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Cholesterol-lowering Efficacy of Unrefined Bran Oil from the Pigmented Black Rice (*Oryza sativa* L cv. Suwon 415) in Hypercholesterolemic Rats

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Abstract The effects of dietary supplementation of the unrefined rice bran oil from 'Suwon 415' pigmented black rice (BRBO) on cholesterol metabolism and cellular antioxidant status were investigated in hypercholesterolemic rats. The significant reduction of total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) concentrations was observed in the plasma of rats fed BRBO. BRBO also decreased plasma and hepatic oxidative stress as a result of increased levels of hepatic thiobarbituric acid reactive substances (TBARS) levels associated with the elevations of hepatic superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities together with increased plasma level of tocopherol. This study indicates that dietary BRBO supplement can leads to the improvement of overall cholesterol metabolism and antioxidant status even more effectively than 'Chuchung' white rice (WRBO). Consumption of BRBO may also protect the liver from oxidative damage caused by lipid peroxidation.

Keywords: rice bran oil, pigmented black rice, hypercholesterolemic action, antioxidant status, lipid peroxidation

Introduction

Cardiovascular disease remains the principal cause of death in countries in the West world and through much of Asia (1). Elevated levels of plasma total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) are among the risk factors strongly associated with the incidence of cardiovascular disease (2). In particular, LDL-C has a significant impact on the pathogenesis of atherosclerosis and atherothrombotic cardiovascular events. Recent studies indicated that LDL is subjected to progressive oxidation and is converted to oxidized-LDL, which then interacts with reactive oxygen species (ROS) generated as byproducts of aerobic respiration process (3). Many studies have demonstrated that oxidized-LDL play various roles in the development of atherosclerosis including the promotion of foam cell formation and induction of vascular endothelial cell damage (4). A number of studies have shown that oils containing particular saturated fatty acids elevate plasma TC, particularly LDL-C, whereas those enriched in unsaturated fatty acids lower LDL-C (5,6).

Recently there has been a renewed interest in the lipid components of dietary oils besides triglycerides, especially after the demonstration of the hypochloesterolemic capacities of rice bran and rice bran oil (7-11). Although rice bran oil is not commonly consumed worldwide, it is believed to be a health food staple in some societies (9). The main components of rice bran oil are reported to be unsaturated fatty acids, triterpene alcohols, phytosterols, tocotrienols, and α-tocopherol. Compared with other highly refined dietary vegetable oils, rice bran oil has been known to contain greater amounts of unsaponifiable, non-triglyceride components such as plant sterols, oryzanol, and tocotrienols, as well as higher levels of unsaturated fatty acids including oleic and linoleic acids (12,13). Several reports demonstrated the cholesterol-lowering effects of rice bran oil by feeding the extracted unsaponifiable fraction alone to animals including primates (5,10,11,14,15). A number of studies performed either *in vitro* or *in vivo* demonstrated that γ-oryzanol, exerts a strong hypolipidemic action compared with unsaturated fatty acid, i.e., the capacity of rice bran oil to lower plasma TC and LDL-C is much greater than predicted upon its fatty acid composition (5,10,11).

Anthocyan-rich pigmented rices species are known as an ancestor type of currently cultivated white rice. White rice (*Oryza sativa* L) is thought to have been developed as a yellowish white mutant through artificial selection over a long period (16). It has been reported that pigmented rices exhibit a more vigorous germinating ability probably due to various physiologically active substances including antioxidants in its bran layer including oryzanol and tocopherol (17,18), plus some pigment molecules such as cyanidin-3-*O*-β-D-glucoside and peonidin-3-*O*-β-D-glucoside, respectively (19). Despite the emerging possibility of using bran oil derived from pigmented rice for predicted hypocholesterolemic effect, there have been no published *in vivo* studies of its hypocholesterolemic potential.

Therefore, the cholesterol-lowering effect of bran oil from pigmented rice on lipid metabolism in rats was evaluated. Rice bran oil from the pigmented black rice, 'Suwon 415', was fed to rats in order to compared the effects of feeding the unrefined bran oil from 'Suwon 415' pigmented black rice (BRBO) on their homeostatic cholesterol metabolism to unrefined rice bran oil derived from 'Chuchung' white rice (WRBO).

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Materials and Methods

Rices and rice bran oil 'Chuchung' white rice, a representative ordinary white rice cultivar, was purchased from at a commercial market (Daegu, Korea). 'Suwon 415', a pigmented black rice cultivar, was obtained as a kind gift of the National Institute of Crop Science, Rural Development Administration (Suwon, Korea).

The unrefined rice bran oil was obtained by hexane extraction of rice bran. Rice bran oil was extracted by refluxing the rice bran with 5 volume of hexane at a mean temperature of 80°C. The hexane was removed from the oil by vacuum distillation with a rotary evaporator. One % butylated hydroxytoluene (BHT), was added to the extracted oil and stored at 4°C untill use.

Animals and diets Thirty male Sprague-Dawley rats (3 weeks old) were purchased from Orient Inc. (Seoul, Korea). Animals were individually housed in stainless steal cages in a room maintained at 20-22°C and 50±10% relative humidity under a 12-hr light and dark alternate cycle. All the rats were fed a commercial chew diet and sterile water *ad libitum*. After 7 days acclimation, the rats were randomly divided into 3 groups (n=10 each), for use in experimental. Three experimental diets were used in this study. The experimental diet for the control group was the same as the basal hypercholesterolemic diet containing 1% cholesterol, based on the AIN-93 standard laboratory diet. The experimental diets for the 'Chuchung' and 'Suwon 415' groups contained 10 weight% each of physically refined bran oil from 'Chuchung' white rice and 'Suwon 415' pigmented black rice in place of soybean oil (Table 2). Table 1 shows the approximate fatty acid contents of the WRBO and BRBO. Food consumption and body weight gain were measured both daily and weekly for all groups.

Tissue preparation At the end of the 6-week experimental period, the rats were anesthetized with intraperitoneal injection of 1% ketamine hydrochloride (2 μL/g) following a 12 hr fast and subsequent weighing. Sacrificed animals were bled with heparinized syringes from their abdominal aorta, and plasma was obtained by centrifugation at 1,500 ×g at 4°C for 20 min. Livers and kidneys were removed and perfused with cold physiological saline. The excised organs were blotted dry, and then weighed. All samples prepared were stored at −70°C until analyzed. This experimental design was approved by the Committee of Kyungpook National University for the care and use of laboratory animals.

Table 1. Fatty acid profiles of dietary oils (%)

Fatty acid		WRBO[1)]	BRBO[2)]
C14:0	Tetradecanoic acid	0.15	0.74
C16:0	Palmitic acid	19.12	20.09
C18:0	Stearic acid	2.21	3.70
C18:1(*n*-9)	Oleic acid	33.02	38.79
C18:2(*n*-6)	Linoleic acid	38.02	28.15
C18:3(*n*-3)	α-Linolenic acid	1.73	1.26
C20:0	Arachdonic acid	3.03	4.67
C20:1	Eicosanoic acid	0.53	0.57

[1)]Rice bran oil prepared from 'Chuchung' white rice.
[2)]Rice bran oil prepared from 'Suwon 415' pigmented black rice.

Measurement of plasma and hepatic lipids The plasma neural lipid (triglyceride, TG) was determined enzymatically using a commercial kit (Asan Pharmaceuticals, Seoul, Korea) based on a modification of the lipase-glycerol phosphate oxidase method (20). The plasma cholesterol (total cholesterol, TC) and high density lipoprotein cholesterol (HDL-C) level were determined using a commercial kit (Asan Pharmaceuticals) based on a cholesterol oxidase method and heparin-$MgCl_2$ precipitation method, respectively, with slight modification (21,22). The plasma LDL-C content was determined through Fridewald's formula calculated upon previously measured values of TG, TC, and HDL-C (23). Spectrometric measurement for plasma glutamic oxaloacetic transaminase (GOT) and glutamic pyruvic transaminase (GPT) activity was also performed using a commercial kit (Asan Pharmaceuticals). The hepatic lipids were extracted following a method described by Folch *et al.* (24). Sufficient volumes of chloroform: methanol mixture (2:1, v/v) was added to frozen liver samples (20 mL/g) to homogenize the tissue. The resultant solvent-extracted fraction was filtrated, and then vacuum dried to produce an exsiccated pellet, which was then weighed to find total hepatic lipid content. To determine the levels of neutral lipid and total cholesterol, ethanol-dissolved exsiccated lipid pellet were analyzed using the commercially available kits (Asan Pharmaceuticals).

Measurement of plasma and hepatic lipid peroxides Plasma and hepatic concentrations of thiobarbituric acid reactive substances (TBARS) were measured to measure lipids peroxidation. For plasma TBARS, the method of Turpeinen *et al.* (25) was used with slight modification. Briefly, 3 mL of trichloroacetic acid and 1 mL of 60 mM TBA was added to 0.5 mL of plasma. Following the incubation at 80°C for 90 min, the mixture was centrifuged at 1,100×g for 15 min to collect the supernatant. the resultant TBARS in supernatants was measured by absorption at 530 nm to express its quantity as malondialdehyde (MDA) equivalent by comparison to a standard TBARS curve using an MDA standard solution. The hepatic TBARS was determined according to the method of Ohkawa *et al.* (26). Five-tenth g of liver tissues was homogenized in a 9-fold volume of 10 mM phosphate buffer (pH 7.0). Then 0.5 mL of the homogenate was mixed with 3 mL of 1% phosphoric acid and 1 mL of TBA, followed by heating in boiling water for 45 min. Addition of 4 mL of *n*-butanol was then added and the mixture was subsequently centrifuged at 2,500×g for 10 min to produce an *n*-butanol layer. TBARS concentration was then determined by measuring the absorbance of the *n*-butanol layer at 532 nm using a spectrophotometer.

Measurement of plasma vitamin A and E contents Plasma retinol and α-tocopherol concentrations were determined by high performance liquid chromatography (HPLC) based on the method of Bieri *et al.* (27). Briefly, 200 μL of plasma was mixed with each 100 μL volume of retinol acetate (20 μg/mL) and tocopheryl acetate (100 μg/

Table 2. Composition of experimental diets (%)

Ingredients	Control	WRBO[1)]	BRBO[2)]
Corn starch	49	49	49
Casein	20	20	20
Sucrose	10	10	10
Soybean oil	10	-	-
Rice bran oil	-	10[3)]	
Pigmented rice bran oil	-	-	10[3)]
Cellulose	5	5	5
Mineral mix.[4)]	3.5	3.30	3.33
Vitamin mix.[5)]	1	1	1
L-Cystin	0.3	0.3	0.3
Choline bitartate	0.25	0.25	0.25
tert-Butylhydroquinone	0.0014	0.0014	0.0014
Cholesterol	1	1	1
Energy (kJ)	1,699.3	1,699.3	1,699.3

[1)]Rice bran oil prepared from 'Chuchung' white rice.
[2)]Rice bran oil prepared from 'Suwon 415' pigmented black rice.
[3)]Contains 1% cholesterol plus each of 10% rice bran oil, respectively.
[4)]Mineral mixture according to AIN-93 (Harlen Teklad, Madison, WI, USA).
[5)]Vitamin mixture according to AIN-93 (Harlen Teklad, Madison, WI, USA).

mL) as internal standards. The plasma lipids were fractionated by extraction with HPLC-grade hexane. The fractionated layer was filtered through 0.45 μm nylon filter into a vial, followed by centrifugation to separate phase containing lipids. Finally, the lipid extract was obtained by drying with nitrogen gas. The resultant extract was dissolved in chromatographic solvent (ether:methanol, 1:1, v/v), and applied to the HPLC apparatus (Waters, Milford, MA, USA) with a C_{18} Bondapak micro column (3.9×300 mm). The mobile phase was a mixture of methanol and water (95:5, v/v), and retinol and α-tocopherol were measured with absorption at 280 nm.

Determination of antioxidant enzyme activities Preparation of the crude hepatic antioxidant enzymes was initiated by homogenizing liver tissues in 0.25 M sucrose. The homogenates were centrifuged at 600×g for 10 min and then consecutively at 10,000×g for 1 hr to recover the mitochondrial fraction for catalase (CAT) assay. The remaining supernatants were re-centrifuged at 105,000×g for 1 hr to recover the cytoplasmic fraction for superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) assays. Catalase activity was determined using the method of Abei (28). The enzyme activity was calculated from the change in absorbance at 240 nm for 5 min following the addition of 10 μL of crude enzyme into 3 mL reaction mixture (1 mM H_2O_2 in 10 mM phosphate buffer, pH 7.0) preincubated at 25°C for 5 min. SOD activity was determined according to the method of Marklund and Marklund (29). Ten μL of crude enzyme fraction was added to 4 mL reaction mixture consisting of 7.5 mM H_2O_2 and 37.5 mM potassium phosphate buffer (pH 7.0), then incubating at 25°C for 5 min. One unit of the enzyme activity was defined as the enzyme amount necessary for 50% inhibition in autoxidation of pyrogallol. The GSH-Px activity was determined using the method of Paglia and Valentine (30) with slight modification. In brief, 100 μL crude enzyme was added to the 250 μL reaction mixture (6 mM GSH, 1.2 mM NADPH, and 1.25 μM H_2O_2 in 20 mM Tris-HCl, pH 7.0) preincubated at 25°C for 5 min. After incubation at 25°C for 5 min, absorbance at 340 nm was read to calculate the reduction rate of absorbance for NADPH coupled with generation of oxidized form of glutathione (GSSG). The activity was expressed as micromole of oxidized NADPH per min per μL of the crude enzyme solution.

Statistical analysis All data are presented as the mean± SD. The data were evaluated by one-way ANOVA using a SPSS program, and the differences between the means assessed using Duncan's multiple range test. Statistical significance was considered at $p<0.05$.

Results and Discussion

Food intakes, body weight, and organ weight There was no significant differences in the food intakes and body weight gain between the groups (Table 3). The addition of BRBO or WRBO had no significant effect on organ weights relative to those of the hypercholesterolemic controls.

Plasma and hepatic lipids composition Table 4 shows the lipid profiles at the conclusion of the 6-week experiment. Plasma TG levels did not differ between all groups. Plasma TC levels in rats fed the BRBO diet were significantly lower than for rats fed a high cholesterol diet. This difference in plasma TC appeared to be direct result of an 18% decrease in LDL-C in BRBO group compared with controls. Moreover, the plasma HDL-C level was about 21% higher in the BRBO group compared with controls. The plasma TC, LDL-C, and HDL-C levels in rats fed

Table 3. Effect of rice bran oil supplements on food intakes, weight gains, and organ weight in high cholesterol fed rats[1)]

	Control	WRBO[2)]	BRBO[3)]
Food intake (g/day)	22.08±0.55[NS]	22.06±0.71	23.01±0.51
Body weight gain (g/day)	7.04±0.18[NS]	7.07±0.27	6.98±0.21
Liver (g)	20.59±0.72[NS]	20.16±0.91	20.90±0.78
Kidney (g)	3.16±0.17[NS]	3.11±0.14	2.89±0.06
Heart (g)	1.43±0.03[NS]	1.42±0.04	1.39±0.05

[1)]Values are expressed as mean±SD (n=10);[NS]no significance between the groups.
[2)]Rice bran oil prepared from 'Chuchung' white rice.
[3)]Rice bran oil prepared from 'Suwon 415' pigmented black rice.

Table 4. Effect of rice bran oil supplements on plasma and hepatic lipids in high cholesterol fed rats[1)]

	Control	WRBO	BRBO
Plasma[2)]			
TG (mg/dL)	38.38±3.58^{NS}	37.68±5.36	34.03±7.41
TC (mg/dL)	62.65±2.99^{b}	61.05±4.27^{ab}	58.10±3.65
HDL-C (mg/dL)	9.69±0.50^{a}	10.91±0.54^{ab}	11.82±1.02^{b}
LDL-C (mg/dL)	50.50±3.41^{b}	47.06±1.65^{ab}	44.24±1.53^{a}
HDL-C/TC (%)	14.88±0.83^{a}	17.11±0.56^{ab}	17.68±0.96^{b}
Atherogenic index	7.79±0.78^{b}	5.05±0.19^{ab}	4.97±0.12^{a}
Liver			
TG (mg/g)	227.10±8.73^{NS}	221.54±11.27	171.68±16.49
TC (mg/g)	94.47±4.18^{NS}	91.46±1.53	90.00±2.24

[1)]Values are expressed as mean±SD (n=10); values in the same row not sharing a common superscript are significantly different at $p<0.05$; ^{NS}no significance between the groups.
[2)]TG, triglyceride; TC, total cholesterol; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol, [LDL-C]= [TC]-([HDL-C]+[TG/5]); Atherogenic index; ([TC]-[HDL-C])/[HDL-C].

WRBO were intermediate to the rats fed BRBO and controls and these levels were not significantly differ from either group. The plasma HDL-C/TC value was significantly higher in BRBO (about 19%) compared to those for rats hypercholesterolemic controls. The atherogenic index of hypercholesterolemic controls, rats fed WRBO and rats fed BRBO was about 7.8, 5.1, and 5.0, respectively. There was no significant difference in hepatic TG levels in between each experimental group. TC concentrations in BRBO appeared to decrease (about 13%) but were not statistically different from the other groups.

Our results showed that the HDL-C/TC ratio in blood was higher in rats fed BRBO than the hypercholesterolemic control. The HDL-C/TC ratio in rats fed WRBO were intermediate to the ratio found in rats fed BRBO because of a significant increase in HDL-C (about 22% increase compared to the control) coupled with a simultaneous decrease in LDL-C (about 8% decrease). Consequently, the atherogenic index in rats fed BRBO was significantly lower than in the control (about 36% reduction). The atherogenic index of rats fed WRBO was also significantly lower than controls but this value was not significantly differentnfrom the BRBO group.

No distinct difference in fatty acids compositions were found between BRBO and WRBO groups for both saturated and unsaturated fatty acids (Table 1). This result indirectly supports the previous notion that the hypocholesterolemic effect of rice bran oil, especially in terms of the enhancement of HDL-C concentrations, is due to its relatively high content of non-fatty acid compounds. It was reported that γ-oryzanol and tocotrienols of rice bran oil might be the critical agents that counteract dietary cholesterol-induced hypercholesterolemia in experimental animals (31). In these reports, however, there were no significant differences in the blood TC or LDL-C between rabbits consuming different rice diets. Our current results are not inconsistent with these previous findings; the plasma TC and LDL-C concentrations did not significantly differ between WRBO and the control. On the contrary, BRBO showed the capacity to lower both TC and LDL-C concentrations to significantly different levels, implying involvement of some minor factors distinctive or relatively abundant in BRBO as compared to WRBO. In this case, the decrease in cholesterol absorption by these factors is one likely explanation of the decline in TC and LDL-C levels in the blood.

A number of studies have demonstrated that feeding dietary plant sterols in 'pharmacological' amounts can decrease plasma cholesterol levels in both humans and experimental animals under conditions in which the dietary fatty acid pattern is kept constant (32,33). Rong *et al.* (34) have shown that feeding γ-oryzanol in the test dose or in all fed diets results in a reduction of cholesterol absorption.

Although γ-oryzanol has understood to be the primary beneficial unsaponifiable compnent of rice bran oil, other unsaponifiable compounds might be actively lower plasma cholesterol. Besides plant sterols and oryzanol, the unsaponifiable components of rice bran oil contains an appreciable amount of β-sitostanol which can interfere with cholesterol absorption in intestine to a greater extent than stanols (35,36). Consequently, one interesting possibility to consider would be that the greater hypocholesterolemic effect of BRBO compared to WRBO is due to the action of the specifically enriched or novel unsaponifiable compounds functionally similar to this sterol.

The current study also showed that hepatic TG concentrations in both BRBO and WRBO groups were significantly higher than in the hypercholesterolemic controls. In contrast, no significant differences could be found for the hepatic total TC between the 3 dietary groups (Table 4). The latter observation could be explained because the soybean oil in the control diet is itself well-known polyunsaturated fatty acid-rich oil with potent hypocholesterolemic effects. A number of studies demonstrated that the alteration in TC concentration in blood and liver is in part affected by the dietary fat type. As reported, unsaturated fatty acids are capable of up-regulating cholesterol esterification, thereby triggering the restoration of suppressed LDL-C receptor activity and cholesterol synthesis in both hepatocytes and enterocytes (37,38). Therefore, similar metabolic rates for cholesterol homeostasis due to the fatty acid composition in dietary oils between the dietary groups examined may explain the lack of significant differences in TC concentration between the experimental groups.

Table 5. Effect of rice bran oil supplements on plasma and hepatic antioxidant status in high cholesterol fed rats[1)]

	Control	WRBO	BRBO
Liver			
SOD (unit/mg)	1.73±0.24[a]	2.06±0.17[b]	2.49±0.03[c]
Catalase (μmol/mg/min)	1.26±0.04[a]	1.52±0.11[b]	1.77±0.09[c]
GSH-Px (nmol/mg/min)	19.85±0.78[a]	21.58±1.24[a]	25.93±1.90[b]
Plasma			
Retinol (μg/dL)	9.38±0.25[ns]	9.47±1.48	10.12±0.45
Tocopherol (μg/dL)	151.53±1.31[a]	165.91±9.35[b]	173.87±0.77[b]

[1)]Values are expressed as mean±SD (n=10); values in the same row not sharing a common superscript are significantly different at $p<0.05$; [NS]no significance between the groups.

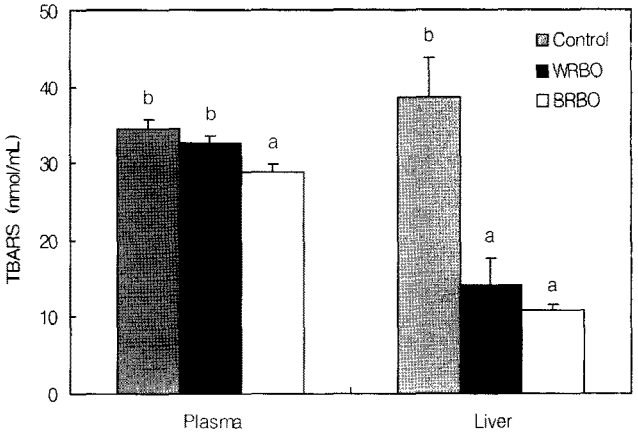

Fig. 1. Effect of rice bran oil supplements on plasma and hepatic TBARS level in high cholesterol fed rats. Values are expressed as mean±SD (n=10); the mean not sharing a common letter are significantly different between the groups ($p<0.05$).

Plasma and hepatic lipid peroxides Plasma and hepatic lipid peroxides levels were assessed by measuring TBARS concentration (Fig. 1). A significant suppression of the hypercholesterolemic diet-induced high level of plasma lipid peroxidation was observed in rats fed BRBO. The TBARS concentration in rats fed WRBO was not significantly different than the hypercholesterolemic control. Similarly, hepatic lipid peroxides levels in both BRBO and WRBO appeared to decrease, but these decreases were not significantly different compared to the controls.

Plasma and hepatic antioxidant status Table 5 lists the plasma content of retinol and tocophrol. Plasma tocopherol levels in both WRBO and BRBO groups were significantly higher than in the hypercholesterolemic controls. However, retinol levels did not differ between these 3 diet groups, although a slight, but insignificant increase was observed in the BRBO group.

The activities of the main hepatic antioxidant enzymes SOD, CAT, and GSH-Px were measured to evaluate the effect of bran oil on hepatic antioxidant status. SOD, CAT, and GSH-Px activities were highest in rats fed BRBO and their activities were significantly higher than in WRBO (>15%), except for GSH-Px (Table 5). The plasma GOT values did not significantly differ between the groups including the hypercholesterolemic control, whereas GPT values in BRBO were lower than in controls, GPT values from rats fed WRBO were significantly different from the other groups (Table 6).

The plasma tocopherol concentration was elevated significantly in both 2 bran oil groups compared to control (up to about 15% increase, Table 5). Nagao *et al.* (39) reported a reduction of plasma and hepatic α-tocopherol concentration in rat fed rice, which led them to hypothesize that consumption of plant sterols in excess leads to lower absorption of lipid-soluble vitamins. The discrepancy for rice bran oil diet-dependent alteration in plasma tocopherol level between the previous reports and our current stydy may be due to the difference in the experimental animals used in each study. Because tocopherols and tocotrienols rich in rice bran oil may improve the oxidative state in the cell milieu, lipid-soluble antioxidants plus certain unsaponifiable compounds such as γ-oryzanol in BRBO or WRBO might contribute to the improvement of *in vivo* antioxidant status.

Besides these antioxidant compounds, another primary antioxidant system is the array of antioxidant enzymes specialized to counteract reactive oxygen species (ROS) generated in the process of aerobic respiration: SOD, CAT, GSH-Px, glutathione reductase, and glutathione transferase (40). ROS levels are the most commonly used parameter of oxidation status in biological systems. We show here that BRBO consumption enhances SOD and catalase activities. Rats fed WRBO also significantly enhance SOD and catalase activities. BRBO also caused the greatest increase in GSH-Px activity in all groups (Table 5). Elevations in 3 major antioxidant enzyme activities might serve as important contributors in lowering plasma and hepatic TBARS levels (Table 5 and Fig. 1).

High cholesterol diet cause an increase in oxidative stress in the liver and results in trigger of molecular oxidation by an enzymatic reaction in liver microsomal cytochrome P450 (CYPs) (41). These oxidized molecules can then consecutive lipid peroxidation of the plasma membrane and endoplasmic reticulum. The peroxidative products induce disturbance of the plasma membrane and eventually cytosolic enzymes such as GOT and GPT leads into the general circulation (42). Therefore, lipid peroxidation of hepatocytes might be a major factor in the incidence of liver injury.

The current study showed that consumption of rice bran oil caused significant suppression of elevated GOT and GPT levels. Specially, BRBO lowered GPT levels by about 37% (Table 6). Significant reductions of TBARS in both blood and liver of rats fed on BRBO diet might support this notion (about 16 and 72% suppression, respectively,

Table 6. Effect of rice bran oil supplements on plasma GOT and GPT in high cholesterol fed rats[1)]

Group	GOT	GPT
	(karmen/mL)	(karmen/mL)
Control	202.83±34.65[NS]	56.56±1.13[a]
WRBO	175.82±19.49	44.95±0.50[ab]
BRBO	159.74±20.05	35.84±9.02[b]

[1)]Values are expressed as mean±SD (n=10); values in the same column not sharing a common superscript are significantly different at $p<0.05$; [NS]no significance between the groups.

Fig. 1), although a significant difference was not found between rats fed BRBO and WRBO.

In conclusion, our study demonstrates the potency of dietary supplement rice bran oils in the improving cholesterol metabolism and antioxidant status *in vivo*. BRBO prepared from 'Suwon 415'-pigmented black rice generally demonstrated more potent bioactivity than WRBO derived from 'Chuchung' ordinary white rice. The large unsaponifible content present in the physically refined bran oil from the pigmented balck rice is likely to be a dominant factor responsible for the hypocholesterolemic property, although the mechanisms involved have not been definitely elucidated. The BRBO-induced metabolic changes may also have been achieved in part due to stimulation of intrinsic antioxidant enzyme activities. A further study to analyze the specific fatty acid constituents as well as the composition of the unsaponifiable fractions from BRBO is needed.

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