Beneficial Effect of 3% Milled-Rice on Blood Glucose Level and Serum Lipid Concentrations in Spontaneously Non-Insulin-Dependent Diabetic Rats

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Summary Three percent milled-rice was evaluated for beneficial effect on blood glucose level and serum lipid concentrations in an experiment with Otsuka Long-Evans Tokushima Fatty (OLETF) rats, used as model animals for spontaneously non-insulin-dependent diabetes mellitus. The same experiment was carried out using Long-Evans Tokushima Otsuka (LETO) rats, the control of the OLETF rats. The results obtained from the rats given a diet containing 3% milled-rice (3% MRD) ad libitum were compared with those from rats given polished rice. During the feeding period of 140 d, body weight of the OLETF rats receiving the 3% MRD was significantly lower than that of the rats fed on the diet containing polished rice (PRD) from the 48th to the 124th days. The body weight of the LETO rats during the both periods of 90 to 104 d and 114 to 140 d was lower than that of the rats receiving the PRD. Though food intakes of the rats receiving 3% MRD were significantly lower in the OLETF and LETO rats during the two periods of days 48 to 124 and days 1 to 140 than in the rats of the PRD group, the feed efficiency of the OLETF or LETO rats did not show significant difference between the 3% MRD and the PRD groups during the same experimental periods. The excretion rate of feces of the OLETF rats receiving the 3% MRD was significantly higher than that of the rats receiving the PRD, both on the 126th day and during the period of days 129 to 131. The fasting blood glucose levels were significantly lower in the OLETF rats receiving the 3% MRD than the rats receiving the PRD on the 84th day, the 105th day and the 127th day, and also lower in the LETO rats receiving the 3% MRD on the 84th day and the 105th day. The incremental areas under the curve of blood glucose concentrations (IAUC-Glc) for 120 min after oral administration of glucose on the 133rd day was lower in the OLETF rats receiving 3% MRD than that of the PRD. The ratio of IAUC-Glc in the 3% MRD to PRD group, after ingestion of diets for 1 h after fasting for 18 h on the 138th day, was 0.89 in the OLETF rats, and 0.74 in the LETO rats. Compared with the PRD group, the amounts of cholesterol and bile acid in the feces of the OLETF rats in the 3% MRD group were significantly higher on days 129–131, and the cholesterol excretion was significantly higher on the 84th day in the OLETF rats in the 3% MRD group. The liver weight, the level of total lipids in liver, and the concentrations of triglyceride and total cholesterol in liver and serum of the OLETF rats on the 140th day were significantly lower in the 3% MRD than those of the PRD group. These results indicate that 3% milled-rice has beneficial effects on blood glucose level and serum lipid concentrations in spontaneously non-insulin-dependent diabetic rats.

Key Words 3% milled-rice, blood glucose, cholesterol, bile acid, OLETF rat

Lifestyle-related disease has been increasing markedly in the Japanese (1). It is well known that obesity can lead to an increase in lifestyle-related disease (2). The prevention of lifestyle-related disease is one of the most important problems for public health. There is a close connection between daily dietary intake and lifestyle-related disease. Several epidemiologic studies reported that diets rich in whole grains decreased the risk of cardiovascular disease (3), stroke (4), type 2 diabetes (5) and certain cancers (6). The bran and germ in whole grains contain fiber, vitamin E, vitamin B6, minerals, antioxidants and phytochemicals that are related to beneficial effects on lifestyle-related disease (7). In the Iowa Women’s Health Study, intakes of total grains, whole grains, dietary fiber, cereal fiber and magnesium showed significant inverse associations with incidence of type 2 diabetes (5). It has also been reported that fiber, vitamin E and magnesium from cereals are associ-
ated with improved insulin sensitivity (8), lower body mass index and blood pressure (9), decreased cardiovascular events and deaths (10), and lower incidence of metabolic syndrome (11). It is possible to indicate that these beneficial effects are related with the reduction of the lifestyle-related disease by the intake of brown rice high in bran component. When streptozotocine-induced diabetic rats were fed pre-germinated brown rice, the elevation of blood glucose and type-1 plasminogen activator inhibitor concentration was lower than those of rats receiving white rice (12). To investigate whether 3% milled-rice, from which 3% of the weight of brown rice is removed and which retains about 70% of the total germ and bran of the brown rice, has beneficial effects against the incidence of type 2 diabetes or not, the present study was designed to examine the beneficial effect of 3% milled-rice on the elevation of fasting blood glucose, levels of serum lipids, excretion of total cholesterol and total bile acid and incremental areas under the curve of blood glucose concentrations (IAUC-Glc) of OLETF and LETO rats. These results were compared with those obtained from rats receiving polished rice.

**MATERIALS AND METHODS**

**Animals.** Four-week-old male OLETF, weighing 63.0±4.10, and LETO rats, weighing 62.3±3.96, were provided from the Tokushima Pharmaceutical (Tokushima, Japan) without charge. They were divided into the PRD (8 OLETF rats and 7 LETO rats) and 3% MRD group (8 OLETF rats and 7 LETO rats), and were housed individually in cages for rats in a temperature-controlled room (20–22°C) with a 12 h interval of light (7:00–19:00) and dark. The rats were fed on the PRD or 3% MRD, prepared as described below, for 140 d. The rats on the 138th day were also allowed to ingesta glucose (2 g/kg body weight) orally to the rats starved for 1 h. Measurement of fasting glucose. The fasting glucose levels of the rats were measured by Antsense III (Horiba, Ltd., Kyoto), using blood obtained from the tail vein on the 42nd day, the 64th day, the 84th day, the 103rd day, the 126th day and the 129th–131st days and dried with a vacuum freeze drier until a constant weight was obtained. The excretion rate of feces of the rats was estimated by dividing the weight of dried feces by the amount of food intake of the rats was recorded every day and every other day during this study, respectively. At the end of the experimental periods, the animals fasted overnight and were immediately sacrificed under ethyl-ether anesthesia after blood was collected via the post-caval vein.

**Preparation of diets.** Brown rice used for this study was grown in a single rice field and was obtained from a rice milling plant in Okayama City. Half of the obtained brown rice was polished to prepare the polished rice for the PRD and a rice milling plant in Okayama, the 3% milled-rice for the 3% MRD was prepared. The polished rice and 3% milled-rice were mechanically ground with a mill, and these powders were used for the diet preparations. The diets were prepared according to the composition of the AIN-93G diet (13), by replacing cornstarch, dextrinized cornstarch and sucrose with the polished rice powder for the PRD or the 3%-milled rice powder for the 3% MRD. The diets contain (in g/kg diet) vitamin free casein (Sigma), 200; soybean oil (no additives), 70; fiber, 50; mineral mixture (AIN-93G-MX), 35; vitamin mixture (AIN-93-VX), 10; l-cystine, 3; choline bitartrate, 2.5; tetra-butylhydroquinone, 0.014. The amount of food intake of the rats was recorded every day during this study.

**Estimation of minerals, vitamins and dietary fiber in polished rice and 3%-milled rice.** The amounts of minerals (Ca, Mg, Zn, Fe, Cu, Na, K) were estimated using an atomic absorption spectrophotometer (SpectrAA-800, Varian, Australia) after digestion with a concentrated HNO₃ (ultra fine grade) in a high-performance microwave digestion unit (mls 1,200 mega, Milestone General, Japan) as described previously (14). The contents of vitamins (B₁, B₂, nicotinic acid, E) were estimated as follows: the thiochrome reaction for vitamin B₁ (15), lumiflavin fluorescence method for vitamin B₂ (16), microbiological assay for nicotinic acid (17), HPLC determination for vitamin E (18). The amount of dietary fiber was prepared according to the method of Prosky et al. (19).

**Estimation of feed efficiency and excretion rate of feces.** The feed efficiency of the rats was calculated based on the amount of food consumption and the body weight gain of the rats during feeding periods from the first to the 47th days, from the 48th to the124th days, from the 125th to the 140th days and from the first day to the final experimental day. The experimental periods to calculate the feed efficiency were divided into two groups, from the 48th to the 124th days during which was observed a significant difference in the body weight of the OLETF rats, and from the first to the 47th days and from the 125th to the 140th days without significant difference between the PRD and the 3% MRD groups. The feces of the rats were collected at 9:00 on the 50th day, the 64th day, the 84th day, the 103rd day, the 126th day and the 129th–131st days and dried with a vacuum freeze drier until a constant weight was obtained. The excretion rate of feces of the rats was estimated by dividing the weight of dried feces by the amount of food intake.

**Measurement of total lipids, triglyceride and total cholesterol in liver.** Total lipids in the liver were extracted with a chloroform-methanol mixture (2:1, v/v). After evaporation of the extracting reagent, total lipids were determined by the weight method, and the concentrations of triglyceride and total cholesterol were estimated by assay kits (Wako Pure Chemical Industries, Ltd., Osaka, Japan) after total lipids dissolved in isopropyl alcohol.

**Measurement of fasting glucose.** The fasting glucose levels of the rats were measured by Antsense III (Hori- ba, Ltd., Kyoto), using blood obtained from the tail vein on the 42nd day, the 63rd day, the 84th day, the 105th day and the 127th day during the feeding periods.

**Oral glucose tolerance test and blood glucose levels after ingestion for 1 h.** An oral glucose tolerance test (OGTT) was performed on the 133rd day by administration of glucose (2 g/kg body weight) orally to the rats starved for 14 h. The rats on the 138th day were also allowed to ingest diets for 1 h after fasting for 18 h. Then blood was taken from the tail vein at 0, 30, 60, and 120 min...
for the OGTT, and at 0, 30, 60, 120, and 180 min for the measurement of blood glucose levels after ingestion for 1 h. The blood glucose concentration was measured as described above, and the IAUC-Glc for 120 or 180 min was calculated.

Measurement of cholesterol and bile acid in feces. The cholesterol and bile acid in the dried feces of the rats on the 84th day and the 129th day were extracted by the method described previously (20) and were determined by assay kits (Wako Pure Chemical Industries, Ltd.).

Measurement of triglyceride, total cholesterol, HDL-cholesterol and insulin. The concentrations of triglyceride, total cholesterol and HDL-cholesterol of the OLETF and LETO rats were determined by assay kits (Wako Pure Chemical Industries, Ltd.). The LDL-cholesterol level was calculated using the calculating formula described by Friedwald et al. (21). Insulin level of the OLETF and LETO rats on the final experimental day was measured by SRL Inc. (medical laboratory for clinical assay, Tokyo, Japan).

Statistical analysis. Statistical analyses were performed with SPSS for Windows (version 10.0. 5J; SPSS Inc, Chicago, IL). All results are expressed as means ± standard deviations. The results of groups were compared statistically using unpaired Student’s t-test. Group differences were considered to be significant when p<0.05.

RESULTS

Nutritive components in the powdered-polished rice and powdered-3% milled-rice

Table 1 shows the nutritive components in the powdered-polished rice and powdered-3% milled-rice used for the preparation of PRD or 3% MRD. The contents of nutritive components of the powdered-3% milled-rice were over 3-fold in vitamin E and dietary fiber, and over 2-fold in the magnesium, nicotinic acid, vitamin B1, iron and potassium compared with those of the powdered-polished rice.

Body growth curve of the rats

Figure 1 shows the changes in the body weight of the OLETF and LETO rats receiving the PRD or the 3% MRD. The body weight of the OLETF or the LETO rats receiving the 3% MRD was significantly lower on the day marked in the growth curve with an asterisk than that of the rats fed with PRD group.

Feed efficiency, excretion rate of feces and intake of nutrients

Table 2 shows the feed efficiency of the OLETF and LETO rats receiving the PRD or 3% MRD. The feed intake of the OLETF rats in the 3% MRD group from the 48th–124th days, and whole experimental period was significantly lower than that of the rats in the PRD group. The body weight gain of the rats showed no difference in the OLETF rats between the PRD and the 3% MRD groups during the experimental period, and was

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Table 1. The contents of dietary fiber, minerals and vitamins in the powdered-polished rice and powdered-3% milled-rice used for preparation of diets.

| Nutrient                  | PR  | 3% MR | 3% MR/PR |
|---------------------------|-----|-------|----------|
| Dietary fiber (g/100 g)   | 0.50| 1.50  | 3.00     |
| Zinc (mg/100 g)           | 1.34| 1.80  | 1.17     |
| Iron (mg/100 g)           | 0.33| 0.73  | 2.21     |
| Copper (mg/100 g)         | 0.13| 0.15  | 1.15     |
| Calcium (mg/100 g)        | 6.01| 7.63  | 1.27     |
| Magnesium (mg/100 g)      | 31.1| 86.4  | 2.78     |
| Sodium (mg/100 g)         | 2.44| 3.37  | 1.38     |
| Potassium (mg/100 g)      | 71.7| 150   | 2.09     |
| Thiamin (mg/100 g)        | 0.11| 0.26  | 2.36     |
| Riboflavin (mg/100 g)     | 0.04| 0.05  | 1.25     |
| Nicotinic acid (mg/100 g) | 1.90| 5.10  | 2.68     |
| Vitamin E (mg/100 g)      | 0.20| 0.70  | 3.50     |

PR, polished rice; 3% MR, 3% milled-rice.
Table 2. The feed efficiency of the OLETF and the LETO rats receiving the PRD or the 3% MRD.

| Feeding period | Rat   | Group | Food intake (g) | Body weight gain (g) | Feed efficiency (%) |
|----------------|-------|-------|----------------|----------------------|---------------------|
| 1–46           | OLETF | PRD   | 1.067±43.6     | 362±16.8             | 34.0±0.781          |
|                |       | 3% MRD| 1.026±40.3     | 343±20.1             | 33.4±1.17           |
|                | LETO  | PRD   | 823±21.6       | 281±8.37             | 34.1±1.02           |
|                |       | 3% MRD| 838±44.8       | 282±17.5             | 33.7±0.817          |

| 48–124         | OLETF | PRD   | 2.047±134      | 271±42.3             | 13.2±1.14           |
|                |       | 3% MRD| 1.80±89.9**    | 238±31.8             | 11.1±1.18           |
|                | LETO  | PRD   | 1.635±56.7     | 212±32.7             | 12.9±1.67           |
|                |       | 3% MRD| 1.543±51.0**   | 185±25.3             | 12.0±1.62           |

| 126–140        | OLETF | PRD   | 313±24.5       | −8.25±9.70           | −2.80±3.4          |
|                |       | 3% MRD| 309±21.1       | −6.93±6.94           | −2.30±2.27         |
|                | LETO  | PRD   | 287±17.5       | −7.47±5.77           | −2.63±1.99         |
|                |       | 3% MRD| 271±9.28       | −6.10±5.03           | −2.25±1.88         |

Table 3. The excretion rate of feces of the OLETF and the LETO rats receiving the PRD or the 3% MRD.

| Rat   | Group | Diet (g) | Feces (g) | RF (%) | Diet (g) | Feces (g) | RF (%) | Diet (g) | Feces (g) | RF (%) |
|-------|-------|----------|-----------|--------|----------|-----------|--------|----------|-----------|--------|
|       | OLETF | PRD 3% MRD | 31.4±3.46 | 2.69±0.68 | 8.00±1.51 | 29.6±2.48 | 1.67±0.46 | 5.62±1.41 | 25.2±2.92 | 1.61±0.30 | 6.47±0.84 |
|       |       | 3% MRD | 27.5±4.27** | 2.19±0.44 | 8.05±1.53 | 25.0±2.92 | 1.61±0.30 | 6.47±0.84 | 19.1±1.18 | 13.1±0.78 | 3.4±0.67 |
|       | LETO  | PRD 3% MRD | 22.7±2.46 | 1.70±0.31 | 7.62±1.70 | 25.4±1.23 | 1.04±0.22 | 4.12±0.93 | 23.5±1.42 | 1.05±0.31 | 4.41±1.13 |
|       |       | 3% MRD | 22.1±0.70 | 2.11±0.42 | 9.52±1.77 | 25.0±1.42 | 1.05±0.31 | 4.41±1.13 | 19.4±0.67 | 14.6±0.67 | 3.9±0.67 |

| Rat   | Group | Diet (g) | Feces (g) | RF (%) | Diet (g) | Feces (g) | RF (%) | Diet (g) | Feces (g) | RF (%) |
|-------|-------|----------|-----------|--------|----------|-----------|--------|----------|-----------|--------|
|       | OLETF | PRD 3% MRD | 33.4±3.91 | 3.63±1.50 | 11.0±5.02 | 26.2±4.61 | 2.01±0.32 | 7.61±0.50 | 28.8±3.63 | 2.61±0.42 | 9.12±1.57* |
|       |       | 3% MRD | 29.9±5.88 | 2.53±0.43 | 8.57±1.00 | 26.2±4.61 | 2.01±0.32 | 7.61±0.50 | 27.3±3.61 | 2.41±0.42 | 8.7±1.57** |
|       | LETO  | PRD 3% MRD | 23.8±2.38 | 2.25±0.77 | 9.53±3.29 | 24.4±3.11 | 1.82±0.30 | 7.53±1.24 | 22.4±3.39 | 2.18±0.34 | 8.98±1.79* |
|       |       | 3% MRD | 22.5±1.60 | 2.15±0.14 | 9.56±0.78 | 22.4±3.39 | 2.18±0.34 | 8.98±1.79* | 20.4±1.60 | 2.01±0.34 | 7.6±1.43 |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet. There is a significant difference at a level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.

Significantly lower in the LETO rats of the 3% MRD group from the first to the 140th days than that of the PRD group. No significant difference in the feeding efficiency was observed in the OLETF and LETO rats between the PRD and 3% MRD groups.

On the other hand, the excretion rate of feces of the rats receiving the PRD or the 3% MRD is shown in Table 3. In the OLETF rats, the food intake of the rats in the 3% MRD group was significantly lower on the 50th day and the 64th day than that of the rats in the PRD group, and the excretion rate of feces of the rats receiving the 3% MRD was significantly higher on the 126th day and the 129th–131st days than those of the rats fed with the PRD. In the LETO rats, the food intake of the rats in the 3% MRD group on the 64th day was significantly lower, and the excretion rate of feces of the rats receiving the 3% MRD on the 126th day was significantly higher than those of the rats in the PRD.

Table 4 shows the intakes of dietary fiber, minerals and vitamins of the OLETF and LETO rats ingesting the PRD or 3% MRD during whole experimental periods. In the OLETF rats, the intakes of copper, calcium, sodium and riboflavin were significantly higher in the PRD group, and the intakes of magnesium, thiamin and niacinic acid were higher in the 3% MRD group. In the LETO rats, the intakes of dietary fiber, magnesium,
Table 4. The intakes of dietary fiber, minerals and vitamins of OLETF and LETO rats receiving the PRD or 3% MRD during the whole experimental period.

| Nutrient          | OLETF rats | LETO rats |
|-------------------|------------|-----------|
|                   | PRD        | 3% MRD    | PRD        | 3% MRD    |
| Dietary fiber (g) | 176±9.04   | 182±8.40  | 141±3.11** | 153±5.05  |
| Zinc (mg)         | 166±8.52   | 157±7.27  | 133±2.93   | 133±4.38  |
| Iron (mg)         | 164±8.42   | 158±7.32  | 131±2.89   | 133±4.40  |
| Copper (mg)       | 23.7±1.22* | 22.0±1.02 | 18.9±0.42  | 18.6±0.61 |
| Calcium (mg)      | 17.53±901**| 16.13±745 | 14.02±310  | 13.58±4.48|
| Magnesium (mg)    | 2.47±1.12**| 1.38±156  | 1.97±43.6**| 2.85±94.1 |
| Sodium (mg)       | 3.65±1.88**| 3.38±156  | 2.92±64.5  | 2.85±94.1 |
| Potassium (mg)    | 14.08±724  | 14.50±670 | 11.27±249**| 12.21±403 |
| Thiamin (mg)      | 19.8±1.02* | 21.1±0.97 | 15.9±0.35**| 17.8±0.59 |
| Riboflavin (mg)   | 21.9±1.13**| 20.1±0.93 | 17.5±0.39* | 17.0±0.56 |
| Nicotinic acid (mg)| 146±7.51** | 198±9.16  | 117±2.58** | 167±5.51  |
| Vitamin E (mg)    | 265±13.6   | 254±11.7  | 212±4.69   | 214±7.05  |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet.
There is a significant difference at the level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.

Table 5. The weight of organs in the OLETF and LETO rats receiving the PRD or the 3% MRD on the final experimental day.

| Rat     | Group   | Liver       |                | Kidneys       |                |
|---------|---------|-------------|----------------|---------------|----------------|
|         |         | Total, g    | g/100 g weight | Total, g      | g/100 g weight |
| OLETF   | PRD     | 22.7±3.04   | 3.25±0.36      | 3.24±0.33     | 0.47±0.06      |
|         | 3% MRD  | 19.0±1.58*  | 2.91±0.13*     | 3.08±0.16     | 0.47±0.02      |
| LETO    | PRD     | 15.3±1.05   | 2.75±0.13      | 2.58±0.08     | 0.46±0.02      |
|         | 3% MRD  | 14.6±0.57   | 2.75±0.11      | 2.46±0.14     | 0.46±0.03      |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet.
There is a significant difference at a level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.

Table 6. The concentrations of total lipids, triglyceride and total cholesterol in the liver of OLETF and LETO rats receiving the PRD or the 3% MRD on the final experimental day.

| Rat     | Group   | Total lipids | Triglyceride | Total cholesterol |
|---------|---------|--------------|--------------|-------------------|
|         |         | mg           | mg           | mg                |
| OLETF   | PRD     | 2.26±4.23    | 686±196      | 127±28.7          |
|         | 3% MRD  | 1.52±1.82*   | 407±61.6**   | 66.3±4.08**       |
| LETO    | PRD     | 7.38±69.6    | 159±26.8     | 47.9±3.91         |
|         | 3% MRD  | 7.10±16.0    | 138±16.2     | 50.3±2.28         |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet.
There is a significant difference at a level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.
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Table 5 shows the weight of organs in the OLETF and LETO rats ingesting the PRD or 3% MRD on the final experimental day. The weight of liver in the OLETF rats and testes in the LETO rats receiving the 3% MRD were significantly lower than those of the rats in the PRD group. Table 6 shows the concentrations of total lipids, triglyceride and total cholesterol in the liver of the OLETF and LETO rats ingesting the PRD or 3% MRD on the final experimental day. In the 3% MRD group, the concentration of total lipids, triglyceride and total cholesterol in the liver of the OLETF rats were significantly lower than those of the rats in the PRD group. Fasting glucose and insulin levels

Figure 2 shows the change in the fasting glucose levels of the rats receiving the PRD or the 3% MRD. OLETF rats receiving the PRD, (○) open circle; OLETF rats receiving the 3% MRD, (●) closed circle; LETO rats receiving the PRD, (□) open square; LETO rats fed on the 3% MRD, (■) closed square. There is a significant different at a level of *p<0.01 and **p<0.05 between the PRD and the 3% MRD group.

Oral glucose tolerance test and blood glucose levels after ingestion for 1 h

Figure 3 shows the change in the blood glucose levels of the OLETF and LETO rats after administration of glucose. OLETF rats receiving the PRD, (○) open circle; OLETF rats receiving the 3% MRD, (●) closed circle; LETO rats receiving the PRD, (□) open square; LETO rats fed on the 3% MRD, (■) closed square.

Table 7. The incremental areas under the curve of blood glucose concentrations (IAUC-Glc) of the OLETF and the LETO rats after administration of glucose.

| Rat   | Group | IAUC      | 3% MRD/PRD |
|-------|-------|-----------|------------|
| OLETF | PRD   | 12,553±3,910 | 0.89       |
|       | 3% MRD| 11,130±2,730 |
| LETO  | PRD   | 6,525±943   | 1.06       |
|       | 3% MRD| 6,947±1,732 |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet. 3% MRD/PRD means the ratio of IAUC-Glc in the 3% MRD to the PRD group.
Table 8. The incremental areas under the curve of blood glucose concentrations (IAUC-Glc) of the OLETF and the LETO rats receiving 1 g of the PRD or the 3% MRD for 1 h.

| Rat  | Group | 1 h IAUC  | PRD | 3% MRD/PRD | 1 g IAUC  | 3% MRD/PRD |
|------|-------|-----------|-----|------------|-----------|------------|
| OLETF| PRD   | 15,201±4.878 |     | 0.80       | 2,536±808 | 0.80       |
|      | 3% MRD| 12,157±5.874 |     |            | 2,030±979 |            |
| LETO | PRD   | 8,595±2.508  | 0.74|            | 1,433±418 | 0.74       |
|      | 3% MRD| 6,336±1.091  |     |            | 1,056±182 |            |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet. 3% MRD/PRD means the ratio of IAUC-Glc in the 3% MRD to the PRD group.

Table 9. The concentrations of cholesterol and bile acid in the feces of the OLETF and the LETO rats on the 84th and the 129th–131st days.

| Rat  | Group | 84 d Feces (g/d) | Cholesterol (µg/d) | Bile acid (µg/d) |
|------|-------|-----------------|--------------------|------------------|
| OLETF| PRD   | 1.65±0.590      | 415±208            | 25.4±7.63        |
|      | 3% MRD| 1.81±0.521      | 833±271**          | 31.3±9.33        |
| LETO | PRD   | 1.05±0.247      | 415±108            | 20.1±5.32        |
|      | 3% MRD| 1.05±0.286      | 392±72.0           | 21.3±2.60        |
|      |       |                 |                    |                  |
|      |       |                 |                    |                  |
| OLETF| PRD   | 5.41±0.686      | 1,603±385          | 107±19.5         |
|      | 3% MRD| 6.12±0.474*     | 2,461±374**        | 134±15.1**       |
| LETO | PRD   | 4.42±0.340      | 1,642±178          | 87.9±10.4        |
|      | 3% MRD| 4.14±1.00       | 1,856±464          | 106±31.6         |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet. There is a significant difference at a level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.

Table 10. The concentrations of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol and insulin in serum of the OLETF and the LETO rats on the final experimental day.

| Rat  | Group | Triglyceride (mg/dL) | Total cholesterol (mg/dL) | HDL-cholesterol (mg/dL) | LDL-cholesterol (mg/dL) | Insulin (ng/dL) |
|------|-------|----------------------|--------------------------|------------------------|------------------------|-----------------|
| OLETF| PRD   | 326±98.2             | 146±7.45                 | 40.5±2.39              | 40.0±17.4              | 3.55±4.65       |
|      | 3% MRD| 212±40.0*            | 132±8.55**               | 37.0±3.73              | 52.3±6.26              | 1.95±1.01       |
| LETO | PRD   | 113±13.5             | 133±17.5                 | 44.0±6.97              | 66.2±15.0              | 3.14±1.85       |
|      | 3% MRD| 108±10.8             | 121±9.41                 | 39.6±4.98              | 59.6±4.75              | 2.23±1.53       |

Results shown as mean±SD. PRD, polished rice diet; 3% MRD, 3% milled-rice diet. There is a significant difference at the level of **p<0.01 and *p<0.05 between the PRD and the 3% MRD group.

ing a maximum at 60 min and decreasing from 60 to 120 min, but the levels of the rats in the 3% MRD group were lower than those of the rats in the PRD group. However, there was no significant difference in the glucose levels between the PRD and 3% MRD. In both groups of LETO rats, the glucose level indicated the same pattern, and there was no significant difference between the two groups.

Table 7 shows the IAUC-Glc of the OLETF and LETO rats after administration of glucose. The ratio of IAUC-Glc of the 3% MRD group to the PRD group was 0.89 in the OLETF rats, and was 1.08 in the LETO rats. Table 8
that 3% milled-rice is digested and absorbed more efficiently between the two groups during the experimental period.

Fecal excretion of cholesterol and bile acid
Table 9 shows the contents of cholesterol and bile acid in feces of the OLETF and LETO rats having received the PRD or 3% MRD. In the OLETF rats, the contents of cholesterol on the 84th day and the 129th–131st days, and the bile acid on the 129th–131st days were significantly higher in the 3% MRD group than those of the PRD group. In the LETO rats, there were no significant differences in the contents of cholesterol or the bile acid between the PRD and the 3% MRD group.

Concentrations of serum lipids and insulin
Table 10 shows the concentration of triglyceride, total cholesterol, HDL-cholesterol, LDL-cholesterol and insulin of the OLETF and LETO rats on the final experimental day. Within the OLETF rats, the concentrations of triglyceride and total cholesterol of the rats in the 3% MRD group were significantly lower than those in the PRD group. In the other components, there were no differences between the two groups. In the LETO rats, there were no differences in any component analyzed in this study between the PRD and 3% MRD groups.

DISCUSSION
It was indicated that high intake of whole grain foods reduces risk of several chronic disease such as cancer (6), type 2 diabetes (5), hypertension (22) and cardiovascular disease (3, 4). Three percent milled-rice, used for this study, was prepared by removing 3% of the weight of brown rice, and could be expected to have beneficial effects on the metabolism of glucose and lipids. The beneficial effects of 3% milled-rice were examined in the blood glucose and serum lipid levels in OLETF rats, and the results were compared with those obtained from rats receiving polished rice. The same experiments were carried out in LETO rats. In the OLETF rats, the body weight and food intake were significantly lower in the 3% MRD group than those in the PRD group, but there was no difference in the feed efficiency between the two groups during the experimental periods except the 124th–140th days in which the growth curve of the PRD group achieved a plateau. The excretion rate of feces did not differ between the 3% MRD and the PRD group during most of experimental period, but it was significantly higher in the 3% MRD group than the PRD group during the later stage of the experimental period in which the food intake began to decrease in the PRD group. In the LETO rats, almost the same results as for the OLETF rats in body weight, feed efficiency and excretion rate of feces were obtained. It is well known that high intakes of whole grains may increase satiety and reduce energy consumption and then lead to weight loss (23, 24). From these results obtained from the present study, it would be assumed that 3% milled-rice is digested and absorbed more slowly, and may increase satiety and decrease food intake more than polished rice, and, thus, contribute to depress the increase of body weight.

It was reported that diets rich in whole grain foods have been linked with a lower prevalence of the metabolic syndrome (25). In addition, several epidemiologic studies have reported that diets rich in whole grains may protect against the incidence of type 2 diabetes (3, 5), and the mortality from cardiovascular disease (4, 25) and stroke (4). In this study, the fasting blood glucose levels were significantly lower in the 3% MRD groups of both OLETF and LETO rats than that in the PRD group after the middle stage of the experimental period. Moreover, the IAUC-Glc of the rats administered glucose and the rats receiving 1 g of the PRD or 3% MRD were lower in the 3% MRD group of OLETF rats than in the PRD group, and also, the IAUC-Glc in LETO rats receiving 1 g of the PRD or 3% MRD was lower in the 3% MRD group than in the PRD group. These results suggest that 3% milled-rice gradually increases blood glucose through slow digestibility and delays the absorption of carbohydrates. Slow digestion and absorption of starch lead to relatively lower insulin and glucose responses (26).

These protective effects of whole grains are due to the presence of dietary fiber, vitamin E, magnesium, folate, and other nutrients and nonnutrients (8, 27). The 3% milled-rice is richer in the nutritive constituents than the polished rice (Table 1). The fecal excretion of cholesterol and bile acid in the final stage of experimental period and cholesterol excretion on the 84th day were significantly higher in the 3% MRD group of the OLETF rats than those in the PRD group, but they were not different in the LETO rats between the PRD and 3% MRD groups. In addition, the liver weight, and the concentrations of total lipids in the liver and triglyceride and total cholesterol in the liver and serum were significantly lower in the 3% MRD group of the OLETF rats than in the PRD. It is not known whether the decrease in total cholesterol in serum of the OLETF rats which ingested the 3% MRD was due to any other lipoproteins in this study. The previous prospective studies consistently showed that dietary fiber decreases the risk of type 2 diabetes (5, 12, 28, 29) and cardiovascular disease (3, 30). These beneficial effects were particularly found in cereal fiber contained in the bran of whole grains (9, 30–32). On the other hand, high fiber diets reduce plasma lipids (3, 30, 33), and increase cholesterol and bile acid excretion (34, 35). From the present study, it was indicated that the protective effect against the increase in serum lipids, and the accelerated effect of fecal excretion of cholesterol and bile acid result from the constituents in bran remaining in 3% milled-rice, but removed during the refining process. Additionally, it was also indicated that the 3% milled-rice contains a large amount of magnesium. The OLETF and LETO rats receiving the 3% MRD consumed a high amount of magnesium during the experimental period (Table 4). It is reported that magnesium is associated with low insulin concentrations (36, 37), and improves insulin resistance (38).
milled-rice is digested and absorbed more slowly and, thus, contributes to depress the increase of body weight. This slow digestion and absorption of carbohydrates, and the constituents such as cereal fiber and magnesium in the bran of the 3% milled-rice suppress the increase in fasting blood glucose and the IAUC-Glc after diet ingestion, and accelerate the fecal excretion of cholesterol and bile acid, and also reduce the concentrations of total lipids in liver, and triglyceride and total cholesterol in liver and serum. 

In conclusion, 3% milled-rice is digested and absorbed more slowly, and, thus, contributes to depress the increase of body weight. This slow digestion and absorption of carbohydrates, and the constituents such as cereal fiber and magnesium in the bran of the 3% milled-rice suppress the increase in fasting blood glucose and the IAUC-Glc after diet ingestion, and accelerate the fecal excretion of cholesterol and bile acid, and also reduce the concentrations of total lipids in liver, and triglyceride and total cholesterol in liver and serum.

REFERENCES

1) Health and Welfare Statistics Association. 2006. Journal of Health and Welfare Statistics, Vol 53, p 84–88. Kosaido, Tokyo (in Japanese).
2) Isomaa B. 2003. A major health hazard: the metabolic syndrome. Life Sci 73: 2395–2411.
3) Ludwig DS, Pereira MA, Kroenke CH, Hnilé JE, Horn LV, Slattery ML, Jacobs DR Jr. 1999. Dietary fiber, weight gain, and cardiovascular disease risk factors in young adults. JAMA 282: 1539–1546.
4) Liu S, Manson JE, Stampfer MJ, Rexrode KM, Hu FB, Rimm EB, Willett WC. 2000. Whole grain consumption and risk of ischamic stroke in women. JAMA 284: 1534–1540.
5) Jacobs DR Jr, Meyer KA, Kushi LH, Folsom AR. 1998. Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women’s Health Study. Am J Clin Nutr 68: 248–257.
6) Levi F, Pasche C, Lucchini F, Chatenoud L, Jacobs DR Jr, La Vecchia C. 2000. Refined and whole grain cereals and the risk of oral, oesophageal and laryngeal cancer. Eur J Clin Nutr 54: 487–489.
7) Jensen MK, Koh-Banerjee P, Hu FB, Franz M, Sampson L, Gronbaek M, Rimm EB. 2004. Intakes of whole grains, bran, and germ and the risk of coronary heart disease in men. Am J Clin Nutr 80: 1492–1499.
8) Slavin JL, Martini MC, Jacobs DR Jr, Marquart L. 1999. Plausible mechanisms for the protectiveness of whole grains. Am J Clin Nutr 70 (Suppl): 459S–463S.
9) Lairon D, Arnauld N, Bertrais S, Planells R, Clero E, Hercberg S, Bourtron-Ruault MC. 2005. Dietary fiber intake and risk factors for cardiovascular disease in French adults. Am J Clin Nutr 82: 1185–1194.
10) Mozaffarian D, Kumanyika SK, Lemaître RN, Olson JL, Burke GL, Siscovick DS. 2003. Cereal, fruit, and vegetable fiber intake and the risk of cardiovascular disease in elderly individuals. JAMA 289: 1659–1666.
11) McKeown NM, Meigs JB, Liu S, Saltzman E, Wilson PWF, Jacques PF. 2004. Carbohydrate nutrition, insulin resistance, and the prevalence of the metabolic syndrome in the Framingham Offspring Cohort. Diabetes Care 27: 538–546.
12) Hagiwara H, Seki T, Ariga T. 2004. The effect of pre-germinated brown rice intake on blood glucose and PAI-1 levels in streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem 68: 444–447.
13) Reeves PG, Nielsen FH, Fahey GC Jr. 1993. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A Rodent Diet. J Nutr 123: 1939–1951.
14) Kikunaga S, Ishii H, Takahashi M. 1995. The bioavail-

ability of magnesium in spinach and the effect of oxalic acid on magnesium utilization examined in diets of magnesium-deficient rats. J Nutr Sci Vitaminol 41: 671–685.
15) Fujiwara M, Matsui K. 1953. Determination of thiamine by the thiochrome reaction. Anal Chem 25: 810–812.
16) Yagi K. 1966. Chemical determination of flavins. In: Methods of Biochemical Analysis, second printing (Glick D, ed). Vol 10, p 319–356. Interscience Publishers, New York.
17) Nimura T. 1957. Microbiological assay of nicotinic acid, pantothenic acid and biotin. Vitamins 12: 106–114 (in Japanese).
18) Ueda T, Igarashi O. 1987. New solvent system for extraction of tocopherols from biological specimens for HPLC determination and the evaluation of 2,2,5,7,8-pentamethyl-6-chromanol as an internal standard. J Micronutr Anal 3: 185–198.
19) Prosky L, Asp NG, Schweizer TF, De Vries JW, Furda I. 1988. Determination of insoluble, soluble, and total dietary fiber in foods and food products: Interlaboratory study. J Assoc Off Anal Chem 71: 1017–1023.
20) Ishibashi G. 2004. Effect of hyaluronic acid on the levels of serum lipids and cecal microflora in rats. Nihon Kasei Gakkaishi (J Home Econ Jpn) 55: 701–706 (in Japanese).
21) Friedwald WT, Levy RT, Fredrickson DS. 1972. Estimation of the concentration of low density lipoprotein cholesterol in plasma without the use of the preparative ultracentrifuge. Clin Chem 18: 499–502.
22) Reaven GM. 1988. Banting lecture 1988. Role of insulin resistance in human disease. Diabetes 37: 1595–1607.
23) Jenkins DJ, Jenkins AL, Wolfever TM, Collier GR, Rao AV, Thomson LU. 1987. Starchy foods and fiber: reduced rate of digestion and improved carbohydrate metabolism. Scand J Gastroenterol Suppl 129: 132–141.
24) Liu S, Willett WC, Manson JE, Hu FB, Rosner B, Colditz G. 2003. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. Am J Clin Nutr 78: 920–927.
25) Sabhoyun NR, Jacques PF, Zhang XL, Juan W, McKeown NM. 2006. Whole-grain intake is inversely associated with the metabolic syndrome and mortality in older adults. Am J Clin Nutr 83: 124–131.
26) Chiasson JL, Josse RG, Leiter LA, Mihić M, Nathan DM, Palmason C, Cohen RM, Wolfever TMS. 1996. The effect of acarbose on insulin sensitivity in subjects with impaired glucose tolerance. Diabetes Care 19: 1190–1193.
27) Manning PJ, Sutherland WHF, Walker RJ, Williams SM, de Jong SA, Ryalls AR, Berry EA. 2004. Effect of high-dose vitamin E on insulin resistance and associated parameters in overweight subjects. Diabetes Care 27: 2166–2171.
28) Liu S. 2003. Whole-grain foods, dietary fiber, and type 2 diabetes: searching for a kernel of truth. Am J Clin Nutr 77: 527–529.
29) Chandalia M, Garg A, Latjohann D, von Bergmann K, Grundy SM, Brinkley LJ. 2000. Beneficial effects of high dietary fiber intake in patients with type 2 diabetes mellitus. N Engl J Med 342: 1392–1398.
30) Wolk A, Manson JE, Stampfer MJ, Colditz GA, Hu FB, Speizer FE, Hennekens CH, Willett WC. 1999. Long-
term intake of dietary fiber and decreased risk of coronary heart disease among women. JAMA 281: 1998–2004.

31) Jenkins DJA, Axelsen M, Kendall CWC, Augustin LSA, Vuksan V, Smith U. 2000. Dietary fibre, lente carbohydrates and the insulin-resistant diseases. Br J Nutr 83 (Suppl): S157–S163.

32) Meyer KA, Kushi LH, Jacobs DR Jr, Slavin J, Sellers TA, Folsom AR. 2000. Carbohydrates, dietary fiber, and incident type 2 diabetes in older women. Am J Clin Nutr 71: 921–930.

33) Jensen MK, Koh-Banerjee P, Franz M, Sampson L, Gronbæk M, Rimm EB. 2006. Whole grains, bran, and germ in relation to homocysteine and markers of glycemic control, lipids, and inflammation. Am J Clin Nutr 83: 275–283.

34) Scheppach W, Luehrs H, Menzel T. 2001. Beneficial health effects of low-digestible carbohydrate consumption. Br J Nutr 85 (Suppl): S23–S30.

35) Zhang JX, Hallmans G, Andersson H, Bosaeus I, Aman P, Tidehag P, Sterling R, Lundin E, Dahlgren S. 1992. Effect of oat bran on plasma cholesterol and bile acid excretion in nine subjects with ileostomies. Am J Clin Nutr 56: 99–105.

36) Paolisso G, Spambato S, Pizza G, Pussariello N, Varricchio M, D’Onofrio F. 1989. Improved insulin response and action by chronic magnesium administration in aged NIDDM subjects. Diabetes Care 12: 265–269.

37) Paolisso G, Spambato S, Gambardella A, Pizza G, Tesauto P, Varricchio M, D’Onofrio F. 1992. Daily magnesium supplements improve glucose handling in elderly subjects. Am J Clin Nutr 55: 1161–1167.

38) Guerrero-Romero F, Tamez-Perez HE, Gonzalez-Gonzalez G, Salinas-Martinez AM, Montes-Villarreal J, Treviño-Ortiz JH, Rodriguez-Morán M. 2004. Oral magnesium supplementation improves insulin sensitivity in non-diabetic subjects with insulin resistance. A double-blind placebo-controlled randomized trial. Diabetes Metab 30: 253–258.