Biochemical and Molecular Action of Nutrients

Red and Black Rice Decrease Atherosclerotic Plaque Formation and Increase Antioxidant Status in Rabbits[1]

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ABSTRACT The influence of white, red and black rice consumption on atherosclerotic plaque formation induced by hypercholesterolemia was investigated in rabbits. Male rabbits ($n = 36$) were divided into five groups. They were fed a normal laboratory purified diet (normal group, $n = 6$), a high cholesterol (0.5g/100g) diet (HC group, $n = 6$), a high cholesterol diet with 30 g/100 g white rice (WR group, $n = 8$), 30 g/100 g red rice (RR group, $n = 8$), or 30 g/100 g black rice (BR group, $n = 8$) for 10 wk. Blood samples were collected for lipid measurements and aorta were removed for assessment of atherosclerotic plaques at the end of the protocol. The oxidant and antioxidant status of blood, erythrocytes, liver and aorta was evaluated. The area of atherosclerotic plaque was 50% lower in rabbits fed the red or black rice diets than in those fed the white rice diet. Compared with the HC and WR groups, serum HDL cholesterol and apolipoprotein (apo) A-I concentration were greater ($P < 0.05$) in the RR and BR groups. Liver reactive oxygen species (ROS) and aortic malondialdehyde (MDA) were significantly lower, and the liver total antioxidative capacity (TAC) and erythrocyte superoxide dismutase (SOD) activity were significantly higher in the RR and BR groups compared with the HC and WR groups. Red or black rice consumption reduced or retarded the progression of atherosclerotic plaque development induced by dietary cholesterol. The enhanced serum HDL cholesterol and apo A-I concentrations, and the increased antioxidant and decreased oxidative status may be mechanisms of the antiatherogenic effect of red or black rice. J. Nutr. 131: 1421–1426, 2001.

KEY WORDS: • *rice* • *atherosclerosis* • *antioxidative status* • *rabbits*

Despite changes in life style and the use of new pharmacologic approaches to lower plasma cholesterol concentration, cardiovascular disease continues to be the principal cause of death in the United States, Europe and much of Asia (Braunwald 1997, Breslow 1997). Major classic risk factors for cardiovascular diseases include hyperlipidemia, elevated levels of LDL cholesterol (LDL-C),[3] decreased HDL cholesterol (HDL-C) and smoking. A number of newer, "nontraditional" cardiovascular risk factors in addition to the classic risk factors have been identified on the basis of recent studies of the pathogenesis of atherosclerosis and atherothrombotic cardiovascular events. Oxidative stress plays a crucial role in the initiation of atherosclerosis (Oparil and Oberman 1999). Reactive oxygen species (ROS) have been implicated in the pathogenesis of atherosclerosis and hypertension (Liao et al.2000, McCall and Frei 1999). Recently, a number of studies indicated that LDL undergo progressive oxidation, becoming oxidized-LDL (ox-LDL) when oxidative stress increases in vivo. Several studies have indicated that ox-LDL play a series of roles in atherosclerotic development by promoting macrophage foam cell formation and inducing endothelial cell damage and smooth muscle cell proliferation (Ross 1999).

A number of studies have shown the importance of antioxidants, including vitamin E and the antioxidant enzymes such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GSH-Px), in protecting animals against the injuries due to oxidative stress, as well as the effect of an enhanced level of antioxidants in ameliorating the oxidant tissue injury (Hennekens et al. 1996, Stephens et al. 1996). Elevated levels of ROS or ox-LDL in vivo could be due to lowered concentrations of antioxidants and activities of antioxidative enzymes.

The treatment of cardiovascular disease with rice diets was suggested several decades ago. More than 50 years ago, it was reported that consumption of white rice decreased blood pressure and lowered hypercholesterolemia in humans (Genest 1986, Kempner 1946). However, there are different types of rice. The most common rice consumed by humans (>85%) is white rice; the rest is colored rice, most of which is red and black rice. Red and black rice are planted mainly in South Asian and other countries such as Italy, Greece and the United States. Europeans eat more black rice than South Asians (Simmons and Williams 1997). Colored rice has long been consumed in China and is considered to be a health food, but there are no studies available concerning the effects of consumption of red or black rice on atherosclerosis or cardiovascular diseases.

An early study by our group showed that red rice consump-

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[3] Abbreviations used: apo, apolipoprotein; C, cholesterol; DCFH, 2′,7′-dichlorodihydrofluorescin; DCFH-DA, 2′,7′-dichlorofluorescin; GSH-Px, glutathione peroxidase; MDA, malondialdehyde; ox-LDL, oxidized LDL; RFI, relative fluorescence intensity; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidative capacity; TG, triglyceride.

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tion (30 g/100 g diet) in rats increased the concentration of plasma HDL-C and activity of blood GSH-Px (Ma et al. 1999). The potentially beneficial effect of red and black rice consumption would be to improve the lipid profile and protect against oxidative stress, thus retarding atherosclerotic formation and development. The present study was designed to investigate the influence of natural red or black rice consumption on atherosclerotic plaque formation or development induced by high cholesterol diet feeding in rabbits and to explore possible mechanisms by which colored rice consumption decreases atherosclerotic plaque formation.

MATERIALS AND METHODS

Animal and diets. Male New Zealand White rabbits ($n = 36$) aged 9 wk and weighing 1.91 kg were obtained from the animal center of Guangzhou, PR-China. Rabbits were housed individually in standard stainless steel cages at 24°C with a 12-h light:dark cycle (lights on, 0630–1830 h). Rabbits had free access to food and tap water. All experiments were performed in accordance with the protocol approved by the standing committee on animals of Sun Yat-sen University of Medical Sciences. Preceding the study, all rabbits consumed the same diet (Harlan Teklad, Madison, WI) for 1 wk. Rabbits were divided into 5 groups and fed one of the following for 10 wk: normal laboratory rabbit diet (normal group, $n = 6$); normal diet with high cholesterol 0.5 g/100 g (HC group, $n = 6$); HC diet with 30 g/100 g white rice powder (WR group, $n = 8$); HC diet with 30 g/100 g red rice powder (RR diet, $n = 8$); HC diet with 30 g/100 g black rice powder (BR group, $n = 8$). The concentrations of protein, carbohydrate and fat as well as other constituents of the white, red and black rice were determined by chemical analysis. The protein levels in the diets with rice supplements were adjusted to the same level as the normal diet (18 g/100 g) by adding casein to compensate the protein shortage from rice powder. The different diets were adjusted to contain 10 g/100 g fat (**Table 1**). The raw material of white and colored rice was husked in the same way (unpolished) so that the rice used underwent the same minimal amount of grinding. The rabbits were weighed every 2 wk during the experiment. At the end of experiment, all rabbits were deprived of food overnight and killed under ether anesthesia. Blood was collected by heart puncture. Whole blood and serum were prepared for laboratory analysis. The major organs and aorta of each rabbit were harvested, washed with ice-cold isotonic saline and weighed. Serum and tissue samples were stored at −20°C until used for analysis.

Assessment of atherosclerotic plaques. After the rabbits were killed, aorta tissue between its origin and bifurcation into the iliac arteries was taken gently, free of adhering tissues. A small part of the arterial specimen at the end of bifurcation without plaques was dissected and then rinsed with ice-cold PBS for tissue malondialdehyde (MDA) measurement. The large part of aorta between its origin and bifurcation into the iliac arteries was opened longitudinally and prepared for plaque assay. Atherosclerotic plaque areas were assessed by a previously described method (Prasad and Kalra 1993). Briefly, the aortic strips were immersed in 10% buffered formalin solution for 24 h and then rinsed in 70% alcohol. The tissue was then immersed in Herxheimer's solution containing Sudan IV (5 g), ethyl alcohol 70% (500 mL) and acetone (500 mL) at room temperature for 15 min and washed in running water for 1 h. Photographs of the intimal surface of the aorta were taken and colored slides were made. The slide was projected on a Caramate Projector Screen with a grid in mm^2 and the total atherosclerotic areas of the intimal surface of the aorta was measured in mm^2. The extent of atherosclerosis was expressed as a percentage of the luminal surface that was covered by atherosclerotic plaques.

TABLE 1

Composition of the rabbit diet[1–4]

Ingredient	Diet				
	Normal	HC	WR	BR	RR
			units/100 g		
Energy, *kJ*	1665.2	1665.2	1665.2	1665.2	1665.2
Protein, *g*	18.0	18.0	18.0	18.0	18.0
Total fat, *g*	10.0	10.0	10.0	10.0	10.0
Corn oil, *g*	7.0	7.0	6.79	6.53	6.58
Lard, *g*	3.0	3.0	2.91	2.80	2.82
Cholesterol, *g*	0	0.5	0.5	0.5	0.5
Total carbohydrate, *g*	59.0	59.0	59.0	59.0	59.0
Cornstarch, *g*	40.0	40.0			
Sucrose, *g*	19.0	19.0			
Salt mixture,[5] *g*	5.0	5.0			
Iron, *mg*	12.2	12.2	8.99	9.02	9.0
Zinc, *mg*	0.298	0.298	0.68	0.75	0.69
Selenium, *μg*	0.90	0.90	1.17	1.52	1.25
Vitamin mixture,[6] *g*	2.0	2.0			
Thiamin, *mg*	0.5	0.5	0.39	0.47	0.37
Vitamin E, *mg*	7.35	7.35	6.53	5.35	5.31
Fiber, *g*	6.0	6.0	4.77	5.04	5.52

[1] Abbreviations used: Normal, rabbits were fed the control diet; HC, normal diet with 0.5 g/100 g cholesterol; WR, HC diet with white rice (30 g/100 g); BR, HC diet with black rice (30 g/100 g); RR, HC diet with red rice (30 g/100 g).

[2] The diet, mineral mix and vitamin mix were supplied by Research Diets (Ralston Purina, St. Louis, MO).

[3] Cholesterol was added without replacement of any nutrient.

[4] Total minerals and vitamins in white, red and black rice were not measured; thus, amounts in the diets are unknown. However, a few minerals and vitamins were determined in the rice, including zinc, selenium, vitamin E and thiamin.

[5] Mineral composition of normal diet (per kg diet): Al, 0.53 mg; Ca, 6.73; Cl, 5.29; Cu, 3.43; F, 0.012 g; I, 1.55 mg; Fe, 0.122 g; Mg, 0.594 g; Mn, 3.64 mg; P, 3.22 g; K, 8.17 g; Se, 9.00 mg; Na, 1.50 g; S, 0.253 g; Zn, 2.98 mg.

[6] Vitamin profile of normal diet (per kg diet): vitamin A, 2.75 mg as all-trans-retinyl palmitate; cholecalciferol, 25 μg; vitamin E, 73.5 mg as all-rac-α-tocopheryl acetate; menadione sodium bisulfite, 2 mg; biotin, 0.2 mg; cyanocobalamin, 10 μg; folic acid, 2 mg; nicotinic acid, 20 mg; riboflavin, 5 mg; calcium pantothenate, 20 mg; pyridoxine 10 mg; thiamin, 5 mg.

Serum lipid profile. Serum cholesterol, triglyceride (TG) and HDL-C concentrations were determined by enzymatic methods using a Hitachi Automatic Analyzer (Tokyo, Japan) and the kits provided by the First Chemical and Pharmaceutical Institute of Japan (Tokyo, Japan). Serum TG concentrations were measured by hydrolyzing the triglycerides and measuring the released glycerol (Bucolo and David 1973). Serum total cholesterol (TC) was determined by using a cholesterol esterase and cholesterol oxidase assay (Allain et al. 1974). Serum concentrations of HDL-C were measured by the same method, as was serum TC after removing LDL-C and VLDL cholesterol (VLDL-C) with magnesium dextran sulfate. Serum LDL concentrations were calculated according to Friedewald formula (Friedewald et al. 1972), which assumes that circulating VLDL consist of 80% triglycerides and 20% cholesterol. Apolipoprotein (apo) B and apo AI were analyzed using commercially available kits (French Bioerieux Sa, RCS Lyon, France), which use the turbidity immunoassay method of Ikeda et al. (1991).

Preparation of tissue fractions for measurement of ROS and antioxidant enzyme activity. The methods for preparation of tissue fractions and measurement of ROS and antioxidant enzymes were as described previously (Prasad et al. 1992). The tissues (liver or aorta) were removed, cleaned of gross adventitial tissue, blotted dry and weighed. They were then homogenized in 5–10 volumes of 50 mmol/L phosphate buffer (pH 7.4) at 4°C for 30 s using a polytron homogenizer. The homogenate was filtered through cheese cloth and the filtrate was centrifuged at 1500 × *g* for 10 min. The resulting supernatants were used for measurement of ROS, total antioxidant capacity (TAC) and antioxidant enzymes.

Preparation of erythrocyte lysate. Erythrocyte lysates were prepared by the method of Minami and Yoshikawa (1979). Briefly, blood was collected in tubes containing EDTA and centrifuged (1500 × *g*) for 15 min at 4°C. The sediment containing the erythrocytes was suspended in normal saline and recentrifuged. This process was repeated twice. Sedimented RBC (0.2 mL/L) were added to 0.8 mL of ice-cold distilled water and mixed thoroughly. This hemolysate was used for estimation of GSH-Px and SOD activities.

ROS level in the liver. The ROS in the liver supernatant were measured by using the fluorescent probe 2′,7′-dichlorodihydrofluorescin (DCFH) (Lebel et al. 1992). Briefly, DCFH was prepared from 2′,7′-dichlorofluorescin (DCFH-DA) by mixing 1.0 mmol/L DCFH-DA (0.5 mL) in methanol with NaOH (0.1mol/L, 2.0 mL). For assaying ROS formation, the reactions were performed in 40 mmol/L Tris-HCl, pH 7.4, in a total volume of 0.4 mL that contained DCFH solution (100 μL). The fluorescence was monitored by a Hitachi F3010 Fluorescence Spectrophotometer with an excitation wavelength of 488 nm and an emission wavelength of 525 nm. The results were expressed as the relative fluorescence intensity (RFI).

TAC in serum and liver. The TAC of serum and liver supernatants were measured using the ability of endogenous antioxidants to scavenge the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) radical cation (Severin et al. 1999).

SOD and GSH-Px activities. SOD activity in the liver supernatant or erythrocyte lysate was measured as described by Oyanagui(1984). Briefly, cytochrome c (10 μmol/L) reduction was measured after 30 s of incubation at 25°C with 50 μmol/L xanthine and 2.5 μmol/L xanthine oxidase in 50 mmol/L potassium phosphate buffer (pH7.8). Absorption at 550 nm was recorded continuously on a Shimazu Multi-Purpose 5000 spectrophotometer (Shimadzu, Kyoto, Japan). GSH-Px activity in liver supernatant was measured using 5,5′-dithiobis (2-nitrobenzoate) as described (Lawrence and Burk 1976). Briefly, the assay mixture consisted of 2.0 mL of 75 mmol/L phosphate buffer (pH 7.0), 50 μL of 60 mmol/L GSH, 0.1 mL of 3 × 10^4 U/L GSH reductase, 0.1 mL of 15 mmol/L disodium salt of EDTA, 0.1 mL of 3 mmol/L NADPH, various amounts (50–200 μL) of supernatant (200–500 μg protein) and 0.3 mL of water. The final volume of the reaction mixture was 3.0 mL. The reaction was started by the addition of 0.1 mL of 75 mmol/L H_2O_2. Conversion of NADPH to NADP was monitored continuously at 340 nm for 4 min.

Determination of MDA. Serum and aorta (lower part of the aorta without plaque) or liver supernatants were analyzed for MDA by the thiobarbituric acid reaction using HPLC with a C-18 column and UV-visible detector (Chen et al. 1998).

Statistical analysis. Results are expressed as means ± SD. The differences among the five groups were analyzed by one-way ANOVA. A Student-Newman-Keuls test was used to determine the significance of differences between any two groups. Differences of $P < 0.05$ were considered significant.

RESULTS

Body weights did not differ among the four groups (data not shown). There were no visible atherosclerotic plaques in aorta of rabbits fed the normal diet, but atherosclerotic plaques of various degrees were visible in aorta of rabbits fed the HC and rice diets. The plaque was much more severe in rabbits fed the HC or the white rice diet than in rabbits fed the red or black rice diet. The plaque or lesion area of aorta in rabbits fed the red or black rice diet was ~50% less than that in rabbits fed the HC or white rice diets ($P < 0.05$) (**Fig. 1**). There were no significant difference in plaque areas of rabbits fed the HC or WR diets.

Rabbits fed the normal diet differed from the other groups in serum lipids. However, there were no significant differences in serum TG, TC, LDL-C and apo B concentrations or the apo AI/apo B ratio among the groups fed the HC and rice diets (**Table 2**). The circulating HDL-C and apo AI concentrations in rabbits fed red or black rice were significantly higher than those in rabbits fed white rice or HC diets ($P < 0.05$). The ratio of LDL-/HDL-C was lower in rabbits fed colored rice than in rabbits fed the white rice or HC diets.

The levels of ROS in liver were higher in rabbits fed the HC or WR diet than in those fed colored rice diets ($P < 0.05$) (**Table 3**). Although there were no differences in MDA of serum or liver among rabbits fed HC or rice diets, MDA levels of aorta of rabbits fed the HC and WR diets were significantly higher than in those fed colored rice diets (Table 3). The liver TAC and erythrocyte SOD in the groups fed red or black rice were significantly elevated compared with those of rabbits fed HC or WR diets ($P < 0.05$) (Table 3). Blood (data not shown) and liver GSH-Px also tended to be greater ($P < 0.07$) in rabbits fed colored rice compared with rabbits fed the HC and WR diets (Table 3).

DISCUSSION

The major finding of this study is that rabbits consuming red or black rice diets (30 g/100 g) with high cholesterol (0.5 g/100 g diet) had dramatically reduced aortic atherosclerotic

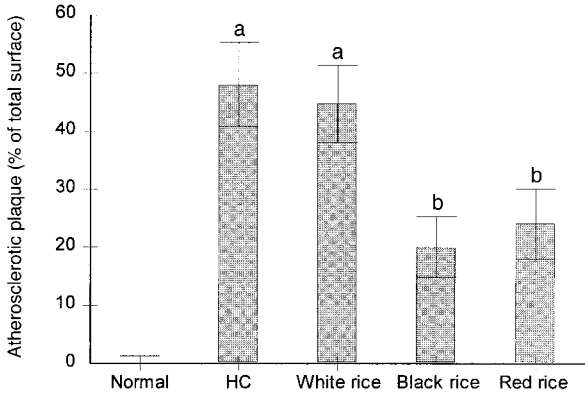

FIGURE 1 Atherosclerotic plaque of aorta in rabbits fed a normal or high cholesterol (HC) diet or the HC diet containing white (WR), red (RR) or black (BR) rice for 10 wk. Values are means ± SD, $n = 6–8$. Bars without common letters are significantly different, $P < 0.05$.

TABLE 2

Serum lipid concentrations in rabbits fed a normal or high cholesterol (HC) diet or the HC diet containing white (WR), red (RR), or black (BR) rice for 10 wk[1]

Variable	Normal	HC	WR	RR	BR
n	6	6	8	8	7
TG, *mmol/L*	0.96 ± 0.50[b]	1.86 ± 0.50[a]	1.48 ± 0.47[a]	1.96 ± 0.94[a]	1.78 ± 0.90[a]
TC, *mmol/L*	4.02 ± 0.07[b]	20.67 ± 3.12[a]	15.72 ± 4.04[a]	16.71 ± 5.52[a]	18.49 ± 1.76[a]
LDL-C, *mmol/L*	2.34 ± 0.05[b]	15.34 ± 4.09[a]	13.19 ± 3.59[a]	13.36 ± 4.63[a]	14.81 ± 1.86[a]
HDL-C, *mmol/L*	0.92 ± 0.04[c]	1.96 ± 0.71[b]	1.87 ± 0.68[b]	2.72 ± 0.80[a]	2.88 ± 0.88[a]
Apo AI, *g/L*	0.37 ± 0.02[c]	0.47 ± 0.07[b]	0.45 ± 0.05[b]	0.57 ± 0.05[a]	0.53 ± 0.06[a]
Apo B, *g/L*	0.17 ± 0.04	0.15 ± 0.05	0.15 ± 0.03	0.15 ± 0.06	0.19 ± 0.07
LDL/HDL	2.54 ± 2.20[c]	8.03 ± 2.23[a]	7.99 ± 2.48[a]	5.14 ± 1.68[b]	5.85 ± 1.93[b]
Apo AI/Apo B	2.17 ± .05[b]	3.13 ± 0.78[a]	3.11 ± 0.62[a]	4.64 ± 2.66[a]	3.19 ± 1.75[a]

[1] Values are means ± S.D. Values in a row with unlike superscripts differ, $P < 0.05$. Abbreviations used: TG, triglyceride; TC, total cholesterol; C, cholesterol; Apo, apolipoprotein.

plaque areas compared with the rabbits consuming white rice (30 g/100 g) or the high cholesterol diet. The plaque areas of the aorta were ~50% less in rabbits fed red or black rice compared with those fed white rice or high cholesterol diets. The basic diet did not differ among the three rice groups except for the different type of rice supplemented. The significant reduction in the atherosclerotic plaque from consumption of colored rice vs. the HC and white rice diets indicated that red rice or black rice can prevent atherosclerotic plaque formation and progression. To our knowledge, this is the first finding of a beneficial effect of red or black rice on prevention of atherosclerosis.

HDL-C is considered to be "good" cholesterol in the circulation (Stein and Stein 1999). It carries the cholesterol or cholesterol esters from peripheral tissues or cells to the liver where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing the cholesterol level in blood and peripheral tissues, and in inhibiting the atherosclerotic plaque formation in the aorta. White rice consumption has been reported to lower blood pressure and plasma lipids (Genest 1986, Kempner 1946). In this study, white rice with 0.5% cholesterol did not significantly affect the lipid profile compared with rabbits fed 0.5% cholesterol only. However, colored rice consumption significantly increased (55%, $P < 0.05$) serum HDL cholesterol and apo AI concentrations compared with white rice consumption. This phenomenon suggests that enhanced HDL-C and apo AI concentrations contributed to the reduction in atherosclerotic lesion areas of aorta in rabbits fed red or black rice diets compared with those fed the white rice diet.

The beneficial effect of rice consumption was studied predominantly from rice bran oil (RBO) or bran fiber. In this study, the beneficial influence of colored rice appeared not to be due to the fiber because the fiber content in the rice diets was less than that in the HC or normal diets. RBO contains oleic, linoleic and linolenic acids as unsaturated fatty acids, and palmitic and stearic acids as saturated fatty acids. In addition, RBO contains unsaponifiable materials including tocopherols, γ-oryzanol, phytosterols, tocotrienols and squalene (Rukmini and Raghuram 1991). A number of studies in humans and animals revealed that RBO consumption lowers serum total TG, TC and LDL-C, and increases plasma HDL-C concentrations (Nicolosi et al. 1991, Purushothama et al. 1995, Seetharamaiah and Chandrasekhara 1989). RBO has hypocholesterolemic activity, and the enhanced HDL concentration is characterized by a relatively high content of non-

TABLE 3

Oxidant and antioxidant status of rabbits fed a normal or high cholesterol (HC) diets or the HC diet containing white (WR), red (RR), or black (BR) rice for 10 wk[1]

	Normal	HC	WR	RR	BR
n	6	6	8	8	8
ROS, RFI (Liver)	98.23 ± 17.29[c]	1211.34 ± 189.32[a]	1139.56 ± 245.77[a]	781.29 ± 125.64[b]	852.18 ± 102.67[b]
TAC Serum, *10³ U/L*	12.21 ± 4.17[c]	56.94 ± 16.31[b]	69.83 ± 26.22[b]	109.29 ± 27.50[a]	101.31 ± 29.11[a]
Liver, *U/mg protein*	2.43 ± 0.76[c]	14.09 ± 2.95[b]	11.84 ± 2.85[b]	16.43 ± 3.54[a]	16.64 ± 2.57[a]
SOD Erythrocyte, *U/g Hb*	467.25 ± 76.89[c]	15,698 ± 984.27[b]	16,001.4 ± 1234.4[b]	19,525.8 ± 2241.3[a]	20,714.5 ± 4990.7[a]
Liver, *U/g protein*	31.10 ± 9.46[b]	166.76 ± 34.68[a]	176.24 ± 25.66[a]	197.28 ± 42.49[a]	209.07 ± 38.85[a]
GSH-Px liver, *U/g protein*	42.12 ± 8.56	37.21 ± 10.33	35.33 ± 9.67	40.53 ± 6.78	38.33 ± 10.1
MDA serum, *μmol/L*	0.12 ± 0.04[b]	0.62 ± 0.19[a]	0.65 ± 0.25[a]	0.82 ± 0.26[a]	0.70 ± 0.23[a]
Liver, *nmol/mg protein*	0.09 ± 0.04[b]	0.19 ± 0.04[a]	0.20 ± 0.04[a]	0.21 ± 0.05[a]	0.19 ± 0.04[a]
Aorta, *nmol/mg protein*	0.43 ± 0.13[c]	5.32 ± 1.03[a]	4.87 ± 0.93[a]	2.73 ± 0.46[b]	2.23 ± 0.42[b]

[1] Values are means ± SD. Values in a row without common superscripts differ, $P < 0.05$. Abbreviations used: GSH-Px, glutathione peroxidase; MDA, malondialdehyde; RFI, relative fluorescence intensity; ROS, reactive oxygen pecies; Hb, hemoglobin; SOD, superoxide dismutase; TAC, total antioxidative capacity.

fatty acid components. It was reported that γ-oryzanol and tocotrienols of RBO participate in its hypocholesterolemic effects (Purushothama et al. 1995, Sugano et al. 1999). In this study, there were no significant differences in serum TC or LDL-C or in apo B concentrations among rabbits consuming different rice diets, in agreement with previous reports (Marsono et al. 1993, Miyoshi et al. 1986). Therefore, the cholesterol-lowering effect of RBO induced by unsaturated fatty acids or unsaponifiable materials appears to be a minor factor in the lowering of atherosclerotic plaque formation in rabbits fed colored rice. However, whether some components in rice bran such as γ-oryzanol and tocotrienols are responsible for the enhancement of serum HDL concentration requires further clarification.

Although a close relationship exists between high blood cholesterol levels and atherosclerosis, it has been suggested that this relationship might be dependent on enhanced oxidative stress (Ohara et al. 1993). Hypercholesterolemia increases the levels of ROS in arterial endothelial cells. Elevated ROS can stimulate the progression of atherosclerotic pathogenesis, in part by promoting endothelial dysfunction, which is the earliest stage of atherosclerosis, and damaging vascular smooth muscle cells (Hogg 1998, Kunsch and Medford 1999,Liao et al.2000). To counteract the oxidants, an important endogenous antioxidant system exists in vivo, which includes antioxidant compounds such as vitamin E and antioxidant enzymes such as SOD, catalase, glutathione peroxidase, glutathione reductase, glutathione transferase and glucose-6-phosphate dehydrogenase (Thomas 1999). It has been reported that supplementation of antioxidant substances such as vitamin E inhibits or retards the development of atherosclerosis in animals (Jialal and Fuller 1995, Steinbrecher 1997). In addition, some minerals such as selenium and zinc may play very important roles in antioxidation (Neve 1995, Oteiza et al. 1995, Torra et al. 1997).

ROS and MDA are the most commonly used markers of oxidation. In this study, supplementation of red or black rice in rabbits significantly lowered liver ROS and aortic MDA, indicating that colored rice supplementation decreased oxidative stress in vivo. Simultaneously, colored rice supplementation increased antioxidant capacity, including enhanced serum and liver TAC and erythrocyte SOD activity. Because consumption of red or black rice leads to improved antioxidation and decreased peroxidation processes, damage from oxidative stress to artery endothelial or smooth muscle cells will be reduced, with fewer atherosclerotic plaques formed in the aorta. Although the present study showed the beneficial effects of red or black rice consumption in improvement of peroxidation and antioxidant processes in vivo, the mechanisms involved have not been determined. The vitamin E concentration in the rice diets was lower than that in the normal and HC diets; thus, plasma vitamin E concentration may be not related to the improvement of antioxidative status in rabbits fed colored rice. But selenium, an antioxidant nutrient, was higher in the colored rice diets than in the white rice or HC diet. This antioxidant nutrient might be responsible in part for the enhancement of antioxidant status in this study. The mechanism by which selenium enhanced the antioxidant status has not been fully elucidated. Selenium is an integral part of the antioxidant enzyme glutathione peroxidase (GSH-Px). In the present study, there were no differences in GSH-Px activities in rabbits consuming different diets with various concentrations of selenium. This is in agreement with a previous study reported by Neve (1995) in which selenium supplementation increased blood selenium concentration; however, there were no significant differences in either plasma or erythrocyte GSH-Px activity.

Because tocopherols and tocotrienols rich in RBO may improve the oxidative state (Rukmini and Raghuram 1991), these compounds derived from colored rice may also have contributed to the improved antioxidant status in rabbits. In addition, other constituents of colored rice such as amino acids, nicotinic acid, riboflavin and minerals may contribute in part to antioxidative improvements because they were higher in colored rice than in white rice.

Alternatively, some special constituents with antioxidative properties such as flavonoids exist in red rice or black rice, but were not identified in the colored rice we used. These constituents may be responsible for the prevention of atherosclerotic plaque formation and improvement in lipid profile as well as antioxidative status. Further studies are required to elucidate the beneficial effect and to identify the constituents responsible for the positive health effect of colored rice consumption.

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