Influence of the Physical Form of Processed Rice Products on the Enzymatic Hydrolysis of Rice Starch in Vitro and on the Postprandial Glucose and Insulin Responses in Patients with Type 2 Diabetes Mellitus

Jae Cheryl Kim,1,2 Jung-In Kim,1,3 Byoung-Wook Kong,1 Min-Jung Kang,1 Myo-Jeong Kim,1,3 and In-June Cha4

1Food Science Institute and School of Food and Life Science, Inje University, Gimhae 621-749, Republic of Korea
2Institute of Basic Sciences, Inje University, Gimhae 621-749, Republic of Korea
3Biohealth Products Research Center, Inje University, Gimhae 621-749, Republic of Korea
4Department of Pharmacology, Inje University, and College of Medicine and Clinical Pharmacology Center, Pusan Paik Hospital, Busan 614-165, Republic of Korea

Received December 5, 2003; Accepted April 23, 2004

The manufacturing processes used determined the physicochemical properties of the three kinds of rice food, garaeduk, bagsulgi, and cooked rice. The initial rate of hydrolysis by porcine pancreatic α-amylase (PPA) was affected by the food form. The firmer structure of garaeduk was apparently responsible for the difficulty in maceration, resulting in less digestion than with easily digestible food for the same maceration time. The initial rate of hydrolysis of each rice product by PPA increased with increasing maceration time in a Waring Blender for all of the processed rice products. The postprandial glucose and insulin responses to the three processed rice products were also studied in ten patients with type 2 diabetes mellitus (4 men and 6 women aged 56.8 ± 2.3 yr; duration of diabetes, 3.6 ± 1.2 yr; body mass index (BMI), 23.7 ± 2.6 kg/m²; fasting serum glucose, 143.9 ± 5.1 mg/dl; serum insulin, 20.8 ± 2.2 μU/ml). Each subject ingested of the three rice foods after a 12-h overnight fast, and the serum glucose and insulin levels were measured over a 0–240 min period. The postprandial serum glucose and insulin levels at 90 min after ingesting bagsulgi and cooked rice were less than those at 60 min, while the levels at 90 min after ingesting garaeduk were higher than those at 60 min. Garaeduk also significantly decreased the incremental responses of glucose and insulin when compared with bagsulgi and cooked rice. The results suggest that garaeduk would be the most unlikely to increase the postprandial serum glucose and insulin levels among the three rice foods. The food form, which eventually differentiated each food by its specific surface area with the same degree of maceration because of the characteristic physical strength, therefore affected the rate of rice starch hydrolysis both in vitro and in vivo.

Key words: rice food; postprandial response; surface area; physical form; type 2 diabetes mellitus

The digestion of starch food that has a particular physical structure is influenced by many factors. The digestion of starch in foods is known to be affected by the physical form of the food, the shape and crystal structure of the starch granule, recrystallization and retrogradation, amylose-lipid complexes, native α-amylase inhibitors, and non-starch polysaccharides. The enzymatic hydrolysis of native starch has been shown to be affected by the granule structure, and especially by the crystal type, granule size, amylose/amylopectin ratio, average molecular weight and presence of lipids and proteins. Efforts have been made to correlate the in vitro hydrolysis of starch food with metabolic responses, and have shown that essentially no significant differences seemed to exist between the in vitro enzymatic hydrolysis of starch and in vivo digestion of starch foods.

The effect of particle size on the enzymatic hydrolysis of native starch and/or starch food is of particular interest in terms of the available surface area per mass (specific surface area) for enzymatic action both in vivo and in vitro. Many studies have shown that the enzymatic hydrolysis of native starch was affected by the size of the starch granules. The porcine pancreatic α-amylase (PPA) activity toward a native starch granule is more accurately described as a function of the surface area of the granules rather than of the substrate concentration. Starches that naturally have a porous surface were more amenable to digestion due to the admission of hydrolytic enzyme molecules to the granule interior. However, the presence of pores in maize granules appeared to significantly affect the
overall rate of digestion after sufficient reaction time, although not at the very early stage of hydrolysis. Since each starch food has its own characteristic physical strength due to the food structure depending on the composition and method of processing (with the exception of dilute starch gruel in which the starch molecules are almost completely dissolved in water), we can speculate that its particle size as a result of mastication in the mouth should affect the action of amylase. The postprandial serum glucose and insulin levels after ingesting each rice product were inversely related to the structural strength of the rice product, resulting from the specific manufacturing process used. Björck et al. have reported that any process that disrupts the physical or botanical structure of a food ingredient will increase the plasma glucose and insulin responses in healthy subjects. The dependence of the particle size of wheat flour and cracked wheat grain samples used for test meals on the postprandial metabolism of carbohydrate food is prominent in the results of Holt & Miller which showed that the smaller the particle size of the raw material of the food, the higher the glycemic-insulin response.

The initial stage of an enzyme reaction on a substrate is the formation of an enzyme-substrate complex. The adsorption of Bacillus subtilis α-amylase to spherulitic starch particles, which are essentially resistant to α-amylolysis at 25 °C, was found to be a prerequisite step for hydrolysis. The amount of enzyme molecules interacting with a particular substrate can be assumed to be dependent on the surface area of the substrate. Double reciprocal plots of the α-amylolysis of native starch granules have well described the dependence of the specific surface area of a substrate rather than the concentration of the substrate on enzyme kinetics.

Glycemic control, which is one of the major goals for the treating diabetes mellitus, is related with preventing or delaying diabetic complications, including cardiovascular diseases, retinopathy, nephropathy and neuropathy. To maintain the fasting glucose level within a desirable range has been the target of glycemic control. However, increasing evidence supports the notion that control of postprandial glucose could be more important in preventing diabetic complications. A starchy food with a low glycemic index is considered beneficial for diabetic patients, since it is digested and absorbed more slowly than a food with a high glycemic index. Rice is a staple for more than 80% of the world population including Korea. Rice is consumed in various processed forms in Korea such as cooked whole-grain rice and rice cake of many varieties depending on the moisture content and preparation method. Since such physicochemical properties as the density, strength, particle size, and degree of gelatinization could each affect the glycemic index, the glycemic and insuclidean responses to different rice products could be different. However, the metabolic responses to the various physical forms of rice products are not completely understood.

We investigated in this study the effect on digestion of the form of different processed rice products both in vitro and in vivo. Patients with type 2 diabetes mellitus ingested each rice product, and the postprandial serum glucose and insulin responses were measured. The effect of particle size on the PPA hydrolysis of each processed rice product was also analyzed. The results of in vitro and in vivo digestion of each rice product were correlated to elucidate the effect of the physical form of the starch food on the retardation of postprandial responses.

Materials and Methods

Materials. Short-grain rice was obtained from a local polishing plant in Busan (Korea). PPA (type VII-A), glucose oxidase, thymersal, and maltose were purchased from Sigma (St. Louis, MO, USA). A radioimmunoassay kit and filter paper were respectively obtained from Linco Research (St. Charles, MO, USA) and Whatman (Maidstone, England). All other chemicals were of analytical grade.

Preparation of the rice products. Three conventional rice products, cooked rice, bagsulgi and garaeduk, were prepared by following the usual procedures applied in Korea. No ingredients other than rice, which was purchased from a local market, were used for the rice products. Descriptions of the three rice products and details of the preparation are given in Table 1. Cooked rice is a sticky rice dish that is usually consumed in Korea. Bagsulgi is a rice cake made with rice flour by steaming in a cage to make a hexahedral shape. Garaeduk is made by extruding steamed rice flour into the form of a round stick. The rice products were used immediately after their preparation to avoid retrogradation.

Subjects. Four males and six females with type 2 diabetes mellitus were selected for determining the postprandial serum glucose and insulin responses after ingesting the rice food. The mean of age and body mass index (BMI) of the patients were 56.8 ± 2.3 yr (mean ± SE) and 23.7 ± 2.6 kg/m², respectively (Table 2). The fasting serum glucose, serum insulin and blood glycated hemoglobin levels were 143.9 ± 5.1 mg/dl, 20.8 ± 2.2 μU/ml and 8.7 ± 0.35%, respectively.

<table>
<thead>
<tr>
<th>Rice product</th>
<th>Description</th>
<th>Preparation procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bagsulgi</td>
<td>Hexahedral shape 15 × 15 × 2 cm</td>
<td>Rice flour steamed at 100 °C in a cage</td>
</tr>
<tr>
<td>Cooked rice</td>
<td>Gelatinized rice grains</td>
<td>Polished rice grains cooked at 100 °C</td>
</tr>
<tr>
<td>Garaeduk</td>
<td>Round stick (diameter 16 mm)</td>
<td>Steamed rice flour successively extruded and soaked in water three times</td>
</tr>
</tbody>
</table>

Table 1. Description and Preparation of the Korean Rice Products
Influence of Physical Form on the Enzymatic Hydrolysis of Rice Starch

Physicochemical properties of the rice products. The moisture content was obtained by drying at 105°C until no weight difference could be observed. The apparent density of the rice products and native starch was measured by adding a weighed amount of starch of known moisture content to a calibrated volumetric flask, making up the volume with water, and obtaining the combined weight of the starch plus water. Each grain of cooked rice was added to a volumetric flask to reject the void volume made by a lump of rice. Subtracting the weight of moisture, fat, protein, crude ash and dietary fiber from the total weight of rice product determined the available carbohydrate. Protein was measured by the Kjeldahl method, and fat was measured by the Soxhlet method. Crude ash was determined by incinerating at 600°C, dietary fiber was obtained by the method of Prosky et al. The strength of each rice product was measured with a rheometer (Fudoh RT-2010DD, Rheotech, Japan), the maximum peak of the recorded force-time curve with adapter #31 being taken as the strength of the rice product. Particle size was measured by a Mastersizer 2000 (Malvern Co., Worcestershire, UK), assuming the shape of the particle to be spherical.

Enzyme hydrolysis. Ten grams of each rice product was sliced into small pieces, and the cooked rice was pulverized with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9). The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9). The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9). The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9). The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9) in a 2 g of available carbohydrate was macerated with a spatula. The rice product equivalent to 2 g of available carbohydrate was macerated with 49.5 ml of a 0.1 M phosphate buffer (pH 6.9).

Postprandial glucose and insulin responses. Each subject was studied on three separate occasions with at least a two-week interval between each, and was successively offered cooked rice, bagsulgi and garaeduk containing 50 g of available carbohydrate with 400 ml of lukewarm water after overnight fasting. The product was consumed in 15 min. Venous samples were collected at 0, 60, 90, 120, 180, and 240 min after the test meal had been eaten and centrifuged at 3,000 rpm for 20 min. Serum glucose and insulin were respectively measured by the glucose oxidase method with a commercial kit (Sigma, St. Louis, MO, USA) and by a radioimmunoassay with a kit (Linco, St. Charles, MO, USA). The serum glucose and insulin values at each time point are expressed as an incremental from the baseline value.

Table 2. Characteristics of the Patients with Diabetes Mellitus

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Duration of diabetes mellitus (yr)</th>
<th>BMI (kg/m²)</th>
<th>Body fat (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56.8 ± 2.3³</td>
<td>3.6 ± 1.2</td>
<td>23.7 ± 2.6</td>
<td>28.4 ± 2.3</td>
</tr>
</tbody>
</table>

³ Mean ± SE, n = 10.

Table 3. Physicochemical Properties of the Rice Products

<table>
<thead>
<tr>
<th></th>
<th>Bagsulgi</th>
<th>Cooked rice</th>
<th>Garaeduk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (wt %)</td>
<td>43.1 ± 0.09</td>
<td>66.0 ± 1.30</td>
<td>47.5 ± 0.06</td>
</tr>
<tr>
<td>Available carbohydrate (wt %)</td>
<td>50.09 ± 0.01</td>
<td>29.92 ± 1.96</td>
<td>46.13 ± 0.09</td>
</tr>
<tr>
<td>Density (g/ml)</td>
<td>1.18 ± 0.02</td>
<td>1.11 ± 0.01</td>
<td>1.20 ± 0.04</td>
</tr>
<tr>
<td>Strength (g)</td>
<td>716 ± 7.7</td>
<td>118 ± 10.0</td>
<td>1152 ± 19.8</td>
</tr>
</tbody>
</table>

¹ Mean ± SE.
gelatinized starch granules can therefore be easily separated by a Waring blender, resulting in higher specific surface area (surface area per mass) than cooked rice at a certain maceration time. Contrary to bagsulgi, although garaeduk is also made of milled rice grains, the extrusion process imparts to the gelatinized starch granules the effect of kneading, resulting in structural continuity between the starch molecules in garaeduk. As a consequence, the strong physical structure of garaeduk makes it more difficult to disrupt the form than with cooked rice and bagsulgi. The firmer structure of garaeduk seems to create difficulty in maceration, resulting in a lower initial rate of hydrolysis which indicates less digestion at a certain maceration time (Table 4). Assuming the power applied to disrupt the form of the rice product is proportional to the maceration time, the particle size of the rice product would become smaller with increasing maceration time. As a consequence, the specific surface area of the rice product is increased with increasing maceration time. Since the enzyme reaction is dependent on the maceration time in a Waring blender it can be expressed as a function of the maceration time. The increased specific surface area of the macerated rice product eventually results in the acceleration of enzymatic hydrolysis. In fact, as particle size decreases with increasing maceration time, less substrate is required to saturate the enzyme. After a long maceration time, all the starch molecules in the three rice products are in such an orientation as that in dilute rice gruel, which eventually results in increased accessibility of the enzyme molecules to the starch molecules during maceration in the Waring blender may have affected the enzymatic hydrolysis. The in vivo digestion of starch food is also affected by milling grade of the raw material and form of the starch food.\textsuperscript{8) In vitro} studies on the factors affecting the rate of hydrolysis of starch in food.

### Table 4. Initial Velocity of Porcine Pancreatic α-Amylase Hydrolysis of Rice Products Macerated for Various Times in a Waring Blender

<table>
<thead>
<tr>
<th>Maceration time (s)</th>
<th>Bagsulgi</th>
<th>Cooked rice</th>
<th>Garaeduk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.52 ± 0.109\textsuperscript{a}</td>
<td>0.93 ± 0.043\textsuperscript{b}</td>
<td>—\textsuperscript{3}</td>
</tr>
<tr>
<td>5</td>
<td>1.87 ± 0.035\textsuperscript{c}</td>
<td>1.39 ± 0.037\textsuperscript{d}</td>
<td>1.19 ± 0.048\textsuperscript{e}</td>
</tr>
<tr>
<td>10</td>
<td>2.16 ± 0.116\textsuperscript{b}</td>
<td>1.75 ± 0.047\textsuperscript{b}</td>
<td>1.65 ± 0.068\textsuperscript{e}</td>
</tr>
<tr>
<td>15</td>
<td>—</td>
<td>—</td>
<td>2.08 ± 0.119</td>
</tr>
<tr>
<td>20</td>
<td>2.78 ± 0.077</td>
<td>2.50 ± 0.039</td>
<td>2.39 ± 0.047</td>
</tr>
</tbody>
</table>

\textsuperscript{1} Mean ± SE. \textsuperscript{2} Values with different superscript letters in the same row are significantly different (P < 0.1). \textsuperscript{3} Not determined. \textsuperscript{4} Values with different superscript letters in the same row are significantly different (P < 0.05).

![Fig. 1.](https://example.com/fig1.png) Hydrolysis of the Rice Products with Porcine Pancreatic α-Amylase as Affected by Maceration. A, bagsulgi; B, cooked rice; and C, garaeduk.\textsuperscript{11} Maceration time in a Waring blender.
have also demonstrated that the particle size played an important role in determining the rate of hydrolysis.\(^{17}\)

**Postprandial glucose and insulin responses**

Figures 2 and 3 respectively show the postprandial increases in serum glucose and insulin. The postprandial serum glucose and insulin levels each reached a peak 60 min after ingestion of bagsulgi and cooked rice, and 90 min after the ingestion of garaeduk. These two responses to cooked rice were significantly lower than those to bagsulgi, and higher than those to garaeduk 60 min after ingestion (\(P < 0.05, n = 10\)). The postprandial serum glucose response to cooked rice and garaeduk was significantly lower than that to bagsulgi 90 min after ingestion (\(P < 0.05\)). Garaeduk also significantly decreased the incremental response for insulin 90 min and 120 min after ingestion, compared with bagsulgi (\(P < 0.05\)).

AUC of the serum glucose response to cooked rice and garaeduk was significantly lower than that to bagsulgi 90 min after ingestion (\(P < 0.05\)). Garaeduk also significantly decreased the incremental response for insulin 90 min and 120 min after ingestion, compared with bagsulgi (\(P < 0.05\)). AUC of the serum glucose response to cooked rice was significantly lower than that to bagsulgi and significantly higher than that to garaeduk (Table 5).

AUC of the response of serum insulin to each of the three rice products was similar to that of serum glucose AUC (Table 5). The results for the postprandial glucose
and insulin responses to the three rice products correspond very well to the results of in vitro α-amylolysis in terms of the effect of particle size with the same maceration time. Therefore, food forms with various degrees of physical strength, and consequently resulting in differences in the particle size of the rice products at the same maceration time, affected the rate of rice starch hydrolysis with the same pattern, both in vitro and in vivo. Björck et al. have also demonstrated that the rate of starch digestion in vitro was a key determinant of the metabolic response to most products.

The initial rate of hydrolysis of the rice products, which had different physical strength, was increased by increasing agitation time in the blender. This indicates that enzymatic hydrolysis of each rice product was highly dependent on the particle size of the fragmented rice food as a consequence of the degree of maceration. This, in turn, was related to the physical structure of the product. The digestion of rice products with characteristic physical forms was highly dependent on the particle size at the time of the enzyme reaction, both in vivo and in vitro. Garaeduk is therefore suggested to have had a beneficial effect on controlling postprandial hyperglycemia in diabetic patients because of its high physical strength and slow digestion rate in the small intestine.

Acknowledgments

This study was supported by a grant from Korea Health 21 R&D project, Ministry of Health & Welfare, Republic of Korea (HMP00-B22000-0147).

References

13) Oosten, B. J., Tentative hypothesis to explain how electrolytes affect the gelatinization temperature of starches in water. Starch, 34, 233–239 (1982).