Water Extract of Defatted Rice Bran Suppresses Visceral Fat Accumulation in Rats

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Rice bran has been reported to inhibit pancreatic lipase activity in vitro. This action shows that administration of rice bran may result in a decrease in plasma triglyceride levels and suppress accumulation of fat in vivo. We administered water extract of defatted rice bran (WED-rice bran) to rats to determine its effects. Single administration of WED-rice bran at a dose of 1 g/kg body weight caused a decrease in plasma triglyceride levels in fat emulsion induced hypertriglyceridemic rats. Four week administration of WED-rice bran suppressed accumulation of visceral fat and body weight gain without influencing food consumption, liver function, and renal function. These results indicate that a reduction of plasma triglycerides and suppression of visceral fat accumulation may be induced by pancreatic lipase inhibition caused by administration of WED-rice bran.

Keywords: *Rice bran; lipase inhibitor; triglycerides; visceral fat*

INTRODUCTION

Threshing rice produces a large quantity of rice bran. Rice bran is utilized as material for oil, animal feed, and fertilizer, but the majority is discarded. Accordingly, we have tried to find an efficient way to utilize rice bran.

Rice bran has been reported to inhibit pancreatic lipase in vitro (Takahashi, 1996). This action indicates that administration of rice bran to animals may cause a reduction in plasma triglycerides and suppress of fat accumulation. However, there are no reports showing that rice bran affects fat accumulation. Hypertriglyceridemia and obesity are risk factors of atherosclerosis (Austin, 1991; Kaplan, 1989). Thus lowering plasma triglyceride levels or suppressing obesity should be of great value in protecting against atherosclerosis. The aim of this study was to ascertain whether rice bran decreases plasma triglycerides and suppresses fat accumulation in rats.

Since rice bran has a very short shelf life, we obtained defatted rice bran by treatment with *n*-hexane for this study. Defatted rice bran is stable in long term. Furthermore, defatted rice bran was extracted by distilled water, and the water extract freeze-dried. This freeze-dried powder of defatted rice bran water extract (WED-rice bran) was used in all studies.

MATERIALS AND METHODS

Materials. Rice bran defatted by using *n*-hexane was obtained from Okayasu Shoten Co., Ltd. (Saitama, Japan). Pancreatic lipase (porcine pancreas, 100 000 units, Type VI-S) was obtained from Sigma, St. Louis, MO. Fat emulsion (Intralipid) was obtained from Otsuka Pharmaceuticals Inc. (Tokyo, Japan).

Preparation of Water Extract of Defatted Rice Bran. Defatted rice bran (1 kg) was added to 10 L of distilled water and stirred for approximately 30 min. Supernatant was obtained via centrifugation (3000 rpm, 10 min). The supernatant was freeze-dried, and this freeze-dried powder (WED-rice bran) was used to all experiments.

Lipase Inhibition Activity of WED-Rice Bran. WED-rice bran was dissolved in distilled water. This solution was then filtrated through a 0.2 μm filter (HLVCDISK25, Kanto Chemicals, Inc., Tokyo, Japan). The filtrated solution was used to determine the lipase inhibition. Pancreatic lipase was dissolved in 2.5% bovine serum albumin−5 mM $CaCl_2$ solution. Lipase activity was measured by a lipase determination kit BMY (turbidity method; substrate, triolein; Tris buffer; pH 9.2, 37 °C; wavelength, 340 nm) from Boehringer Mannheim GmbH (Mannheim, Germany).

Animal Experiments. Male Sprague Dawley rats were obtained at 4 weeks of age from Charles River Japan (Yokohama, Japan) and were used at 6 weeks. The animals were maintained on a 12-hour light/dark cycle at a constant temperature of 23 ± 2 °C. All animal experiments were approved by the local animal ethics committee of Otsuka Pharmaceutical Factory, Inc.

Single Administration of WED-Rice Bran. Rats were stratified by body weight and divided into four groups of 10 rats each. Food was withheld for 15 h. Intralipid was administered orally to rats at 2 mL/kg. WED-rice bran was suspended in water and administered orally to the rats at 0.5 and 1.0 g/mL/kg. The rats in the control group received water alone at 1 mL/kg. Two hours later, blood samples were obtained from the abdominal aorta using a heparinized syringe under ether anesthesia.

Four Week Administration of WED-Rice Bran. Rats were stratified by body weight and divided into two groups of six rats each. Rats were housed individually cages. WED-rice bran group was fed chow (CRF-1; Oriental Yeast, Tokyo, Japan) containing 10% WED-rice bran for 4 weeks. The control group was fed a placebo diet that did not contain WED-rice bran. Contents of WED-rice bran and CRF-1 are shown in Table 1. Body weight and food consumption were recorded weekly. At the end of the experimental periods, the animals were killed by exsanguination under ether anesthesia. Blood samples were collected from the abdominal aorta for plasma biochemical parameter measurements. The visceral fat was removed and weighed.

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Table 1. Contents of WED-Rice Bran and CRF-1 Diets

	WED-rice bran (g/100 g)	CRF-1 (g/100 g)
carbohydrate	54.9	53.5
protein	25.1	23.1
lipid	6.8	5.9
nitrogen	4.2	3.9
ash	11.2	6.5
water	2.0	7.7

Table 2. Effects of WED-Rice Bran on Lipase Activity[a]

WED-rice bran (mg/dL)	lipase activity (IU/L)	WED-rice bran (mg/dL)	lipase activity (IU/L)
0	965	40	636
20	833	80	271

[a] Substrate concentration, 0.3 mM.

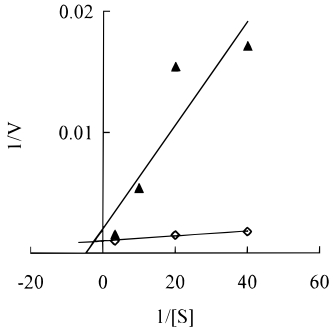

Figure 1. Inhibition of lipase activity by WED-rice bran. Experiments were carried out as described in Materials and Methods. S, concentration of substrate, Triolein (mM); V, velocity, lipase activity U/L: ▲, with WED-rice bran 1.25%; ◇, without WED-rice bran.

Determination of Plasma Biochemical Parameters. Plasma total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), nonesterified fatty acid (NEFA), and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used in the case of TC, the Nescote HDL-C kit N (heparin calcium precipitation; Nippon Shoji, Osaka, Japan) for HDL-C, the NEFA C-test Wako for NEFA, and glucose C-II Wako for glucose. Plasma total protein (TP), albumin (Alb), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine (Cr) were determined with an automatic analyzer (Fuji Drichem, Tokyo, Japan).

Respiratory Quotient (RQ) and Oxygen Consumption (VO_2). The RQ and VO_2 of rats was measured at the end of the 4 week feeding period. Two rats each were placed in glass metabolic chambers with sufficient food and water for 24 h. Air was drawn through the chambers at a rate of 1.5 L/min. Outflow air composition was measured using an automated open-circuit respirometer (OXYCON GAMMA, Fukuda Electrics, Inc, Tokyo, Japan).

Statistical Analysis. The results are expressed as means ± SD. Comparison between two groups was analyzed for statistical significance by Student's *t*-test or Aspin−Welch's *t*-test. Comparisons among more than two groups were analyzed using the one way analysis of variance, followed by the Dunnett's test.

RESULTS

Effects of WED-Rice Bran on Pancreatic Lipase. As shown in Table 2, WED-rice bran inhibited pancreatic lipase activity dose dependently. The inhibition of pancreatic lipase by WED-rice bran was mixed (Figure 1).

Table 3. Effects of Single Administration of WED-Rice Bran on Plasma Lipid Levels[a]

		plasma lipids (mg/dL)	
	n	cholesterol	triglycerides
normal rats	10	45 ± 8	54 ± 8*
intralipid treated rats			
control	10	44 ± 6	63 ± 10
rice bran 0.5 g/kg	10	46 ± 5	61 ± 10
1.0 g/kg	10	44 ± 5	53 ± 10*

[a] Data are expressed as means ± SD ($n = 10$). Significantly different from the value in the respective control rats: $*P < 0.05$.

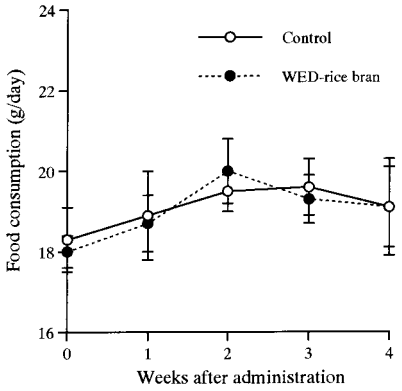

Figure 2. Effects of 4 week administration of WED-rice bran on food consumption in rats. Rats were divided into two group of six rats each. The rats were fed food containing 10% WED-rice bran for 4 weeks. The control group was fed a diet that did not contain WED-rice bran. All rats were fed ad libitum. Data are expressed as means ± SD.

Effects of Single Administration of WED-Rice Bran on Plasma Lipid Levels in Intralipid-Induced Hyperlipidemia Rats. Administration of Intralipid increased plasma triglyceride levels, but did not affect plasma cholesterol levels in fasted rats.

Administration of WED-rice bran at a dose of 1.0 g/kg of body weight decreased plasma triglyceride levels in Intralipid-treated rats. However, WED-rice bran did not affect plasma total cholesterol levels (Table 3).

Effects of 4 Week Administration of WED-Rice Bran on Food Consumption, Body Weight, and Visceral Fat Weight in Rats. There were no differences in food consumption between the WED-rice bran group and the control group (Figure 2).

Body weight changes in the WED-rice bran group and the control group are shown in Figure 3. Body weight gain in the WED-rice bran group was lower than for the control group at 4 weeks.

Visceral fat weight of the WED-rice bran group was lower than for the control group (Figure 4).

Effects of 4 Week Administration of WED-Rice Bran on Biochemical Parameters in Rats. There was no difference between the WED-rice bran group and the control group with regard to plasma TC, HDL-C, TG, NEFA, glucose, AST, ALT, BUN, Cr, TP, and Alb (Table 4).

Effects of 4 Week Administration of WED-Rice Bran on RQ and VO_2 in Rats. There was no difference in RQ (WED-rice bran, 0.884 ± 0.013; control, 0.878 ± 0.016) and VO_2 (WED-rice bran, 0.01082 ± 0.00063 mL/min; control, 0.01083 ± 0.00046 mL/min) between the WED-rice bran group and the control group.

Table 4. Plasma Biochemical Parameters in Rice Bran Treated Rats[a]

	n	TC (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)	NEFA (μequiv/L)	glucose (mg/dL)	AST (U/L)	ALT (U/L)	BUN (mg/dL)	Cr (mg/dL)	TP (g/dL)	Alb (g/dL)
control	6	54 ± 12	49 ± 5	281 ± 31	286 ± 56	130 ± 6	75 ± 16	37 ± 11	26.6 ± 2.3	5.7 ± 0.2	5.9 ± 0.4	4.0 ± 0.4
rice bran	6	53 ± 7	51 ± 4	287 ± 28	281 ± 31	122 ± 12	70 ± 24	32 ± 4	25.8 ± 2.3	5.5 ± 0.4	5.9 ± 0.4	4.2 ± 0.4

[a] Rats were divided into two groups of six rats each. WED-rice bran group was fed chow containing 10% WED-rice bran for 4 weeks. The control group was fed diet that did not contain WED-rice bran. At the end of the experimental, blood samples were collected from abdominal aorta for plasma biochemical parameter measurements. Data are expressed as means ± SD.

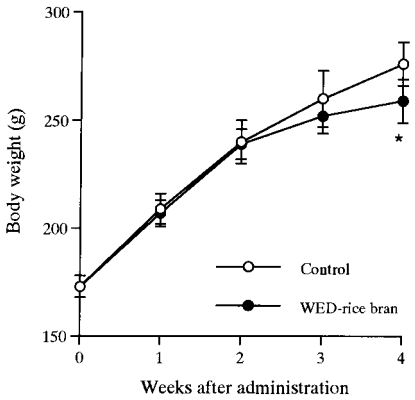

Figure 3. Effects of 4 week administration of WED-rice bran on body weight in rats. Rats were divided into two group of six rats each. The rats were fed food containing 10% WED-rice bran ad libitum for 4 weeks. Data are expressed as means ± SD. Significantly different from the value in the respective control rats: $*P < 0.05$.

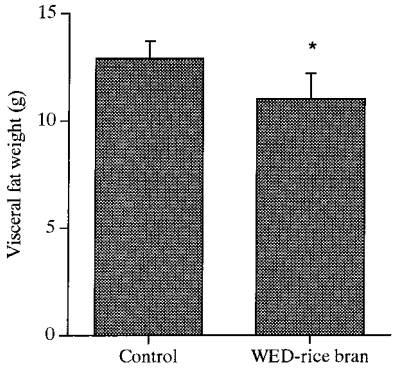

Figure 4. Effects of 4 week administration of WED-rice bran on visceral fat weight in rats. Rats were divided into two group of six rats each. The rats were fed food containing 10% WED-rice bran ad libitum for 4 weeks. Data are expressed as means ± SD. Significantly different from the value in the respective control rats: $*P < 0.05$.

DISCUSSION

Seeds of various plants are rich in fat. Furthermore, these seeds contain lipase inhibitors among others (Satouchi et al., 1974; Kirst et al., 1971). However, the roles of these enzyme inhibitors in their original plant seed have not been elucidated. Rice bran has been reported to inhibit pancreatic lipase (Takahashi, 1996). Rice bran is produced by threshing rice. The majority of rice bran, however, is discarded. We therefore attempted to find an efficient way to utilize rice bran.

Dietary triglycerides are hydrolyzed in the intestine by pancreatic lipase to fatty acids and monoglycerides, which are reesterified to form triglycerides in intestinal mucosal cells. Triglycerides are assembled with other lipids and proteins to form the core of nascent chylomicrons. After completion of assembly in the Golgi apparatus, nascent chylomicrons are secreted into interstitium of intestinal villi and enter lacteals (Scriver et al., 1995). Therefore, inhibition of lipase causes a reduction of plasma triglyceride. If rice bran inhibits lipase activity in vivo, rice bran may be useful as a functional food.

In this study, water extract from defatted rice bran (WED-rice bran) was used. The diet compositions of WED-rice bran did not differ from the ordinary rat diet CRF-1 (Table 1). This indicates that WED-rice bran can be used for breeding rats.

We confirmed in vitro that WED-rice bran possesses a lipase inhibitor. The type of inhibition of WED-rice bran appeared to be mixed inhibition (Murray et al., 1993). However, we have not yet determined the true inhibition type at this time.

Single oral administration of WED-rice bran decreased plasma triglyceride levels in Intralipid-treated rats. This indicates that the lipase inhibitor in WED-rice bran suppresses the hydrolysis of the triglycerides contained in Intralipid and suppresses absorption of these triglycerides.

Four weeks of WED-rice bran administration suppressed body weight gain and visceral fat weight compared to the control group. Food containing 10% WED-rice bran did not affect plasma levels of total cholesterol, TG, TP, Alb, and glucose (indicators of nutritional condition). Furthermore, it did not affect AST and ALT (indicators of liver function) and BUN and Cr (indicators of renal function). These results indicate that suppression of body weight gain and visceral fat weight did not result in malnutrition, hepatic disorder, and renal disorder. There was no difference in RQ and VO_2 between the WED-rice bran group and the control group. These result indicate that WED-rice bran did not affect total energy expenditure. Therefore, administration of WED-rice bran may cause suppression of body weight gain and suppression of accumulation of visceral fat by lipase inhibition.

In this study, we did not determine fecal fat contents. Knowing these data will give us more detailed information on the relation between fat excretion and WED-rice bran. We feel that the purification of the lipase inhibitor from WED-rice bran may be necessary for the treatment of hypertriglyceridemia and obesity in humans. We plan on performing experiments to determine this in the future.

In summary, we confirmed that WED-rice bran possesses a pancreatic lipase inhibitor and suppresses accumulation of visceral fat in rats. Therefore, WED-rice bran may be beneficial for the treatment of hypertriglyceridemia and obesity in humans.

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