Mini Review

Bioactive Ingredients in Rice (Oryza sativa L.)
Function in the Prevention of Type 2 Diabetes

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Summary Diabetes mellitus (DM) greatly impacts human health worldwide as over 400 million patients suffer from DM-related symptoms. Type 2 DM accounts for more than 90% of DM and is caused mainly by unhealthy lifestyles, such as high-calorie and high-fat diets. Such undesirable eating habits induce resistance to insulin resulting in high blood sugar levels that cause induction of various symptoms and complications of DM. Therefore, management of blood sugar levels is important for preventing DM. Our group has recently found that rice (Oryza sativa L.) contains anti-diabetes compounds. Here, we summarize the effect of the bioactive ingredients in rice on preventing type 2 DM.

Key Words rice, diabetes, blood glucose level, γ-oryzanol, albumin, globulin, hydrolysate

Diabetes mellitus (DM) is one of the most common and serious metabolic disorders, with the number of patients estimated to be 422 million in 2014 according a report conducted by the World Health Organization (1). The number of patients is predicted to increase, reaching 592 million by 2035 worldwide (2). Borderline diabetics or prediabetics who experience no obvious symptoms, but are at high risk for developing DM. The number of borderline diabetics is thought to be more than that of diagnosed DM patients (3). The common symptoms of DM, such as retinopathy, nephropathy, neuropathy, and angioopathy, appear suddenly; thus, asymptomatic people, including prediabetics, should focus on preventing DM. Complications induced by DM often reduce the health-related quality of life of DM patients due to blindness, renal failure, and foot ulcers.

DM is classified into type 1 and 2; more than 90% of DM patients are classified as type 2. Type 2 diabetes is often caused by heredity, high-calorie diets, high-fat diets, and/or exercise shortage. The contribution of the latter three causes can be reduced by making lifestyle changes and appropriately controlling postprandial blood glucose concentrations. However, if people continue these unhealthy lifestyles, insulin resistance can occur, which is caused by the endoplasmic reticulum stress within the hypothalamus (4). High blood glucose concentrations for a long-term period can cause various complications including heart disease, stroke, diabetic retinopathy, and renal failure. These complications may lead to blindness and the need for artificial dialysis and limb amputation due to decreased blood flow. Postprandial hyperglycemia has a greater influence on the risk of cardiovascular disease than fasting hyperglycemia according to a previous study (5). Therefore, for prevention and treatment of type 2 diabetes, it is important to improve diet and exercise and manage postprandial blood glucose concentrations.

Blood glucose concentrations during normal conditions are elevated after meals and then decrease to pre-meal concentrations via a homeostatic system in the human body. Ingested starch and oligosaccharides are hydrolyzed in vivo by α-amylase and glucosidases, such as maltase, to glucose. Glucose is absorbed by small intestinal epithelial cells via the sodium-dependent glucose cotransporter (SGLT1) and then delivered to blood vessels. Elevated glucose concentrations are detected by β cells of the islets of Langerhans present in the pancreas that then secrete insulin. Insulin reduces blood glucose levels by promoting glucose uptake from blood into skeletal muscle cells by translocating GLUT4 to the cell membrane. Secretion of insulin is promoted by incretins such as glucagon-like peptide-1 (GLP-1), a gut hormone secreted by L cells mainly located at the ileum in response to feeding (6). Secreted incretins are rapidly inactivated by peptidyl peptidase-IV (DPP-IV) in the plasma to maintain homeostasis (7). Therefore, major targets for suppressing postprandial blood glucose concentrations include i) inhibition of digestive enzymes such as α-amylase and glucosidases to retard the hydrolysis of starch and sucrose, ii) suppression of glucose absorption by adsorbing glucose in the gut and promoting its excretion or by inhibiting the function of glucose transporters, and iii) enhancement of insulin secretion by promoting incretin secretion or inhibiting DPP-IV activity.

Recently, bioactive ingredients that suppress postprandial blood glucose concentrations have been identified in cereals. Cereal seeds often contain α-amylase inhibitor (α-AI) in the water-soluble protein (albumin) fraction possibly to defend against threats such as insects (8). Well-studied cereal seeds include wheat (Triticum aestivum L.), rice (Oryza sativa L.), barley (Hordeum vulgare L.), rye (Secale cereal L.), maize (Zea mays L.), and kidney beans (Phaseolus vulgaris L.) (9–17). Wheat α-AI shows strong inhibitory effects against mammals by suppressing postprandial hyperglycemia in rats, dogs, and humans (18–20). In addition, wheat α-AI shows thermal stability and resistance to digestion by pepsin and trypsin (21). Therefore, wheat α-AI has been used as a
bioactive compound for suppressing postprandial blood glucose concentrations in Food for Specified Health Uses (FoSHU) in Japan (22). Recently, we reported that buckwheat α-AI also suppresses postprandial hyperglycemia in rats, but through a different mechanism from that of wheat α-AI (23).

Rice is an important source of not only carbohydrates, but also protein (24, 25), and has been consumed worldwide since ancient times. Harvested rice grains are coated with inedible husks, and the husks are removed resulting in edible brown rice. Brown rice is mainly composed of rice bran and endosperm, the removal of rice bran produces white rice. Recent studies have shown that brown-rice diets improve insulin sensitivity and prevent the occurrence of diabetes compared with white-rice diets (26, 27). Because many people eat rice and do not show severe allergic reactions such as anaphylaxis and the compounds found in rice may be effective for the prevention and treatment of diabetes, rice compounds could be used as food additives or medicine. In this review, we introduce bioactive ingredients contained in rice bran and endosperm that show preventive and therapeutic effects against DM.

γ-Oryzanol
γ-Oryzanol prevents and improves type 2 diabetes by reducing pancreatic islet dysfunction and preference for high-fat diets (HFDs) (28, 29). γ-Oryzanol is a physiologically active compound unique to rice that is extracted and purified from rice bran oil or rice germ oil (30) being a mixture of ferulic acid esters of phytosterols.

The following experiments were conducted to examine the effect of γ-oryzanol on DM (28, 29). Eight-week-old C57BL/6J male mice were allowed free access to a high-fat diet (45% of energy as fat). γ-Oryzanol (20, 80, or 320 µg/g body weight−1 day−1) dissolved in water was administered orally in the stomach every day for 13 wk. Blood glucose concentrations, body weights, blood insulin concentrations, and mRNA levels of endoplasmic reticulum stress response genes (Chop, ERdj4, and Xbp1s) in the hypothalamus were measured. The postprandial blood glucose concentrations of the γ-oryzanol-administered mice (γ-oryzanol group) were significantly lower than the vehicle group where phosphate-buffered saline alone was administered, although body weights and insulin secretion levels did not vary between groups. The expression levels of Chop, ERdj4, and Xbp1s in the γ-oryzanol group were lower than those of the vehicle group.

Another animal experiment to examine the effect of γ-oryzanol was conducted. γ-Oryzanol was administered to eight-week-old male C57BL/6J mice for 2 wk and then streptozotocin, which induces endoplasmic reticulum stress of pancreatic β-cells, was administered. The concentration of plasma insulin levels of the γ-oryzanol group was statistically lower than that of the vehicle group.

Taken together, these results indicate that absorbed γ-oryzanol in the body ameliorates endoplasmic reticulum stress on the hypothalamus. Pancreatic islet dysfunction was reduced by γ-oryzanol decreasing pancreatic endoplasmic reticulum stress, and glucose-responsive insulin secretion of pancreatic β-cells was enhanced. Therefore, γ-oryzanol may be efficacious for the prevention and improvement of diabetes.

Alkali-Extracted Rice Protein (AE-RP)
Proteins extracted from rice endosperm with alkali aqueous solution delay the progression of diabetic nephropathy (31, 32). AE-RP was extracted as follows (33). Rice flour of Oryza sativa L. was stirred in 0.2% NaOH aqueous solution for 1 h at room temperature and allowed to stand overnight. The mixed solution was centrifuged at 3,000 ×g for 10 min and the supernatant was recovered. The precipitate was extracted again and the supernatant after centrifugation was also recovered and combined. The supernatant was adjusted to pH 6.0 with 1 N hydrochloric acid, and the resulting precipitate was recovered. Distilled water was added to the precipitate, followed by centrifugation at 3,000 ×g for 10 min, and the obtained precipitate was washed three times. Thereafter, the precipitate was freeze-dried, and the resulting protein was designated as AE-RP.

The following experiments were conducted with AE-RP. Seven-week-old male Goto-Kakizaki (GK) rats, a non-obese type 2 diabetes model, were fed a high sucrose diet with 20% AE-RP for 10 wk (31). Urine was recovered and urine albumin, an early marker of diabetic nephropathy, was measured during the last 3 d of the experimental period. Kidneys were collected to determine the mesangial matrix scores, a marker of nephropathia. The urine albumin and mesangial matrix scores of rats fed AE-RP were lower than that of the control group where casein was administered instead of AE-RP. These effects are thought to be related to the amino acid composition of AE-RP and blood adiponectin concentrations. Since AE-RP is richer in arginine than casein and since arginine is used as a substrate for nitric oxide synthesis in vivo, AE-RP may improve nitric oxide production. Soy protein reduces nephropathy by increasing nitric oxide production through a mechanism similar to that of AE-RP (34). In addition, blood adiponectin levels increased in the AE-RP group. Since adiponectin is a regulator of urinary albumin excretion (35), the increase is also considered to have ameliorated the progression of nephropathy.

AE-RP was also administered to Zucker diabetic fatty (ZDF) rats, an obese diabetes model (32). Seven-week-old male ZDF rats were fed with a diet containing 20% AE-RP or casein as a control for 8 wk. ZDF rats developed hyperglycemia, hyperinsulinemia, and hyperlipidemia by week 13. In this case, AE-RP showed a suppressive effect on diabetic nephropathy, likely due to its high arginine content. In addition to the direct effect of AE-RP on diabetic nephropathy, AE-RP significantly decreased hemoglobin A1c levels and increased carnitine levels, leading to the reduction of lipid accumulation. AE-RP also increased the rate of glycolysis and the pentose phosphate pathway. Since the reduction of lipid
accumulation and the activation of glycolysis and the pentose phosphate pathway suppress diabetic nephropathy, the renoprotective effect of AE-RP is also mediated indirectly.

**Rice Endosperm Protein Hydrolysate (REPH) and Rice Bran Protein Hydrolysate (RBPH)**

REPH and RBPH promote the secretion of GLP-1 and prevent the degradation of GLP-1 by DPP-IV, thereby moderating increases in blood glucose concentrations (36).

REPH and RBPH used for cellular and animal experiments were prepared as follows. REPH was obtained by hydrolyzing AE-RP with pepsin for 30 min. RBPH was similarly obtained by hydrolyzing rice bran protein (RBP) with pepsin for 30 min.

REPH or RBPH were orally administered to 6-wk-old male Sprague Dawley (SD) rats (0.1–2 g·kg body weight$^{-1}$) together with glucose (2 g·kg body weight$^{-1}$). REPH and RBPH lowered glycemic responses such as blood glucose and insulin concentrations. The gastric emptying, known to be delayed by GLP-1, in the REPH and RBPH groups was suppressed. When REPH or RBPH were orally administered to rats and glucose was subsequently intraperitoneally administered, the GLP-1 concentrations in the portal and tail vein were significantly increased compared to the control group where water was administered instead of REPH and RBPH. In addition, when REPH or RBPH were administered directly to the ileum of rats, DPP-IV activity in the blood was inhibited in the REPH and RBPH groups. These results suggest that REPH and RBPH promote GLP-1 secretion and inhibit DPP-IV activity, resulting in delayed glucose absorption in the small intestine and reduced blood glucose concentrations due to the incretin effect.

**16-Kda Rice Albumin (RA)**

RA is a water-soluble protein derived from the endosperm of rice. RA inhibits the absorption of glucose by adsorbing glucose itself, thus suppressing postprandial blood glucose levels (37).

RA was extracted as follows. Milled white rice was immersed overnight in a 5-fold weighted 100 mM citric acid aqueous solution (pH 6.0) and centrifuged at 15,000 $\times g$. Supernatants were heated for 20 min and centrifuged at 15,000 $\times g$. Supernatants were concentrated using ammonium sulfate, and the precipitate was recovered after centrifugation at 15,000 $\times g$. The recovered precipitate was dialyzed against distilled water, and the supernatant obtained by centrifugation was used as the albumin fraction. The albumin fraction was further purified by gel filtration chromatography, and the obtained fraction was lyophilized to result in a 16-kDa albumin fraction.

RA was orally administered to 7-wk-old male Wistar rats (1 g·kg body weight$^{-1}$) together with starch or glucose (50–200 mg·kg body weight$^{-1}$). Distilled water was administered instead of RA for the control. Blood glucose and insulin concentrations were measured. Administration of RA significantly suppressed the increase in blood glucose and insulin concentrations compared to the control group in both cases of starch and glucose loading. RA adsorbs glucose in the gut and promotes its excretion, thus suppressing blood glucose elevation.

The glucose adsorption ability of RA in vitro was evaluated by a system using a semipermeable membrane, which was originally designed for evaluating glucose adsorption onto water-soluble dietary fiber developed by Ou et al. (38). The glucose adsorption ability of RA was lower than that of guar gum, but it was equivalent to carboxymethyl cellulose. Furthermore, we also found that RA is not susceptible to hydrolysis by digestive enzymes. These unique features of RA are probably due to the tight structure of RA with five intramolecular disulfide bonds as determined by LC-MS/MS. Thus, RA can adsorb glucose in vivo similar to water-soluble dietary fiber to prevent uptake of glucose from the small intestinal epithelium.

The various anti-diabetes compounds derived from rice described here demonstrate that rice is not only a source of carbohydrates and protein, but also a source of bioactive ingredients for preventing diabetes. These compounds should be focused on by researchers searching for anti-diabetes therapies. Furthermore, these compounds show potential for use as new materials in the food and medicinal industry. Although rice has been reported to have a few anti-diabetes agents, these agents are quite unique in their mechanism of action and are worth being investigated further.

**Disclosure of State of COI**

No conflicts of interest to be declared.

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