Effects of Long-Term Administration of Rice Predigested with Pancreatin or Diastase on the Growth of Streptozotocin-Induced Diabetic Rats

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The effects were investigated of predigested rice on diabetic rats induced by streptozotocin. Rice treated with pancreatin or diastase was not always appropriate for the diabetic rats, there being no difference in the serum glucose level between the groups fed on rice treated with and without the enzymes. The group fed on the diastase-treated rice had a higher serum fructosamine level than the other groups. Rapid absorption of the glucose did not suppress the appetite of the rats, so it may be useful for aged persons, sportsmen and patients who need an adequate energy supply over a short period.

(Received September 26, 1996)

**Keywords:** rice, digestive enzyme, diabetes mellitus, rat, pancreatin, diastase.

**INTRODUCTION**

In the previous study on healthy rats given rice treated with digestive enzymes (Higashino et al. 1997), we found that rice treated with pancreatin or diastase was more readily absorbed than untreated rice; thus, the blood glucose level was rapidly elevated, and such lipids as triglyceride and cholesterol ester were synthesized with high efficiency. In addition, rice treated with digestive enzymes did not affect the liver function, and rice treated with diastase required a shorter large intestine than normal. Therefore, rice treated with these enzymes may be an appropriate food for aged persons who have a low function in the digestive system, sportsmen who have a high energy demand for a short period, and for patients with alimentary tract disorders, exhaustive diseases such as carcinoma, infectious diseases, or metabolic diseases such as diabetes mellitus. Rice has historically been a staple food in Japan. Since it has a light, simple taste, too much rice is often consumed. Overeating causes obesity, which accelerates the atherosclerotic disorders involved in diabetes mellitus, hyperlipidemia, hypertension and hyperuricacidemia, and often causes ischemic heart diseases such as myocardial infarction and angina pectoris. Rice cultivated in Japan contains ca. 80% carbohydrate, 6-8% protein, and a small percentage of fat (Okuno 1994). Therefore, since rice is an important calorie source in Japanese food, we considered the blood glucose level would be promptly elevated by the consumption of predigested rice, and that overeating might thereby be prevented through stimulating the satiety center in the hypothalamus, especially in diabetic patients, and thus decreasing the calorie intake. We therefore studied the effects of a long-term administration of predigested rice on the growth of diabetic rats treated with streptozotocin.

**MATERIALS AND METHODS**

**Diabetic rats induced by streptozotocin and feeding**

Male Sprague-Dawley (SD) rats aged 6 weeks and weighing 170-180 g were purchased from Japan SLC (Shizuoka, Japan). After being acclimatized for 1 week, the diabetic animals were prepared by intravenously injecting a streptozotocin (ICN Biochemicals, Cleveland, OH, USA) solution (35 mg/ml in a 50 mM citrate buffer at pH 4.5) at a dose of 35 mg/kg of body weight through the tail vein. One week after this pretreatment, these rats with a 200-400 mg/dl blood glucose level when hungry were selected as the diabetic animals. The animals were divided into 4 groups, each consisting of 9 rats, as follows: the S group given a powdered SP diet (Funahashi Farm, Chiba, Japan), the C group given a powdered SP diet
mixed 1:1 with powdered polished rice (Suisho-mai®, Daiichi-Shokuryo Co., Japan), the P group given powdered SP chow mixed 1:1 with polished rice powder that had been treated with pancreatin, and the D group given a powdered SP diet mixed 1:1 with polished rice powder treated with diastase. The animals were fed for 8 weeks. Each diet contained the number of calories and nutrients indicated previously (Higashino et al. 1997) (372 kcal, 20.8 g of protein, 4.5 g of fat, and 58.6 g of glucose in 100 g of powder for group S; 364 kcal, 13.0 g of protein, 2.5 g fat and 72.2 g of glucose in 100 g of powder for group C; 367 kcal, 11.5 g of protein, 2.4 g of fat and 74.7 g of glucose in 100 g of powder for group P; 363 kcal, 12.0 g of protein, 2.5 g of fat and 73.0 g of glucose in 100 g of powder for group D). The rats were given tap water ad libitum under the conditions of 22 ±2°C and 60±5% humidity.

Preparation of rice digested with enzymes

The preparation method for the diets has been described previously (Tajiri 1995). In brief, polished rice (Suisho-mai®) was purchased from the market. Rice treated with pancreatin or diastase was processed by soaking polished rice in a solution containing 5.0% pancreatin (from porcine pancreas, containing trypsin, carboxypeptidase, lipase, amylase, ribonuclease and residues; Kanto Chemical Industries, Tokyo, Japan) or 5.0% diastase (β amylase, from barley; Kanto Chemical Industries) for 30 min at 40°C and then for 120 min more without the solution. After being dried with hot air (40°C for 24 h), the rice was ground to powder by a flour mill (Wiley-Mill 1029-A, Daizen Industries Co., Tokyo, Japan). Each powder was mixed half-and-half with a powdered SP diet and given to the rats in C, P and D groups. Rice treated with pancreatin was predigested into 70% carbohydrate, 69% protein and 59% fat, and diastase-treated rice was predigested into 74% carbohydrate, 58% protein and 34% fat (our laboratory data).

Measurement of parameters related to growth

Food intake was measured every day, and body weight and body length from the proximal point of the neck to the proximal point of the tail (neck-to-tail length) were measured every week. From these data, the growth rate (%)(Δ body weight (BW)/whole BW ×100) and food efficiency (%)(Δ BW/food intake×100) were calculated every 4 weeks. Values for glucose, fructosamine, total protein, total (T)-cholesterol, triglyceride, glutamic oxaloacetic transaminase (GOT), glutamic pyruvate transaminase (GPT) in the serum, and blood cell counts in the whole blood were measured by an autoanalyzer every 4 weeks in 0.3 ml of blood drawn from the tail vein in the afternoon. The glucose content in excreted urine was measured for urine collected in a metabolic cage, and the rectal temperature was measured with a thermistor for mice (Natsume, Tokyo, Japan) every 4 weeks. The tip of the thermistor was inserted 3 cm into the rectum of the manually held rat, and the temperature was read 30 s after insertion. Photographs of ocular fundus were taken every 4 weeks with an RC-2 fundus camera (Kowa, Osaka, Japan) and were used to determine the hypertensive (H) and sclerotic (S) arterial changes and transparency for cataracts by the criteria according to the Scheie classification (0-4 scores) with some modifications.

Statistical analyses

Each result is expressed as the mean ± SEM and was analyzed by Student’s unpaired t-test after confirming the same distribution of the data between two groups by the F-test. p values less than 0.05 are considered statistically significant.

RESULTS

Comparison of food intake

Food intake per day by the four groups is shown in Fig.1. There was no significant difference in quantity among three groups (C, P and D) until the 5th week of the experiment, although the food intake of the S group was slightly lower than that of each other group. After the 6th week, the food intake of each group decreased significantly. The results are shown in Fig.1.

Fig. 1. Comparative food intakes

○, S group (100% commercial feed (SP)); ●, C group (50% untreated rice + 50% SP); △, P group (50% rice treated with pancreatin + 50% SP); ▲, D group (50% rice treated with diastase + 50% SP).
group was restricted to the dose given to the S group. Therefore, the food intake of the C, P and D groups after the 6th week each decreased by about 20% compared with that of the 5th week. The calorie intake in the 1st week was 53.8±0.4, 53.3±0.4, 52.9±0.4, and 51.0±0.4 kcal/100 g BW by the S, C, P and D groups, respectively. The figures for the 5th week were 38.5±1.7, 44.6±1.5, 46.2±2.0, and 44.1±1.8 kcal/100 g BW in the S, C, P and D groups, respectively, and those for the 8th week were respectively 33.9±1.3, 33.1±1.3, 32.9±1.3, and 32.1±1.2 kcal/100 g BW.

Effects of differing food intake on the body weight and neck-to-tail length

In the diabetic, no body weight increase after 1 week was apparent. Although no significant difference in value among the 4 groups was observed, the body weight of the P and D groups tended to be lower than that of each of the other 2 groups after the 3rd week (Fig. 2). Similar findings to the body weight change in the values for the neck-to-tail length could be identified, except for the following values which increased with age: in the S group, 168±2 mm at the initial stage (0W), 174±3 mm after 4 weeks (4W), and 184±5 mm after 8 weeks (8W); in the C group, 168±2 mm at 0W, 175±3 mm at 4W, and 185±4 mm at 8W; in the P group, 169±1 mm at 0W, 173±4 mm at 4W, and 180±6 mm at 8W; and in the D group, 168±2 mm at 0W, 171±2 mm at 4W, and 180±4 mm at 8W.

Growth rate and food efficiency

The growth rate and food efficiency calculated from the data on body weight and food intake are shown in Fig. 3. There was no significant difference in the growth rate between the four groups during the periods of 1–4 weeks and 1–8 weeks (Fig. 3, top). There was also no significant difference in the food efficiency among the four groups during the periods of 1–4 weeks and 5–8 weeks (Fig. 3, bottom), although the D group tended to have a lower than that of each other group.

Effect on rectal temperature of feeding rice

The rectal temperatures of the four groups during the observation period ranged from 37.4–38.0°C without any significant difference (data not shown).

Fig. 2. Body weight gain of the diabetic rats

○, S group (100% commercial feed (SP)); ●, C group (50% untreated rice + 50% SP); △, P group (50% rice treated with pancreatin + 50% SP); ▲, D group (50% rice treated with diastase + 50% SP).

Fig. 3. Growth rate (top) and food efficiency (bottom)

□, S group (100% commercial feed (SP)); □, C group (50% untreated rice + 50% SP); ■, P group (50% rice treated with pancreatin + 50% SP); ■, D group (50% rice treated with diastase + 50% SP). Growth rate (%) (Δ body weight (BW)/whole BW×100) and food efficiency (%) (ΔBW/food intake×100) were calculated every 4 weeks.
Effect of feeding rice on the glucose and fructoseamine concentrations in the serum

The serum glucose level during the 1st to 5th week when food was given ad libitum to the rats gradually increased with time and then decreased during the 6th to 8th week when food was restricted. The C, P and D groups showed slightly lower values without any significant difference than the value for the S group (Fig. 4, top). There was no significant difference in fructosamine level at the 4th and 8th weeks, although the D group showed slightly higher values than the other groups (Fig. 4, bottom).

Effect of feeding rice on glucose excreted in the urine

The glucose excreted in the urine was measured, and the results are shown in the Fig. 5. There was no significant difference in values among the four groups at the 1st, 4th, or 8th week of the experiment.

Effect of feeding rice on the T-cholesterol and triglyceride concentrations in the serum

There was no significant difference in the values for T-cholesterol and triglyceride among the four groups at the 4th and 8th weeks (Fig. 6).

Effect of feeding rice on the GOT and GPT activities in the serum

Up to the 4th week, there was no significant difference in GOT and GPT activities between the four groups. However, both activities for the P group were higher, although not significantly so, than those for the C group at the 8th week (Fig. 7).

Effect of feeding rice on the total protein concentration in the serum and on the general blood cell analysis

Total protein in the serum, and the hemoglobin concentration, cell number of erythrocytes, leukocytes, and platelets in the blood were not significantly different among the four groups at stage (data not shown).

Ocular fundus findings at the 4th and 8th weeks

Ocular fundus determined by the modified Scheie classification were not significantly different in the scores for H (hypertensive) or S (sclerotic) change in the retinal arteries. Although there was no significant difference in the scores for cataracts, those derived from the P and D groups tended to be slightly lower (Fig. 8).

Fig. 4. Serum glucose (top) and fructosamine (bottom) levels

O, S group (100% commercial feed (SP)); ●, C group (50% untreated rice + 50% SP); △, P group (50% rice treated with pancreatin + 50% SP); ▲, D group (50% rice treated with diastase + 50% SP).

Fig. 5. Amount of glucose excreted in the urine

O, S group (100% commercial feed (SP)); ●, C group (50% untreated rice + 50% SP); △, P group (50% rice treated with pancreatin + 50% SP); ▲, D group (50% rice treated with diastase + 50% SP).
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Macroscopic findings, tissue weights of the organs, and small and large intestinal lengths in the four groups at the 8th week of feeding

There were no significant macroscopic disorders or weight difference in such organs as the brain, pituitary, heart, lung, thymus, aorta, liver, pancreas, kidney, adrenal gland, and testis among the four groups by autopsy (data not shown). Although there was no difference in the small intestinal length between the four groups, the large intestine derived from the D group was shorter than those of the other groups (Fig. 9).

DISCUSSION

Our previous study (Tajiri 1995) suggested that instant food prepared from artificially predigested rice was suitable for aged people because it was easier to swallow. We also proposed that rice treated with enzymes would be useful for sportsmen and for patients with alimentary tract disorders, metabolic diseases like diabetes mellitus, and so on, because rice predigested with pancreatin or diastase was readily absorbed and maintained good liver function during the growth of rats (Higashino et al. 1997). We thus investigated the effects of predigested rice on the growth and physiology of diabetic rats.

The food intake by three groups (C, P and D) tended to be higher than that by the S group, but it was not significantly different during the 6 weeks of ad libitum feeding. Despite the higher calorie intake during this period, the growth rate ascertained by body weight and neck-to-tail length was lower in each of the groups given predigested rice (P and D). Therefore, the food efficiency of each of the P and D groups was also lower than that of the S and C groups. The present study on diabetic rats did not

Fig. 6. Total cholesterol (top) and triglyceride (bottom) levels

□. S group (100% commercial feed (SP)); □. C group (50% untreated rice +50% SP); □. P group (50% rice treated with pancreatin +50% SP); □. D group (50% rice treated with diastase +50% SP).

Fig. 7. GOT (top) and GPT (bottom) activities

□. S group (100% commercial feed (SP)); □. C group (50% untreated rice +50% SP); □. P group (50% rice treated with pancreatin +50% SP); □. D group (50% rice treated with diastase +50% SP).
show any body weight increase after 1 week, in contrast to the results observed for normal rats (Higashino et al. 1997). This finding suggests that a very high glucose excretion rate into the urine due to insulin deficiency disturbed the body weight increase in the diabetic rats. Although the serum glucose level in each of the C, P and D groups was lower than that of the S group, serum fructosamine, which reflects chronic hyperglycemia, and urine glucose in the D and P groups (especially in the D group) were each higher.

These data taken together with the previous findings (Higashino et al. 1997) for normal rats suggest that rice treated with enzymes was more readily absorbed from the gut and more effectively transformed to lipids than the untreated rice in normal rats; therefore, the serum glucose level in the afternoon could have been at a lower level due to higher glucose excretion into the urine and the higher T-cholesterol level in the pancreatin- and diastase-treated rice groups when compared with the C and S groups. Furthermore, hypoglycemic substances may have been contained in the rice because the serum glucose level tended to decrease not only in the P and D groups, but also in the C group.

In the present experiment, the serum GOT and GPT activities were higher in the P group than those in the other groups. Since both GPT and GOT activities vary widely depending on age (Tajima et al. 1989), the precise effect of the rice digested with pancreatin on the liver function remains unclear. We hypothesize that rapid absorption of digestive materials such as amino acids, amines and fatty acids in
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the insulin-deficient status may disturb the membrane function in the liver, because rice treated with pancreatin did not disturb the liver function in normal rats (Higashino et al. 1997).

The fundus photographs taken at the 8th week of the experiment revealed nonsignificant effects of the enzyme-treated rice on the retinal vessels. On the other hand, diastase- and pancreatin-treated rice groups tended to show lower cataract changes, as detected by transparency to light, than the other groups. Thus, rapid processing of the nutrients might have had a favorable effect on protein metabolism against degeneration of the lens. Neither rice with nor without the enzyme treatment caused any organ damage or disorder in the diabetic rats, like the findings obtained for normal rats. The large intestine of the D group was shorter in both the diabetic and normal rats, indicative of the rapid absorption of nutrients without the normal need for intestinal flora.

Although there was no difference in the small intestinal length between the 4 groups, the large intestinal length of the D group was shorter than that of the other groups, as was also observed with normal rats (Higashino et al. 1997)(Fig. 9).

These findings indicate that rice treated with resolving enzymes was not always appropriate for diabetic rats as we had expected. When the food was given ad libitum, the food intake of predigested rice was higher than that of untreated rice. Rapid absorption of glucose did not suppress the appetite of the rats. There was no difference in the serum glucose level between the groups fed on rice treated with and without the enzymes, and the group fed on diastase-treated rice had a higher serum fructosamine level than the other groups.

There are many people who have an allergy to rice. Lower-allergen rice can be made by predigesting 16-kDa allergenic protein with protease (Arai 1993) or by using mutant rice with low in allergen content (Nishio and Iida 1993). Therefore, rice predigested with pancreatin or diastase may also be useful for people allergic to rice. Since rice treated with enzymes has a distinctive odor and lower quality, rice treated with enzymes should be applied as instant rice, rather than rice that need to be boiled or steamed (Tajiri 1995). We also propose that rice treated with the enzymes can be powdered and mixed with wheat flour for baking.

We conclude from our studies on normal and diabetic rats that since rice predigested with digesting enzymes was rapidly and effectively absorbed and transformed to such compounds as lipids, it will be useful for aged persons who have reduced function in the digestive system, for sportsmen who have a high energy demand for a short time, and for patients with alimentary tract disorders, exhaustive diseases such as carcinoma, infectious diseases, and rice allergies.

REFERENCES


パンクレアチンまたはジアスターゼで前処理した米の長期投与が
ストレプトゾトシン誘発糖尿病ラットに与えた影響

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平成８年９月26日受理

酵素であらかじめ処理した米は、ラットの生長に影響することなく素早く吸収され、脂質
に変換代谢されることを前回の実験で明らかにした。その興味ある結果から、代謝の面で欠
陥を持った糖尿病ラットを用いて同様の実験を試みたところ、次のような結果と推論を得た。
パンクレアチンやジアスターゼで前処理した米を投与されたラットの血糖値は非処理米群よ
り低くならず、ジアスターゼで処理した米を食したラットのフルクトシミン値は非処理米群
よりむしろ高値であった。したがって、処理米投与群で白内障の進展が抑制される傾向が見
られたものの、糖尿病ラットにとって酵素処理米は決して適した飼料ではなかった。しかし、
その優れた易吸収性から高齢者や、スポーツ選手や、消化器または消耗性疾患患者にとって
適した食物になるのではないと考ええた。

キーワード：米、消化酵素、糖尿病、ラット、パンクレアチン、ジアスターゼ。