediabetic subjects, aged between 30-60 years, were recruited in the study. Inclusion criteria included: prediabetic subjects, who were treated by diet; HbA1c level ≤ 6.5%; BMI ≤ 35 kg/m2, absence of a history of gastric surgery or current gastric disease. Subjects were not scheduled for test sessions during the week of their menstrual cycle to avoid hormonal effect on blood glucose (Escalante and Alpizar., 1999). All the subjects had given their informed consent before entering into the study and the protocol was approved by the Human Ethics Committee of Mahidol University Institutional Review Board (MU-IRB 2014/039.1703), which conformed to the Helsinki Declaration.

Test meals

The energy level of test meals was adjusted based on each participant's estimated resting metabolic rate (Mifflin *et al*., 1990) and 3-day food records. Food composition and nutritional characteristics of the test meals were shown in Table 5. The food ingredients in LGI-LGL and HGI-LGL were similar, except for the difference in types of rice. The GI and GL of vegetables and fruit were obtained from previous study (Atkinson *et al.,* 2008 and Bunprakong, L., 2012), calculated as according to the following formulae (Galgani *et al.*, 2006):

Meal GI = {[$GI_{Food\ A}$ × g available carbohydrate (avail CHO) $_{Food\ A}$] +($GI_{Food\ B}$ × g avail CHO) Food B) + …}/total g avail CHO

Meal GL = ∑foods {[GI × g avail CHO]/100}

Experimental procedure in test meals

This study was a single blind, randomized-controlled trial consisting of LGI-LGL and HGI-LGL, which was separated by 1- week washout period. Subjects were instructed to consume the diet within 15 minute. Blood sample for glucose and insulin estimation were collected at 0, 15, 30, 45, 60, 90, 120, 180 and 240 minute. Then, plasma glucose and serum insulin were measured by glucose oxidase method using an automatic analyzer (Hitachi P800 Modular Chemistry analyzer, Roche diagnostics Ltd, Thailand) and chemiluminescence immunoassay (CLIA; Analyzer: Liaison XL, Manufacturer by Diasorin Ltd, Italy), respectively. Blood sample for GLP-1 and GIP analysis was collected at 0, 30, 60, 90, 120, 180 and 240 minute in K2EDTA tube and centrifuged immediately at 3000 rpm at 4°C for 10 minute. The plasma samples were frozen and stored at -80°◦C until analysis. Total GLP-1 and GIP concentrations were measured using GLP-1 (7-36 and 9-36) and GIP ELISA kit (Merck, Millipore®, Billerica, MA, USA) with a lower limit of sensitivity of 1.5 pmol/l and 1.0 pmol/l, respectively.

Statistical analysis

In phase I, results were expressed as mean ± SD and mean ± SEM. For GI study, those with intra-individual variability (%CV) were greater than 30% for reference glucose and were considered as outliers. GI values that were greater than ±2SD of the group mean GI were considered to be outliers and were excluded from the analysis. Mean differences between MIPG, GR and GI among rice varieties were analyzed using one-way analysis of variance (ANOVA) and post hoc by Bonferroni comparison. Pearson's correlation coefficients were applied to determine the relationships between GI values and amylose content, and between GI values and dietary fiber of rice. In phase II, paired t test was used to compare the blood glucose, insulin, GLP-1 and GIP response between test meals. Results were considered significant at $P<0.05$.

Results and Discussion

Phase I

Subjects

The baseline characteristics were shown in Table 1. Mean age was 25.9±5.0 years. BMI, FBS, HbA1c, total cholesterol, LDL, HDL and triglyceride concentration were in the normal ranges.

Table 1. Baseline characteristics of study subjects

Subject characteristic	Value (n= 22; Mean±SD)
Age (years)	25.9± 5.0
BMI (kg/m^2)	21.1 ± 2.4
FBS (mmol/L)	4.9 ± 0.3
Fasting Insulin (pmol/L)	25.0±14.6
HbA1C (%)	5.2 ± 0.3
Total cholesterol (mmol/L)	4.6±0.9
Triglyceride (mmol/L)	0.8±0.3
LDL (mmol/L)	2.6±0.7
HDL (mmol/L)	1.7±0.3

Data expressed as the mean±SD
BMI, body mass index; FBS, fasting blood sugar; LDL, low density lipoprotein; HDL, high density lipoprotein

Nutritive values of rice varieties.

The nutritive values of cooked rice (per 100 g) were shown in Table 2. It was found that dietary fiber in PK+4#117A08 and PK+4#66B09 was greater than that of other varieties ($P<0.05$); whilst fat content was not detected in PK+4#66B09. Amylose content of four new breeding rice varieties were ranged from 26.2%-29.6%, indicating that these varieties had high amylose content (Juliano, 1992).

Glycemic index, glycemic response and maximum increase in plasma glucose of tested rice

As shown in Table 3, according to the classification of GI value (Wolever *et al.,* 2006), PK+4#20A09 (GI= 48.1) and PK+4#1_E06 (GI=54.6) were referred as low GI, whilst PK+4#117A08 (GI= 63.8), PK+4#66B09 (GI= 66.1) and basmati rice (GI= 66.2) were categorized as a medium GI. The mean GI value for the jasmine rice (GI= 90.7) was significantly greater than those of PK+4#1_E06 and PK+4#20A09. Our results revealed that GI value of basmati and jasmine rice varieties in the present study was similar to the previous reports as 58-77 and 72-116, respectively (Ranawana *et al*., 2009; Kataoka *et al*., 2012; Truong *et al*., 2014). The low-medium GI of the new breeding rice was attributable to high amylose content, which resulted in difficult and slower digestion. This might be due to intestinal amylase enzyme which has less pronounced effect on rice with high amylose content, thereby slowly increasing the blood glucose after rice digestion.

Our results found significant negative correlation between amylose content and GI of rice, while dietary fiber did not show any correlation with GI value (data not shown). Similar to previous studies, it has been suggested that the composition and type of dietary fiber affect the regulation of blood glucose and insulin response rather than the amount of dietary fiber consumed per serving. Results from the studies by Panlasigui *et al*. (1991) and Burton *et al.* (2011) indicated that beside the amylose content, there might be other factors such as the structure of the starch component, the amount of resistant starch, the degree of starch damage through food processing or other physiochemical properties.

Phase II

Subjects

Average participant age and BMI were 44.0±10.3 years and 28.0±4.0 kg/m^2, respectively. Mean FBS of subjects was 5.8±0.4 mmol/L and HbA1c was 6.2±0.3%. Mean total cholesterol (5.8±1.0 mmol/L), LDL (4.2±0.8 mmol/L) and triglyceride (1.7±0.6 mmol/L) concentrations were higher than the normal values.

Test meals

As shown in Table 4, two test meals (LGI-LGL vs. HGI-LGL) provided relatively the same amount of energy (407±1 vs. 411±15 kcal), protein (23.0±0.3 vs. 25.3±4.3 g), fat (8.6±0.0 vs. 8.3±0.5 g) and total carbohydrate (59.5±0.6 vs. 58.7±2.8 g). Although amount of rice content was similar (110 g per meals), GL differed substantially between the two test meals (GL = 21.3 and 35.8 in LGI-LGL and HGI-LGL, respectively).

Effect of test meals in prediabetic subjects

As shown in Figure 1, blood glucose and insulin levels were lower following the LGI-LGL meal, as compared to the HGI-LGL meal. Our results were similar to previous studies (Liu *et al*., 2012, Runchey *et al.*, 2013). Generally, the homeostasis of postprandial blood glucose is controlled not only by the direct stimulating insulin secretion for absorption of nutrients but also through the secretion of incretin hormones (Kim and Egan, 2008). Regarding GLP-1, it is an incretin hormone released by enteroendocrine-L cells predominantly in the ileum and colon in response to food intake. A higher GLP-1 concentration in response to the LGI-LGL test meal could lower postprandial glucose and insulin responses. Our findings were consistent with previous finding which showed that GLP-1 concentration was greater in the LGI-LGL than the HGI-LGL test meals (Runchey *et al*., 2013). Previous study specified that the effect of a low GL meal, as compared to a high GL meal on postprandial concentrations of GLP-1 were similar to effects of alpha-glucosidase inhibitors on incretin secretion (Aoki *et al*., 2010). Our findings were in

Table 2. The nutrient composition of cooked rice (g/100 g)

Test rice	Moisture g	Energy Kcal	Protein g	Fat g	Ash g	Total CHO g	DF g	Available CHO g	Amylose g
Jasmine rice	62.7±1.6^{c}	150±7	3.1±0.1	0.2±0.0	0.1±0.0	33.9±1.6	0.9±0.0	33.0±1.7	-
Dry basis	-	402±0^{a}	8.3±0.5^{d}	0.5±0.0^{ab}	0.3±0.0^{d}	90.8±0.4^{a}	2.4±0.2^{c}	88.5±0.7^{a}	15.6±0.5^{d}
Basmati rice	66.5±0.6^{b}	134±2	3.5±0.1	0.2±0.0	0.3±0.0	29.5±0.6	1.3±0.1	28.2±0.6	-
Dry basis	-	397±0^{c}	10.5±0.4^{ab}	0.6±0.0^{ab}	0.8±0.0^{b}	88.1±0.4^{cd}	3.9±0.1^{b}	84.2±0.3^{b}	23.7±0.7^{c}
PK+4#1_E06	64.6±0.9^{c}	140±4	3.1±0.2	0.1±0.1	0.3±0.0	31.8±1.0	1.5±0.1	30.3±1.0	-
Dry basis	-	396±0^{d}	8.7±0.5^{cd}	0.3±0.2^{c}	1.0±0.1^{a}	89.9±0.5^{ab}	4.3±0.3^{b}	85.6±0.7^{b}	29.6±1.0^{a}
PK+4#20A09	65.5±1.0^{b}	137±4	3.3±0.4	0.2±0.0	0.4±0.0	30.6±0.7	1.6±0.1	29.0±0.7	-
Dry basis	-	396±0^{d}	9.7±0.8^{bc}	0.5±0.1^{b}	1.0±0.1^{a}	88.8±0.7^{bc}	4.5±0.3^{b}	84.3±0.5^{b}	29.6±0.7^{a}
PK+4#66B09	67.1±3.1^{ab}	131±12	3.8±0.5	ND	0.2±0.0	28.9±2.9	2.8±0.1	26.1±2.9	-
Dry basis	-	398±0^{b}	11.6±1.4^{a}	ND^{d}	0.5±0.0^{c}	87.9±1.4^{cd}	8.6±0.8^{a}	79.3±2.0^{c}	26.2±0.8^{b}
PK+4#117A08	68.9±0.8^{a}	124±3	3.6±0.2	0.2±0.0	0.2±0.0	27.0±0.9	2.6±0.1	24.4±0.9	-
Dry basis	-	397±0^{c}	11.5±0.7^{a}	0.7±0.1^{a}	0.8±0.1^{b}	87.1±0.7^{d}	8.4±0.5^{a}	78.6±1.0^{c}	29.5±0.2^{a}

Value as mean±SD, derived from duplicate analysis of 3 individual composite samples of each test food based on the AOAC method (2005).
Means difference in each group, followed with the same letter in each column were not significantly different (P>0.05) when tested using ANOVA.
Energy derived based on 1 g protein = 4 kcal, 1 g fat = 9 kcal and 1 g carbohydrate = 4 kcal.
PK+4, Pinkaset+4; DF, dietary fiber; Total CHO, total carbohydrate; Available CHO, Available carbohydrate; ND, Not Detected

Table 3. Maximum increase in plasma glucose (MIPG), glycemic response (GR) and glycemic index (GI)) for each test rice.

Test foods	MIPG	GR	GI	
	(mmol/L)	(mmol.min/L)	(%)	classification
Reference food (glucose)	3.1±1.7	178±41^{a}	100	High
Jasmine rice	2.4±0.6	164±28^{a}	90.7±12.0^{a}	High
Basmati rice variety	2.6±1.0	111±21^{ab}	66.2±8.0^{ab}	Medium
PK+4#1_E06	2.1±0.8	100±21^{b}	54.6±6.5^{b}	Low
PK+4#20A09	2.1±1.1	92±24^{b}	48.1±6.2^{b}	Low
PK+4#66B09	2.1±1.0	136±31^{ab}	66.1±11.0^{ab}	Medium
PK+4#117A08	2.0±0.9	114±25^{ab}	63.8±12.5^{ab}	Medium

Data expressed as the mean±SEM.
MIPG, Maximum increase in plasma glucose; GR, glycemic response; GI, glycemic index
Subjects exceeding ±2SD were excluded from the group. Total subject for GI calculation = 9 subjects each.
GI is calculated as the ratio of incremental area under the blood glucose curve for 2 h after rice is eaten and the corresponding area after glucose is eaten, multiplied by 100%.
GI values are categorized as either low (≤55), medium (55-69) or high (≥70).
Means difference in each group, followed with the same letter in each column were not significantly different (P>0.05) when tested using ANOVA.

Table 4. Food composition and nutritional characteristics of the test meals

Nutrients	LGI-LGL	HGI-LGL	Recipes
Energy (kcal)	407±1	411±15	- Stir fried rice (110 g rice*, 50 g breast chicken, 25 g egg, 7 g oil, 75 g mixed vegetable and 8 g seasoning)
Protein (g)	23.0±0.3	25.3±4.3	
Fat (g)	8.6±0.0	8.3±0.5	
Dietary fiber (g)	9.0±0.2	7.3±0.1	- Chicken soup (30 g breast chicken, 35 g winter melon and 2 g soy sauces)
Total CHO (g)	59.5±0.6	58.7±2.8	
GI (%)	41.7	70.3	- Guava 120 g
GL	21.3	35.8	

* LGI-LGL, PK+4#20A09; HGI-LGL, Jasmine rice

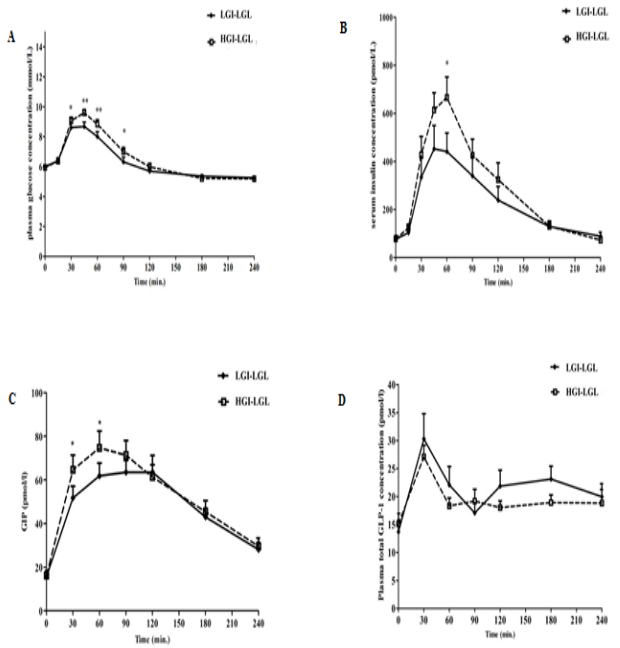

Figure 1. Plasma glucose (A), serum insulin (B), gastric inhibitory polypeptide; GIP (C) and total glucagon-like peptide-1; GLP-1 (D) of subjects fed with LGI-LGL; Low GI-Low GL (solid line with closed diamonds) and HGI-LGL; High GI-Low GL (dotted with open squares) test meals during 240 minute. Data was expressed as mean ± SEM (n=12). $^{*}P<0.05$; $^{**}P<0.01$, significant differences in blood glucose, insulin and GIP were observed between LGI-LGL and HGI-LGL test meals. No significant mean difference in total GLP-1 response among test meals were observed, at $P<0.05$ by paired student's t-test.

agreement with other studies which revealed that greater blood glucose and GIP secretion were found after HGI-LGL meal consumption. High GI diet moves rapidly through the stomach, therefore food is rapidly absorbed at proximal of small intestine. The enteroendocrine K-cells also localize mainly in the proximal intestine and play a key role on producing or secreting GIP (Wachters-Hagedoorn *et al*, 2006).

Higher intake of white rice was associated with the increased risk of type 2 diabetes; due to the high GI value (Hu *et al*., 2012). All newly developed breeding rice varieties, particularly, PK+4#20A09 white rice variety was considered as potential alternative rice source for people who are at risk of type 2 diabetes.

Conclusion

In this study, low GI was found in PK+4#20A09 and PK+4#1_E06 rice varieties, whereas PK+4#66B09 and PK+4#117A08 were categorized as medium GI food. Low GI rice (PK+4#20A09) showed the beneficial effect on lowering the glycemic response in prediabetic subjects.

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