Replacing White Rice with Pre-Germinated Brown Rice Mildly Ameliorates Hyperglycemia and Imbalance of Adipocytokine Levels in Type 2 Diabetes Model Rats

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**Summary** Pre-germinated brown rice (PR) has been developed industrially in order to enhance the nutritional functions of its source material, brown rice (BR). The present study was aimed at clarifying the effect of PR on the type 2 diabetes mellitus. We employed Otsuka Long-Evans Tokushima Fatty (OLETF) rats as a model of type-2 diabetes mellitus. OLETF rats were fed on either PR or white rice (WR) from the age of 4 to 35 wk. Age-matched male Long-Evans Tokushima Otsuka (LETO) rats as a non-diabetic control were also fed on WR. The HbA1c level in OLETF rats was significantly higher than that in LETO rats. However, the level was lower in PR-fed OLETF rats than in WR-fed OLETF rats. The plasma concentrations of TNF-α and PAI-1 in OLETF rats were higher than those in LETO rats. However, both elevated levels were decreased by the PR-feeding, but not by the WR-feeding. On the other hand, the plasma adiponectin concentration in OLETF rats was lower than that in LETO rats. The decrease in adiponectin level of OLETF rats was ameliorated by PR-feeding. The size of adipocytes in PR-fed OLETF rats was smaller than that in WR-fed OLETF rats. In summary, intake of PR instead of WR ameliorates both insulin resistance and imbalance of the levels of plasma adipocytokines leading to diabetic complications.

**Key Words** pre-germinated brown rice, diabetes, adipocytokine, adiponectin, PAI-1

The number of patients with type 2 diabetes mellitus has recently been abruptly increased in Asian countries (1, 2). Especially in the highly industrialized countries, type 2 diabetes is a major risk factor of myocardial and cerebral infarctions, which are the second and the third causes of death, respectively, following cancer (3). Type 2 diabetes is mainly caused by a decreased insulin response, which instantiates as insulin-signaling via the insulin receptor in peripheral tissues (4). In addition to genetic background, excess energy intake and the modern life-style including the daily use of energy-saving electrified transportation systems such as elevators and escalators, are thought to be major causes of type 2 diabetes. To prevent type 2 diabetes and its complications, control of the blood glucose concentration by an appropriate energy intake with the well-balanced daily diet is highly recommended (5).

Pre-germinated brown rice (PR) has recently been widely served in Japan. PR has been developed industrially in order to enhance the nutritional functions of its source material, brown rice (BR). PR is produced by soaking BR in water until it germinates slightly. Amounts of some constituents including γ-aminobutyric acid (GABA) are remarkably increased in PR; 3–5-fold increase in GABA is produced in PR from BR (6). GABA is known to regulate blood pressure and affect the nervous system as a neurotransmitter, and also to potentiate insulin secretion from the pancreas (7–9).

We have reported that the feeding of streptozotocin-induced type 1 diabetic rats with a PR diet ameliorated the elevation of blood glucose concentrations, type-1 plasminogen activator inhibitor (PAI-1) and lipid peroxide concentrations in comparison with rats fed a white rice (WR) diet (10). These results strongly suggest that, instead of WR, intake of PR is effective for the prevention of diabetic vascular complications including macrovascular complications such as myocardial infarction, and microvascular complications such as retinopathy and nephropathy. It has been reported that the postprandial glucose concentrations of volunteers who took PR were significantly lower than those of subjects who took WR; however, there was no significant difference in the glucose concentrations between the PR and WR groups (11). These results suggest that there is no PR-specific component responsible for lowering the postprandial glucose level, and the component(s) common to both PR and BR, but not to WR, should be the principle.

In the present study, we examined the effect of PR on
the fasting blood glucose, plasma adiponectin, TNF-α and PAI-1 concentrations in type 2 diabetes mellitus. For the purpose, we employed Otsuka Long-Evans Tokushima Fatty (OLETF) rats as a model of type 2 diabetes mellitus. OLETF rats have been well characterized by obesity, hyperinsulinemia, late onset of hyperglycemia, and hyperplastic foci of pancreatic islets (1, 2). The pathological features of glomerulosclerosis observed in OLETF rats are similar to those seen in human type 2 diabetes mellitus (1-3); thus these animals have been widely used as a model for the diabetic complications of human diabetes mellitus including nephropathy.

The cytokines secreted by adipocytes are called adipocytokines, and these plasma concentrations are closely related to the differentiation and maturation of adipocytes in the adipose tissues. Production and secretion of PAI-1 and TNF-α by adipocytes are known to be upregulated by obesity (14), but that of adiponectin, a negative adipocytokine, is known to be downregulated by obesity (15). TNF-α causes insulin resistance (16), and adiponectin increases the insulin sensitivity in peripheral tissues including adipose, muscle and liver (15). Thus either the upregulation of TNF-α or downregulation of adiponectin, or a combination of these changes in adipose tissues, is thought to be causative of type 2 diabetes.

In the present study, the effect of PR food on the type 2 diabetes model rats. OLETF has been examined in terms of plasma adipocytokine levels in comparison with that of WR food. We now report the amelioration of type 2 diabetes in OLETF rats with PR.

**MATERIALS AND METHODS**

**Animal and diets.** All animal experiments were performed in accordance with the Guidelines for Animal Experiments of the College of Bioresource Sciences, Nihon University. Male OLETF rats, a model of type 2 diabetes mellitus, were obtained from Tokushima Research Institute (Otsuka Pharmaceutical Co. Ltd., Tokushima, Japan). Male Long-Evans Tokushima Otsuka (LETO, obtained from the same company at Tokushima) rats were employed as a non-diabetic control. Both OLETF and LETO rats were raised in our animal facility from the age of 4 to 35 wk. The rats were kept in stainless wire cages in the animal facility with a 12 h:12 h light/dark cycle and a temperature main

![Table 1: Composition of the pre-germinated brown rice and white rice powder used in this study.](https://example.com/table1)

| Major composition/100 g | Pre-germinated brown rice powder | White rice powder |
|------------------------|---------------------------------|-------------------|
| Water                  | 14.7 g                          | 15.8 g            |
| Protein                | 6.9 g                           | 5.7 g             |
| Fat                    | 2.5 g                           | 1.7 g             |
| Carbohydrate           | 72.0 g                          | 75.7 g            |
| Dietary fiber          | 2.7 g                           | 0.6 g             |
| Ca                     | 8.8 mg                          | 4.7 mg            |
| Mg                     | 117 mg                          | 32.2 mg           |
| Thiamin                | 0.37 mg                         | 0.11 mg           |
| Total tocopherols      | 1.3 mg                          | 0.3 mg            |
| γ-amino butyric acid   | 15 mg                           | 3 mg              |
| Oryzanol               | 30.9 mg                         | 4.1 mg            |

PR and WR were produced from the same kind of Japonica rice (Hoshinoyume) grown in Hokkaido, Japan. The major components of these powders are listed in Table 1.

During the experiment, the amount of food intake was recorded every day, and body weight and blood glucose concentration were measured once a week. Plasma insulin concentration was measured once a month. The levels of hemoglobin A1c (HbA1c) and plasma adiponectin, TNF-α and PAI-1 concentrations were assayed at the end of the experimental period as described below.

**Assay of blood glucose, plasma insulin and blood HbA1c.** The blood glucose concentration was measured by DEXTER-Z II (Bayer Medical, Leverkusen, Germany). Rats were starved for 20 h prior to the measurement, and the blood was collected from the tail vein.

The plasma insulin concentration was assayed by using a commercial ELISA kit (High sensibility insulin concentration analysis kit, Morinaga Co., Tokyo, Japan). HbA1c was measured by use of the DCA 2000 system (Bayer Medical).

**Plasma PAI-1, TNF-α, and adiponectin concentrations.** The rats were sacrificed under anesthesia with pentobarbital at 35 wk of age, and blood samples were withdrawn by cardiac puncture with a heparinized syringe. Plasma was separated from the blood by centrifugation at 3,000 rpm for 15 min at 4°C, and stored at −80°C until analysis.

Plasma PAI-1 concentration was measured by ELISA as we described previously (10). Plasma TNF-α and adiponectin concentrations were also measured by ELISA using commercial kits (for TNF-α, BioSource International, Camarillo, CA, USA; and for adiponectin, Otsuka Pharmaceuticals, Tokyo, Japan).

**Histology of adipose tissues.** After the collection of blood, the epididymal adipose tissue was excised from each rat, and weighed. A small part of the tissue was fixed with 4% paraformaldehyde and embedded with paraffin for preparing tissue sections for microscopic observation. The sections (5 μm thick) were placed on a slide glass precoated with 3-aminopropyltrithoxysilane, and stained with hematoxyline/eosin (HE). The
cellular size of adipocytes was measured by using an imaging software program (Axiossvision 3.1, ZEISS Co., Germany). Practically, approximately 5 cells in a microscopic view were picked up and their major and minor axes were measured. Three different views of one tissue section were randomly chosen for the measurement, and these were triplicated, and the cellular sizes of 3 to 5 animals a group were averaged finally.

Statistical analysis. All data are expressed as means±SE. All statistical analyses were performed using StatView Version 11 software (SAS Institute, Inc., NC, USA). One-way analysis of variance (ANOVA) and subsequent Tukey-Kramer multiple comparisons test were used to compare groups. The p values less than 0.05 were considered to be significantly different.

RESULTS

Changes in body weight and total food intake

We started the feeding of OLETF and LETO rats with PR and WR diets at 5 wk of age. The amount of food eaten by the OLETF rats was much larger than that by the LETO rats (WR/LETO, 3,604 g vs. WR/OLETF, 5,186 g; PR/OLETF, 5,386 g). In good consistency with the amount of food intake, the increase in body weight of the OLETF rats was higher than that of the LETO rats at 6 wk of age (WR/LETO, 504 g vs. WR/OLETF, 739 g; PR/OLETF, 755 g, Table 2). The OLETF rats preferred the pre-germinated brown rice diet to the white rice diet, but body weight-gain rates was almost identical between the OLETF and LETO rats regardless of diet (WR/LETO, 0.140 g/g diet; WR/OLETF, 0.143 g/g diet; and PR/OLETF, 0.140 g/g diet).

Blood glucose, HbA1c, and plasma insulin concentrations

Figure 1 shows changes in the fasting blood glucose concentration during the experimental period. The blood glucose concentration in OLETF was significantly higher than that in LETO (LW vs. OW) at 10, 15, 20, 25 and 30 wk. The dietary pre-germinated brown rice suppressed the increases in the blood glucose concentration in OLETF (OP) and the value at 15 wk was significantly lower than that of WR-fed OLETF rats (OW).

HbA1c in PR-fed OLETF rats (OP) was also significantly lower than that in WR-fed OLETF rats (OW) (p<0.05; Table 2). Plasma insulin concentration in OLETF rats at 31 wk old was higher than that in LETO, suggesting that insulin resistance and subsequent hyperinsulinemia are caused in OLETF rats. The hyperinsulinemia observed in OLETF rats was slightly ameliorated when these rats were fed with PR.

Plasma PAI-1, TNF-α and adiponectin concentrations

The adiponectin concentration in WR-fed OLETF rat plasma was lower than that in WR-fed LETO rat plasma; however its concentration in PR-fed OLETF rats was kept higher than that in WR-fed OLETF rats (p=0.08, WR/OLETF vs. PR/OLETFOP, Fig. 2A).

On the other hand, plasma TNF-α and PAI-1 concentrations of OLETF rats were higher than those of LETO rats (Fig. 2B and C). The intake of PR was likely to ameliorate the increase in these concentrations in obese OLETF rats, although its effect was statistically insignificant.

Table 2. Total food intake, body weight and biochemical parameters in the blood during experimental period.

|                | LW | OW      | OP      |
|----------------|----|---------|---------|
| Total food intake (g) | 3,604±36.0a | 5,186.8±124.1b | 5,386.8±50.0b |
| Body weight (g)       | 75.23±2.6 | 70.13±2.5 | 70.4±2.3 |
| Initial               | 504.02±6.9a | 739.94±15.9b | 755.44±11.4b |
| Epididymal adipose tissue weight (g) | 8.29±0.34 | 18.86±1.00 | 18.78±0.71 |
| Plasma insulin (ng/mL) | 1.48±0.29 | 2.27±0.23 | 2.26±0.37 |
| 16 wk old             | 31 wk old | 2.01±0.29a | 4.74±0.35b | 3.84±0.47b |
| HbA1c (%)             | 2.67±0.0a | 4.51±0.5b | 3.32±0.2a |

LW, LETO rats fed white rice diet; OW, OLETF rats fed white rice diet; OP, OLETF rats fed pre-germinated brown rice diet. Each value represents the mean±SE of 9–10 rats. Values not sharing a common letter are significantly different at p<0.05 by Tukey-Kramer multiple comparisons test.
Histological examination of adipose tissues from OLETF rats fed either PR or WR diet

Plasma adipocytokine concentrations are known to be closely related to maturation and growth of adipocytes in the adipose tissues. Because the increased plasma adiponectin, TNF-α and PAI-1 concentrations were ameliorated in PR-fed OLETF rats, we next examined the histology of adipose tissues to see if the architectural change of the tissues could be observed (Fig. 3).

The size of adipocytes in OLETF rats was observed to be much larger than that of LETO rats (Fig. 3C); however, the increased size of adipocytes of OLETF rats was reduced slightly by feeding them with PR (see PR vs. WR above OLETF, Fig. 3C).

DISCUSSION

Diabetes mellitus has increased quite recently in developed countries. Especially in the Asian countries, people have changed their life-styles by preference for a western style diet. Thus, the population with diabetes has abruptly increased (2). Dietary fiber is well known as a food component to ameliorate diabetes (17). Water-soluble dietary fiber makes fast the gastrointestinal transition of food stuff, and reduces nutrient absorption, resulting in the suppression of postprandial glucose increase (18, 19). In contrast, water-insoluble fiber is said to be less effective for lowering the blood glucose level, but its chronic intake can ameliorate a high blood glucose level at fasting (18, 19).

PR is produced by soaking unpolished brown rice in the water to promote slight germination, and it is known to contain higher amounts of GABA, calcium, magnesium, vitamin B1 and E as well as dietary fiber in comparison with polished white rice (Table 1). Thus, PR has been used as a functional food in Japan.

Because the diabetic disorder develops gradually in OLETF rats through their daily eating habit for as long as 35 wk, both PR and WR were used to feed OLETF rats ad libitum from the age at 5 wk old to 35 wk old.

There was no significant difference in the fasting blood glucose concentration between OLETF rats fed PR and WR at 31 wk, but a significantly lower level emerged in OLETF fed PR than OLETF fed WR at 15 wk (Table 2). The HbA1c in OLETF rats was significantly lower in the PR-fed group than in the WR-fed group, suggesting that PR food suppresses the average level of blood glucose, including the postprandial blood glucose level (Table 2). In our previous study, we obtained the result that PR depleted both water-soluble and oil-soluble fractions but still had a post-prandial blood glucose-
lowering effect, which suggested that the higher content of dietary fiber in PR than in WR might be one of the principles responsible for the effect (11).

Type 2 diabetes sometimes accompanies obesity, and the size of adipocytes in the white adipose tissue is expanded as obesity develops. The secretion of adipocytokine from the adipocytes is known to be greatly influenced by the maturation status of the adipose tissue and its volume (14). There was no significant difference between the weights of body and adipose tissue of OLETF rats fed either the PR or WR diet (Table 2). Plasma concentrations of TNF-α and PAI-1 in OLETF rats were higher than those of LETO rats, and reciprocally adiponectin concentrations in OLETF rats were lower than those in LETO rats (Fig. 2). PR ameliorated the increase in both plasma TNF-α and PAI-1, and the decrease in adiponectin in OLETF rats. These results suggest that PR may have the effect of ameliorating the imbalance of adipokineton secretion in diabetes mellitus. TNF-α causes insulin resistance by phosphorylating a serine residue of the insulin receptor substrate 1 (IRS-1) (20). PAI-1 is well recognized as a fibrinolytic inhibitor triggering thrombotic diseases (21). PAI-1 also causes insulin resistance through its binding with vitronectin, a potentiator of insulin signaling (22). Adiponectin potentiates the insulin sensitivity of muscles through the activation of PI3-kinase associated with IRS-1 signaling (23), and at the same time, adiponectin suppresses the production of TNF-α (24). Although no significant difference was observed in the total white adipose tissue weights between the PR-fed and WR-fed groups of OLETF rats (Table 2), the sizes of their adipocytes were histologically different; i.e., those of PR-fed rats were smaller than those of WR-fed rats (Fig. 3). Thiazolidinedione (TZD), a synthetic agonist for nuclear receptor PPARγ, is known to stimulate adipocyte proliferation and bring down the size of adipocytes in adipose tissues, and then ameliorate the insulin resistance (25). Thus the PR that has size-reducing activity might alleviate the insulin resistance, although the mechanisms through which the PR and the agent exhibit their functions must be quite different.

PR contains a higher amount of oryzanol and GABA. Oryzanol is a potent anti-oxidant which can suppress the reactive oxygen species generated under a high blood glucose concentration. Thus the intake of PR is thought to be preventive against diabetic complications such as nephropathy through its oxidative stress-decreasing effect. GABA is not only a neurotransmitter, but also an agent to decrease blood pressure or to increase insulin secretion (7, 9). Taken together, PR containing larger amounts of oryzanol and GABA than WR may prevent the progression of diabetic complications. To explore the possibility, we performed the measurement of renal excretion of proteins and observation of renal histology, and found that proteinuria and glomerular sclerosis were tended to be ameliorated in the PR-fed OLETF rats (data not shown).

In summary, intake of PR as a replacement for WR for a certain period of time ameliorates HbA1c levels, insulin resistance and imbalance in the levels of plasma adipocytokines, which are parameters leading to diabetic complications such as nephropathy. White rice is the staple food in some Asian countries including Japan. To replace the principle food white rice with pre-germinated brown rice may contribute to the reduction of type 2 diabetes in these countries.

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