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Rice Bran Fractions Improve Blood Pressure, Lipid Profile, and Glucose Metabolism in Stroke-Prone Spontaneously Hypertensive Rats

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Effect of dietary supplementation of two types of rice bran fraction on blood pressure (BP), lipid profile, and glucose metabolism in stroke-prone spontaneously hypertensive rats was studied. Male 4-week-old rats were divided into one group fed the AIN-93M-based control (C) diet and two groups fed diet supplemented with 60 g/kg of Driselase and ethanol fractions (DF and EF, respectively) of rice bran. After 8 weeks feeding, the BP decreased in the DF and EF groups in comparison with the C group ($p < 0.01$). Plasma ACE inhibitory activity, BUN, BUN/creatinine ratio, albumin, triglyceride, and glucose levels were lower in the DF and EF groups than in the C group ($p < 0.01$). Plasma nitric oxide and urinary 8-hydroxy-2′-deoxyguanosine levels were lower in the DF and EF groups than in the C group ($p < 0.01$). Rice bran fractions appear to have a beneficial dietary component that improves hypertension, hyperlipidemia, and hyperglycemia.

KEYWORDS: Rice bran; Driselase fraction; ethanol fraction; blood pressure; nitric oxide; ACE inhibitory activity; 8-OHdG; lipid profile; glucose level.

INTRODUCTION

Hypertension, a manifestation of cardiovascular disease, has continued to be a major cause of morbidity and death. Pharmacological treatment using drugs such as enalapril, losartan, and ramipril has shown that these agents have the capability to act as antihypertensive drugs (*1*−*3*). Their antihypertensive actions are due to their ability to inhibit the angiotensin-1-converting enzyme (ACE).

Presently, much effort is being invested in detecting bioactive components in foods that can contribute to a decreased risk of cardiovascular diseases. There is an increasing interest in the use of dietary antioxidants, including vitamin E, vitamin C, carotenoids, and polyphenols, in the prevention of cardiovascular diseases and their risk factors (*4*). Consumption of various edible plants, including vegetables, fruits, and cereals, has been regarded as a preventive factor against these chronic diseases, and antioxidative constituents present in these foods are important because of their scavenging property.

Among these edibles, rice bran is a byproduct of the rice milling process, and it contains various antioxidants that impart beneficial effects on human health. It is well-known that a major rice bran fraction contains 12−13% oil and highly unsaponifiable components (4.3%). This fraction contains tocotrienol, γ-oryzanol, and β-sitosterol; all of these constituents may contribute to the lowering of the plasma levels of the various parameters of the lipid profile (*5*−*7*). Rice bran also contains a high level of dietary fibers (β-glucan, pectin, and gum) (*8*). In addition, it also contains 4-hydroxy-3-methoxycinnamic acid (ferulic acid), which may also be a component of the structure of nonlignified cell walls. Ferulic acid is a unique bifunctional element that can cross-link heteroxylans in cereal tissues (*9*). Various studies have indicated that purified ferulic acid has a protective effect against β-amyloidal peptide toxicity (*10*); this has been described as a photoprotective (*11*) or antioxidative effect (*12*). It also decreases the blood glucose levels in streptozotocin-induced diabetic rats (*13*) and reduces the blood pressure (BP) in spontaneously hypertensive rats (SHR) (*14*).

A recent report showed that the outer layer fraction of black rice inhibited atherosclerotic plaque formation, lowered the aortic 8-hydroxy-2′-deoxyguanosine (8-OHdG), and decreased malondialdehyde levels in serum and aorta that were induced by hypercholesterolemia in rabbits (*15*). The present experiment was conducted to determine whether rice bran supplementation has physiological effects on spontaneous hypertension from the viewpoint of dietary manipulation by using naturally occurring foods. We used two types of rice bran fraction as a dietary component in the prevention or treatment of hypertension in

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Table 1. Composition of Experimental Diets (g/kg)

ingredients	C	DF	EF
tert-butylhydroquinone	0.008	0.00752	0.00752
L-cystine	1.8	1.692	1.692
choline bitartrate	2.5	2.35	2.35
vitamin mixture	10	9.4	9.4
mineral mixture	35	32.9	32.9
soybean oil	40	37.6	37.6
cellulose	50	47	47
sucrose	100	94	94
casein	140	131.6	131.6
cornstarch	620.69	583.45	583.45
test substance	-	60	60

stroke-prone spontaneously hypertensive rats (SHRSP), a species that has a hereditary predisposition to hypertension and stroke. The results further demonstrate the health-promoting potential of rice bran fraction.

MATERIALS AND METHODS

Materials. Reagent kits for measuring blood urea nitrogen (BUN), creatinine, albumin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C), glucose, and ACE from bovine lung were purchased from Wako Pure Chemical Co. (Osaka, Japan). NO kits (NO_2/NO_3 Assay kit-C II Colorimetric) were purchased from Dojindo (Kumamoto, Japan). Hippuryl-L-histidyl-L-leucine (HHL) and Driselase from *Basidiomycetes* sp. were purchased from Sigma Chemical Co. (St. Louis, Missourie). The 8-OHdG enzyme-linked immunosorbent assay (ELISA) kit was purchased from the Institute for the Control of Aging (Shizuoka, Japan).

Rice Fractions. In this study, we used two types of rice bran fraction as the test substances—the Driselase fraction (DF) and the ethanol fraction (EF). First, 500 g of the outer layer of rice was agitated in 1.0 L of 70% ethanol for 2 h and filtered; this yielded two fractions (the solid and filtered fractions). The DF was derived from the solid fraction that was obtained from the layer of the whole grain of rice (rice bran). Driselase is a commercial plant cell wall-degrading enzyme mixture containing cellulase, xylanase, and laminarinase; however, it is esterase-free. The solid fraction was dried at room temperature and then suspended in 10 mM acetate buffer (500 mL) containing Driselase (0.2 mg/L) from *Basidiomycetes* sp. (Sigma Chemical Co., St. Louis, MO). After overnight treatment with Driselase at 37 °C, the suspension was filtered and finally lyophilized; the final product was the DF. Finally, the EF derived from the filtered fraction was evaporated and then lyophilized.

Animals and Treatment. In this study, we used 4-week-old male SHRSP/Izumo rats of the same body weight that were bred in our laboratory. They were housed in individual stainless steel cages in a room maintained at 23 ± 2 °C with 50% ± 10% humidity and a 12-h light−dark cycle (8:00 pm−8:00 am). The rats had free access to fresh diet and drinking water for 8 weeks. The composition of the AIN-93M-based experimental diets is shown in **Table 1** (*16*), and it was supplemented with 60 g/kg of the DF and EF as the test substances; *tert*-butylhydroquinone (TBHQ), L-cystine, choline bitartrate, and soybean oil were purchased from Wako Pure Chemical Co. (Osaka, Japan). The vitamin and mineral mixtures, cellulose, sucrose, and casein were obtained from Oriental Yeast Co. (Tokyo, Japan). The food intake was recorded every day, and the body weight was measured once a week. The basal diet (C) was supplemented with the DF and EF at a dosage of 60 g/kg, as described by Zhao et al. (*17*). On the fifth day before sacrifice, the rats were transferred to metabolic cages for the collection of urine for 24 h. Urine was filtered and stored at −20 °C until it was required for analysis. After 16 h of fasting, the rats were sacrificed under light diethyl ether anesthesia, blood was collected from the abdominal aorta, and plasma was separated for the estimation of BUN, creatinine, albumin, TC, TG, HDL-C, and glucose.

Blood Pressure (BP) Measurements. The BP was measured once a week using the tail cuff method with a BP meter without warming (MK-2000, Muromachi Kikai, Tokyo, Japan). A minimum of six BP measurements were obtained for each rat. The average value of four consistent readings of systolic BP was regarded as the individual systolic BP.

Table 2. Effect of Diets on Food Intake, Final Body Weight, and FER[a,b]

	C	DF	EF
food intake	24.90 ± 0.41 a	23.42 ± 0.60 b	21.24 ± 0.32 c
final body wtt	255.58 ± 3.68 b	249.40 ± 7.13 b	227.75 ± 4.96 a
FER[c]	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01

[a] Values are given as means ± SEM. [b] Different letters in the same row represent significant difference ($p < 0.05$). [c] Ratio of gram final body weight gain per gram diet that consumed over experimental period.

Biochemical Analysis. Plasma levels of BUN, albumin, TC, TG, glucose, and HDL-C levels were determined by enzymatic colorimetric methods (Wako Pure Chemical Co., Osaka, Japan). LDL-C was calculated according to the Friedewald formula: LDL-C = (TC − HDL-C) − (1/5 × TG). The plasma and urinary levels of creatinine were also determined by enzymatic colorimetric methods (Wako Pure Chemical Co., Osaka, Japan). The ACE inhibitory activity was determined according to the method of Chusman and Cheung (*18*) with slight modifications. One hundred and fifty microliters of 5 mM HHL in borate buffer (100 mM borate and 100 mM NaCl, pH 8.3) was preincubated at 37 °C for 10 min; 60 μL of the sample and 10 μL of ACE (0.5 mU/μL) were added, and the mixture was incubated at 37 °C for 30 min. The content of hippuric acids liberated from HHL by the enzymatic reaction of ACE was spectrophotometrically measured at 228 nm after ethyl acetate extraction. One unit of ACE inhibitory activity was defined as the amount of enzyme that cleaved 1 mol of substrate/min. The plasma and urinary NO levels were determined by the Griess method [NO_2/NO_3 Assay kit-C II (Colorimetric), Dojindo, Kumamoto, Japan]. The plasma and urine NO levels were assayed after ultrafiltration using centrifugal filter devices (Amicon 100 UFC 3 LGC 00, Millipore, Billerica, MA). The absorbance of the samples was measured at 540 nm in a 96-well plate using a Spectra Microplate Autoreader (Bio-Rad Model 680, Hercules, CA).

Analysis of 8-OHdG. The urinary 8-OHdG levels were determined by indirect competitiveELISA. (New 8-OHdG Check; Institute for the Control of Aging, Shizuoka, Japan). The sample was pretreated and assayed according to the instruction manual. The absorbance was measured at 450 nm using a Spectra Microplate Autoreader (Bio-Rad Model 680, Hercules, CA). The concentration of 8-OHdG in the urine samples was interpolated from a standard curve that was drawn with the assistance of logarithmic transformation. The results were expressed as ng/mg of creatinine.

Total Phenolic Content (TPC). The TPC of crude rice bran fractions was determined using the Folin-Ciocalteau reagent (*19*, *20*). The reaction mixture contained 100 μL of crude rice bran at three concentrations, 500 μL of the Folin-Ciocalteau reagent, and 1.5 mL of 20% sodium carbonate. The final volume was made up to 10 mL with pure water. After 2 h of reaction, the absorbance at 765 nm was measured and used to calculate the phenolic contents using gallic acid and catechin as standards. Reactions were conducted in triplicate.

Expression of Results and Statistical Analysis. Statistical analysis was performed by the repeated measurement of one-way analysis of variance (ANOVA) followed by the Fisher's test. A probability of $p < 0.05$ and 0.01 was considered to indicate a significant difference between the means. Values are presented as mean ± SEM.

RESULTS

Food Intake, Body Weight, and Food Efficiency Ratio (FER). The food intake, final body weight, and FER are shown in **Table 2**. Feeding rats on rice bran significantly decreased their food intake. Similar to food intake, the body weight of the EF diet group was significantly lower than that of the DF and C diet groups. However, the FER (**Table 2**) and the relative

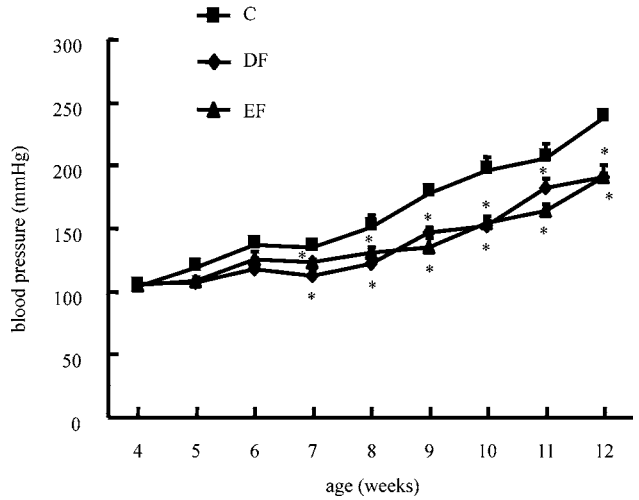

Figure 1. Effect of experimental diets on the systolic blood pressure in rats. Each point represents the mean ± SEM. *Significant difference from control ($p < 0.01$). Key: C (control) diet; DF (Driselase fraction) diet; EF (ethanol fraction) diet.

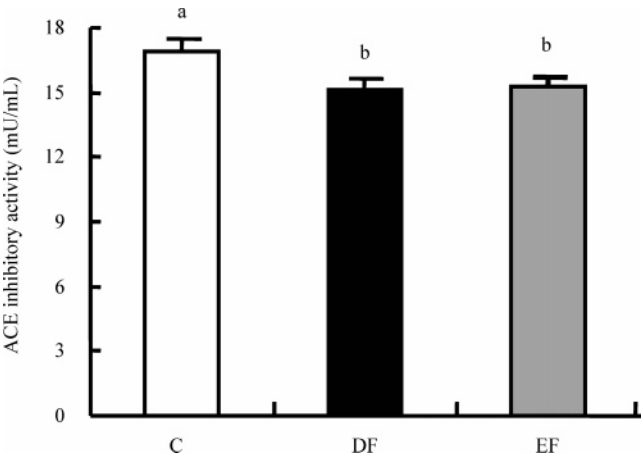

Figure 2. Effect of experimental diets on the plasma ACE inhibitory activity in rats. Each bar represents the mean ± SEM. Different letters represent significant differences ($p < 0.05$). Key: C (control) diet; DF (Driselase fraction) diet; EF (ethanol fraction) diet.

weight of the liver, heart, adipose tissue, and kidney were similar across the three groups (data not illustrated).

Blood Pressure. The major purpose of this study was to clarify whether both the rice bran fractions (DF and EF) have a BP-lowering activity in SHRSP. The systolic BPs of rats fed the experimental diets is illustrated in **Figure 1**. SHRSPs fed the DF- and EF-containing diets showed a significant decrease in the BP when compared with the rats fed the C diet from 7 weeks of age until the end of the experimental period. At the end of the experimental period, the systolic BP was 240.17 ± 2.70, 190.83 ± 2.65, and 190.33 ± 10.50 mmHg for the C, DF, and EF diet groups, respectively.

Biochemical Parameters. Figure 2 shows the ACE inhibitory activity in plasma and indicates that the activity in the DF and EF diet groups was significantly lower ($p < 0.05$) than that in the C diet group. Both the DF and EF diets lowered the ACE inhibitory activity by 10.83% and 9.85%, respectively. **Table 3** summarizes the plasma and urinary levels of the parameters of the lipid profile and kidney functions and the glucose levels. The plasma TC, LDL-C, and TG levels in the DF diet group were significantly lower than those in the C diet group, and there was no difference in the TC and LDL-C levels between the EF and C diet groups. The average HDL-C/LDL-C ratio in the DF diet group was higher than that in the other diet groups, although there was no significant difference. At the end of the experiment, in comparison with the C diet group, the fasting plasma glucose concentrations in both the DF and EF diet groups were lower by 20.46% and 26.08%, respectively ($p < 0.05$). Both of the supplemented diets showed a significant reduction in the plasma BUN, BUN/creatinine ratio, and albumin levels. The urinary creatinine levels were significantly lower in the EF diet group, but there was no difference between the DF and C diet groups. However, the same urea concentration level was observed in rats fed on the three diets.

NO and 8-OHdG Levels. The plasma and urinary NO levels of rats fed the experimental diets are shown in **Figure 3**. The plasma NO levels of the rats in the DF and EF diet groups were significantly lower than that of rats in the C diet group; however, there was no significant difference in urinary NO excretion across the groups. Urinary 8-OHdG has been widely used as a sensitive marker of the in vivo oxidative DNA damage and total systemic oxidative stress. **Figure 4** shows the effect of the C, DF, and EF diets on the urinary 8-OHdG level. The rice bran fraction-containing diet significantly decreased the urinary 8-OHdG levels when compared with the C group diet.

Total Phenolic Content. The TPC of the samples of both the rice bran fractions was reported as gallic acid equivalent (GAE) and catechin equivalent (**Table 4**). The EF diet had a higher phenolic content than the DF diet.

DISCUSSION

The main purpose of the present study was to determine the effect of the rice bran fraction-supplemented diet on the BP, lipid profile, and glucose levels of SHRSP/Izumo. Indeed, we also determined the kidney function, ACE inhibitory activity, NO level, and urinary 8-OHdG level as markers of oxidative stress. In addition, we attempted to determine that phenolic content of the rice bran fraction contributed to the decrease in the BP, lipid profile, and glucose levels. In the present study, we showed that rice bran fractions could decrease the BP, plasma ACE activity, BUN, BUN/creatinine ratio, albumin, TG, and glucose levels. Further, we showed that rice bran fractions decreased the plasma NO and urinary 8-OHdG levels. In this study, we have used SHRSP as an animal model of hyperlipidemia (*21*, *22*), severe hypertension, multisystem end-organ damage with prominent involvement of the kidney (proteinuria) (*23*), and higher oxidative stress in the brain (*24*).

In comparison with the C diet, feeding the EF diet resulted in a decrease in the food intake and body weight (**Table 2**). However, there are no significant differences with regard to the FER and relative weight of the liver, heart, adipose tissue, and kidney (mg/100 g) across the three groups. The calculated FER refers to the ratio gram final body weight gain to gram diet consumed over the experimental period. Since no significant difference was observed with regard to the FER across the three groups, we considered that significant difference in the food intake, due to variation in the rat's appetites for rice bran, and its effect on body weight would not be contributing factors to the biochemical parameters and BP.

Hypertension is one of the risk factors for stroke, coronary heart disease, and renal vascular disease. The present study showed that the development of BP was significantly inhibited in SHRSP consuming the rice bran fraction-containing diet. The BP progressively increased over time during the feeding period; we observed that the rice bran fraction-containing diet significantly decreased the BP from 3 weeks of feeding to the end of

Table 3. Effect of Diets on Plasma and Urinary Biological Parameters[a,b]

biochemical parameter	C	DF	EF
plasma			
total cholesterol (mg/dL)	70.83 ± 2.80 a	50.91 ± 1.58 b	62.65 ± 6.45 a
HDL cholesterol (mg/dL)	22.81 ± 3.91	21.57 ± 1.27	20.93 ± 3.10
LDL cholesterol (mg/dL)	34.63 ± 3.73 a	19.48 ± 0.90 b	32.48 ± 7.64 a
HDL-C/LDL-C	0.73 ± 0.16	1.12 ± 0.10	0.82 ± 0.23
triglyceride (mg/dL)	66.97 ± 1.75 a	49.33 ± 2.85 b	46.20 ± 5.51 b
glucose (mg/dL)	201.09 ± 17.02 a	159.94 ± 5.24 b	148.65 ± 4.29 b
BUN (mg/dL)	14.81 ± 1.30 a	11.52 ± 0.93 b	11.41 ± 0.97 b
creatinine (mg/dL)	0.88 ± 0.04	0.87 ± 0.02	0.87 ± 0.05
BUN/creatinine	16.72 ± 1.10 a	13.31 ± 1.10 b	13.12 ± 0.98 b
albumin (mg/dL)	3.38 ± 0.08 a	2.67 ± 0.11 b	2.90 ± 0.13 b
urine			
creatinine (mg/dL)	11.14 ± 1.16 a	8.76 ± 0.57 ab	7.68 ± 0.65 b
urea (g/dL)	0.28 ± 0.03 ab	0.29 ± 0.0 a	0.23 ± 0.01 b

[a] Values are given as means ± SEM. [b] Different letters in the same row represent significant difference ($p < 0.05$).

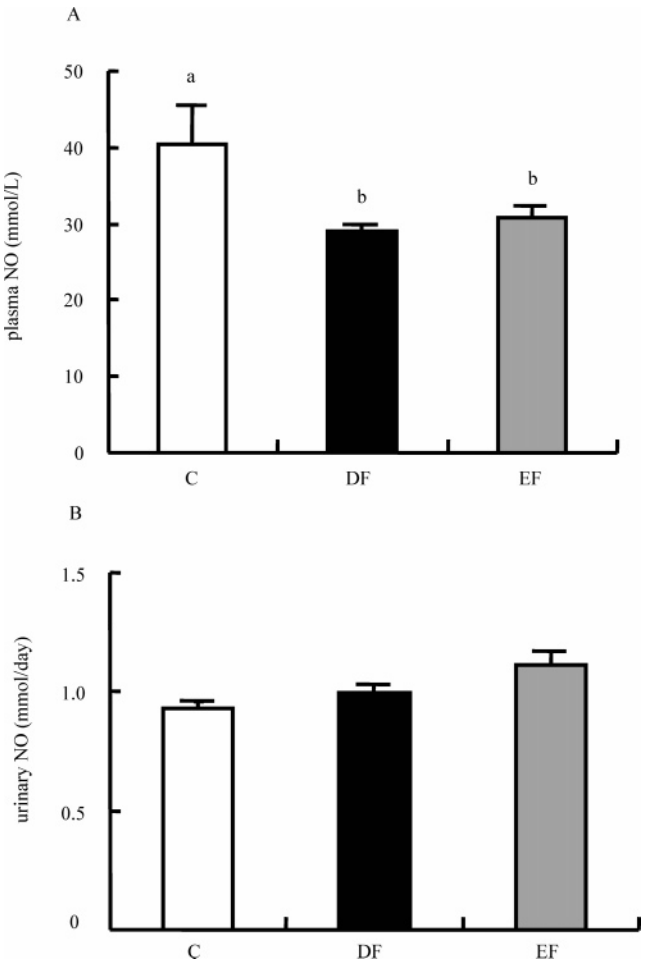

Figure 3. Effect of experimental diets on the plasma (A) and urinary (B) NO levels in rats. Each bar represents the mean ± SEM. Different letters represent significant differences ($p < 0.05$). Key: C (control) diet; DF (Driselase fraction) diet; EF (ethanol fraction) diet.

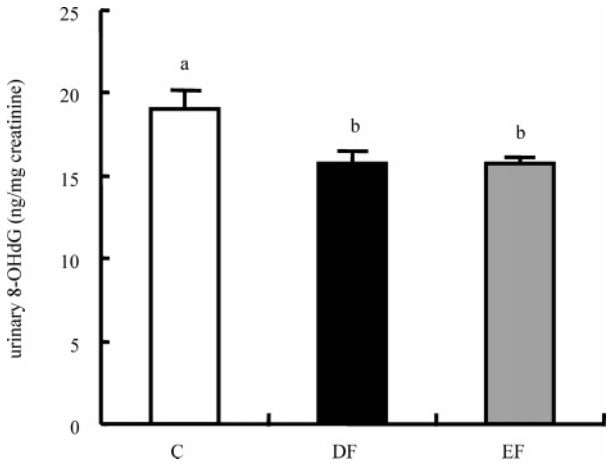

Figure 4. Effect of experimental diets on the urinary 8-OHdG levels in rats. Each bar represents the mean ± SEM. Different letters represent significant differences ($p < 0.05$). Key: C (control) diet; DF (Driselase fraction) diet; EF (ethanol fraction) diet.

Table 4. Total Phenolic Content of Rice Bran Fraction[a]

rice bran	GAE[b] (mg/g)	CE[c] (mg/g)
DF	64.74 ± 0.90	62.61 ± 0.90
EF	139.60 ± 2.21	137.47 ± 2.74

[a] Values are given as means ± SEM. [b] Gallic acid equivalent. [c] Catechin equivalent.

experiment (**Figure 1**). At the end of the experimental period, a 50-mmHg decrease in BP was observed in both the EF and DF diet groups when compared with the C diet group. The present results clearly demonstrate that administration of rice bran fractions decrease the BP. This is the first report that showed that rice bran has the potential to decrease the BP in rats. The ferulic acid content in both the rice bran fractions might possibly be a factor responsible for the decrease in the BP in SHRSP (*14*). Our research group has analyzed the ferulic acid content in the DF and EF (0.19 and 9.12 mg/100 g samples, respectively). Recently, it has been reported that rice bran contains approximately 0.1% ferulic acid (*10*); ferulic acid is known to be a type of phenolic acid compound distributed in cereals and plants. Another study has reported that when free ferulic acid (50 mg/kg) was orally administered to SHR, the BP was lowest after 1 h, and the values returned to basal levels at 6 h (*14*). In addition, when SHR were administered 10 or 50 mg/kg/d ferulic acid in the control diet for 6 weeks, the BP decreased significantly in comparison with that of SHR administered only the control diet (*14*).

Several specific ACE inhibitors such as captopril and enalapril have proved to be useful as antihypertensive drugs; however, several adverse side effects have been reported. In recent years, many ACE inhibitory peptides from food protein sources have been isolated and reported, such as Indonesian dried-salted fish (*25*), sardine muscle (*26*), tofuyo fermented soybean food (*27*), and porcine skeletal muscle (*28*). In addition, there have also

been a few reports on the nonpeptidyl ACE inhibitors present in foods such as mushroom (*29*), ashitaba (*30*), and garlic (*31*). The in vitro analysis conducted in the present study has indicated that the antihypertensive effect of rice bran was due to the inhibition of ACE inhibitory activity in plasma. Although it was an in vitro analysis, we believe that the ACE-inhibitory substances in both the rice bran fractions could be responsible for the decrease in BP; this observation is in agreement with the results obtained with the nonpeptidyl ACE-inhibitory substance in garlic (*31*). Moreover, we investigated the effect of both the rice bran-derived DF and EF on the kidney function in SHRSP. The kidney is the main target organ for hypertensive damage (*32*). Therefore, it is worthwhile to evaluate whether rice bran exerts a protective effect against the development of the kidney disease. The results of the present study showed that administration of both the rice bran fractions significantly ameliorated the kidney function parameters such as the plasma BUN, albumin, total protein, and BUN/creatinine levels (**Table 3**). Based on these findings, it is suggested that both the crude rice bran fractions may have antihypertensive effects in SHRSP that are due to amelioration of the kidney function.

Oxidative stress plays an important role in the initiation and progression of cardiovascular diseases, including hypertension, type II diabetes, hypercholesterolemia, atherosclerosis, and heart failure (*33*). Reactive oxygen species (ROS) are ubiquitous and occur in many organs, and these arise from both exogenous and endogenous sources. Excessive production of ROS is implicated in the development of hypertension or coronary heart disease at different stages, including vascular endothelial cell damage, foam cell formation, vascular smooth muscle cell proliferation, and gene expression of blood pressure-related factors, impaired vasomotor reactivity, and plaque instability *(4*, *34*). In the present study, administration of both the rice bran fractions resulted in lower plasma NO concentrations than that in the C diet group (**Figure 3**). Several studies have reported that potent antioxidant therapy for ameliorating hypertension lowered urinary NO metabolite excretion and that increases in $O_2^{\bullet -}$ generation in SHRSP could contribute to the decreased availability of basal NO (*35*, *36*). The results presented here did not show a significant difference with regard to the urinary NO levels. However, another study has shown significant differences in the urinary NO levels after feeding SHRSP with black and green tea polyphenol for 3 weeks (*37*). The systolic and diastolic BP as well as NO levels was significantly lower in the black and green tea polyphenol-fed group (*37*). Indeed, our results showed that urinary 8-OHdG increased in the C diet group; after 8 weeks of the DF and EF administration, the urinary 8-OHdG level was significantly decreased (**Figure 4**). Urinary 8-OHdG has been reported to serve as a sensitive biomarker of both oxidative DNA damage and oxidative stress. In another study (*15*), supplementation with the outer layer of black rice significantly lowered the aortic 8-OHdG when compared with administration of a high-cholesterol diet, and the level with the supplemented diet was the same as that of the normal diet. Our data indicated that the supplementation of rice bran fractions decreased oxidative stress (**Figure 4**). Since oxidative stress plays an important role in the development of cardiovascular diseases, the decreased DNA oxidative damage due to rice bran supplementation could contribute to the inhibition of the progression of hypertension in SHRSP.

It has been established that elevated plasma TC, specifically LDL-C, is a factor associated with atherosclerosis and cardiovascular diseases. The results of our experiment confirmed that oxidative damage occurs in parallel with the progression of hyperglycemia and hyperlipidemia. The results of the present study showed that feeding with either of the rice bran fraction contributed to a reduction in the fasting plasma glucose levels in SHRSP (**Table 3**). Moreover, a significant decrease in the plasma TC, LDL-C, and TG was observed in the DF diet group when compared with the C diet group; the effect of the DF diet was better than that of the EF diet. The mechanism that underlies the hypolipidemic action of rice bran remains unknown. The lipid-lowering properties of rice bran and its bioactive compounds have been demonstrated by a number of investigators (*5*−*7*, *38*). Tocotrienol, one of the major compounds present in rice bran, is effective in lowering the serum TC and LDL-C levels and acts by inhibiting the activity of the hepatic enzyme β-hydroxy-β-methylglutaryl coenzymeA (HMG-CoA) reductase through a posttranscriptional mechanism (*39*). The high urinary 8-OHdG and plasma albumin levels in the C diet group investigated in this study suggested that increased oxidative stress has a primary role in the pathogenesis of diabetes. The urinary 8-OHdG level was significantly increased in obese diabetic KKAy mice in comparison with C57BL mice, which were nondiabetic mice (*40*). Recent reports have revealed an increased systemic oxidative stress in diabetic patients and in diabetic animal models (*41*). Antioxidants such as tocopherol, tocotrienol, γ-oryzanol, and polyphenols and their components are present in both of the rice bran fractions and might be able to maintain glucose levels by exerting their effect on glucose absorption, utilization, and excretion. These compounds are free radical scavengers and can reduce the complications of diabetes (*6*, *8*, *41*). From this viewpoint, the results of our study clearly demonstrated that both of the rice bran fractions can decrease the urinary 8-OHdG levels.

Phenolic compounds were considered as a major group of substances that contribute to the antioxidant activities of these fractions. The phenolic contents of the DF and EF were 64.74 and 139.60 mg/g GAE, respectively, and 62.61 and 137.47 mg/g of catechin equivalent, respectively; these values are higher than the reported TPC of some commercial varieties of rice bran available in Pakistan (*43*). The presence of antioxidant properties in rice has already been reported in relevant studies (*44*−*46*). The antioxidant properties of the aqueous ethanol fractions of rice bran, the role of dietary pigmented rice in providing protection against lipid peroxidation in rat kidneys, and suppression of ROS by pigmented rice in an in vitro assay have been reported (*47*).

We used two rice bran fractions that were derived from the outer layer of rice after ethanol extraction or enzymatic treatment with Driselase. Rice bran is composed of the aleurone layer of the rice kernel and some part of the endosperm and germ and rich sources of protein, lipid, vitamin, and trace minerals, including antioxidants such as tocopherol, tocotrienol, γ-oryzanol, polyphenol (*5*, *8*). The synergistic effect of bioactive components contained in the DF and EF is responsible for lowering the levels of the parameters studied here. We determined that EF contained a large amount of the lipid component of rice bran, whereas the DF contained the nonlipid components. To date, we have determined that ferulic acid and TPC present in both the fractions contributed to the improved BP, lipid profile, and glucose metabolism in SHRSP. Ferulic acid is a ubiquitous plant constituent present in seeds and leaves in its free form and is covalently linked to lignin and other biopolymers through its phenolic nucleus, which accounts for its potent antioxidant properties (*48*). Since the decrease of BP (**Figure 1**), plasma lipid, and glucose levels (**Table 3**) by both the DF and EF diets was the same in this study, future studies

are required for elucidating the detailed mechanism underlying these similarities.

The present data suggest that our rice bran fractions are excellent functional food derived from plants and can be used for the purpose of dietary manipulation by naturally occurring foods, in the absence of therapeutic agents. In addition, we showed for the first time that the DF of rice bran prevents BP, hyperlipidemia, and hyperglycemia. Further, we described the effects of various other components of rice bran and determined whether they were as beneficial as the unsaponifiable component of rice bran. Future studies will elucidate the detailed mechanism by which rice bran fractions regulate hypertension and lipid metabolism at the level of gene expression.

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NOTE ADDED AFTER ASAP PUBLICATION

The original posting of January 27, 2006, contained an error in Figure 1. This has been corrected with the posting of February 7, 2006.

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