

## A Rice Bran Oil Diet Increases LDL-Receptor and HMG-CoA Reductase mRNA Expressions and Insulin Sensitivity in Rats with Streptozotocin/Nicotinamide-Induced Type 2 Diabetes<sup>1</sup>

Chia-Wen Chen\* and Hsing-Hsien Cheng<sup>†2</sup>

\*Graduate Institute of Pharmacy and <sup>†</sup>School of Public Health and Nutrition Science, Taipei Medical University, Taipei, Taiwan, ROC

**ABSTRACT** A rice bran oil (RBO) diet can reduce plasma lipids; this was attributed to the specific components,  $\gamma$ -oryzanol and  $\gamma$ -tocotrienol, which individually were shown to be hypocholesterolemic; however, the mechanism of their effects on diabetic hyperlipidemia and the development of diabetes is not known. Rats with streptozotocin/nicotinamide-induced type 2 diabetes were divided into control, RO10, and RO15 groups, and fed cholesterol-free diets containing 0, 10, and 15 g RBO with 0, 352, and 528 g  $\gamma$ -oryzanol and 0, 6.0 and 9.0 mg  $\gamma$ -tocotrienol/100 g diet for 4 wk. Diabetic rats fed the RBO diet had greater insulin sensitivity ( $P = 0.02$ ) than rats fed the control diet. Diabetic rats fed the RBO diet also had lower plasma triglyceride ( $P = 0.003$ ), LDL cholesterol ( $P = 0.028$ ), and hepatic triglyceride concentrations ( $P = 0.04$ ), as well as greater fecal neutral sterol and bile acid excretion than those fed the control diet. After 4 wk, there was an  $\sim 100\%$  ( $P < 0.001$ ) increase in the abundance of hepatic cholesterol  $7\alpha$ -hydroxylase, an 89% ( $P < 0.001$ ) increase in the hepatic LDL-receptor, and a 50% ( $P < 0.001$ ) increase in hepatic 3-hydroxy-3-methylglutaryl coenzyme A reductase mRNA in rats fed the RBO diet compared with those fed the control diet. These findings support the conclusion that a rice bran oil-containing diet can significantly suppress hyperlipidemic and hyperinsulinemic responses in diabetic rats. The high contents of  $\gamma$ -oryzanol and  $\gamma$ -tocotrienol in RBO can lead to increased fecal neutral sterol and bile acid excretion, via upregulation of cholesterol synthesis and catabolism. *J. Nutr.* 136: 1472–1476, 2006.

**KEY WORDS:** • rice bran oil • type 2 diabetic rats • HMG-CoA reductase • LDL-receptor • insulin sensitivity

Several studies in humans (1,2) and animals (3,4) have established that the ingestion of a rice bran oil (RBO)<sup>3</sup> diet reduces serum cholesterol and triglycerides. From the results of human and animal studies, researchers speculated that the hypocholesterolemic effect of RBO is attributed to its specific components,  $\gamma$ -oryzanol and  $\gamma$ -tocotrienol. The major components of  $\gamma$ -oryzanol were identified as ferulic acid esters of triterpene and phytosterols (5).  $\gamma$ -Oryzanol was shown to decrease plasma cholesterol in rats (3,4). An amount of  $\gamma$ -oryzanol  $> 0.2\%$  (weight percent of the diet) decreased serum and liver cholesterol levels in hypercholesterolemic rats induced by additional dietary cholesterol. The mechanism of the hypocholesterolemic effect of RBO and  $\gamma$ -oryzanol is through decreasing cholesterol absorption in the intestines and increasing fecal cholesterol excretion (6).

RBO is also rich in tocotrienols; the major components are  $\beta$ - and  $\gamma$ -tocotrienols. It is postulated that tocotrienols, especially  $\gamma$ -tocotrienol, the most hypocholesterolemic potent of all of the tocotrienols, regulates cholesterol production in mammalian cells by inhibiting 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase activity, and decreasing liver cholesterol levels and plasma total cholesterol (TC) and LDL cholesterol (LDL-C) concentrations (7).

Much research has focused on the ability of RBO to decrease plasma levels of TC and LDL-C in both hypercholesterolemic and normal animals. However, it is important to determine whether changes in plasma lipids and insulin resistance in diabetes after administration of an RBO diet are related to modifications in gene expressions of cholesterol  $7\alpha$ -hydroxylase (CYP7A1), the LDL-receptor, and HMG-CoA reductase, major regulators for maintaining whole-body cholesterol homeostasis.

The regulation of cholesterol homeostasis is associated with its effect on LDL-receptor levels and on the activity of regulatory enzymes such as HMG-CoA reductase. Thus, the coordinated control of HMG-CoA reductase and LDL-receptor mRNA levels reflects their common regulation by shared transcriptional activation (8). The LDL-receptor is also a major regulator of plasma LDL-C concentrations; we were interested in evaluating whether the removal of LDL-C from the circulation contributes to lower plasma cholesterol levels after RBO consumption.

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<sup>2</sup> To whom correspondence should be addressed. E-mail: chenghh@tmu.edu.tw.

<sup>3</sup> Abbreviations used: BW, body weight; CYP7A1, cholesterol  $7\alpha$ -hydroxylase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HDL-C, HDL cholesterol; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-coenzyme A reductase; LDL-C, LDL cholesterol; MUFA, monounsaturated fatty acid; NEFA, nonesterified fatty acid; RBO, rice bran oil; RO10 group, diabetic rats fed a 10% rice bran oil-containing diet; RO15 group, diabetic rats fed a 15% rice bran oil-containing diet; SFA, saturated fatty acid; STZ, streptozotocin; TC, total cholesterol.

Furthermore, CYP7A1 is a liver-specific enzyme, which catalyzes the first and rate-limiting step in the classical bile acid synthesis pathway (9). The regulation of CYP7A1 activity seems to be important because bile acid synthesis is the major pathway responsible for maintaining whole-body cholesterol homeostasis (10,11). However, the mechanisms by which  $\gamma$ -tocotrienol and  $\gamma$ -oryzanol in rice bran oil achieve the lowering of LDL-C remain unclear in diabetes. A few studies have dealt with the effects of consuming an RBO diet on plasma insulin and hepatic lipid concentrations, and its association with the hepatic mRNA expressions of HMG-CoA reductase, CYP7A1, and the LDL-receptor.

On the basis of these observations, the aims of this study were to investigate the effect of RBO rich in  $\gamma$ -tocotrienol and  $\gamma$ -oryzanol on plasma lipids and the transcriptional activation of hepatic HMG-CoA reductase, CYP7A1, and the LDL-receptor in rats with streptozotocin/nicotinamide-induced type 2 diabetes.

## MATERIALS AND METHODS

**Animals and diets.** Male Wistar rats [ $n = 32$ ; aged 7 wk with a body weight (BW) of  $200 \pm 10$  g] were obtained from the Animal Center of Taiwan University Medical College, Taipei, Taiwan. Rats were housed individually in wire-bottomed stainless steel cages in an air-conditioned room ( $21 \pm 2^\circ\text{C}$ , 50–70% relative humidity) with a 12-h light:dark cycle and free access to the basal diet and water for 1 wk before diabetes was induced. Diabetes was induced by an i.p. injection of streptozotocin (STZ; 45 mg/kg BW) followed 15 min later by an injection of nicotinamide (200 mg/kg BW). After 2 d, this step was repeated using the modified Masiello method (12). Nicotinamide and STZ were freshly prepared in a 0.9% (wt:v) sodium chloride solution. A rat was considered to be diabetic when its fasting plasma glucose concentration was  $>10$  mmol/L 14 d after the last induction date. At this point, baseline blood samples were collected from the tail vein of rats after anesthetization with ether gas; the rats then began to consume the experimental diets.

RBO was extracted by the supercritical  $\text{CO}_2$  fluid extraction method (13). The high-fat diet contained 150 g of fat (control, soybean oil; RO10, 1:2 soybean oil: rice bran oil; RO15, rice bran oil), 590 g cornstarch, 200 g casein, and 10 g  $\alpha$ -cellulose as fiber/kg diet. Choline, cysteine, minerals, and vitamins were added as described in AIN-93 (14). Diabetic rats were divided into control, RO10, and RO15 groups ( $n = 8$ /group) and were fed cholesterol-free diets containing 0, 10, and 15 g RBO with 0, 35.2, and 52.8 g  $\gamma$ -oryzanol and 0, 60, and 90 mg  $\gamma$ -tocotrienol/100 g of diet for 4 wk.

After consuming the diets for 4 wk, the rats were deprived of food overnight ( $\sim 14$  h); then they were anesthetized with 1 g/L sodium pentobarbital in a 0.9% (wt:v) sodium chloride solution and killed by exsanguination from the abdominal aorta. Blood was centrifuged at  $1200 \times g$  at  $4^\circ\text{C}$  for 10 min, and the plasma was collected. The livers of rats in all groups were removed. Fecal samples of the rats in each group were collected for 48 h at the end of the experimental period. All samples were frozen at  $-70^\circ\text{C}$  until analysis. All animal experimental procedures followed published guidelines (15) and were approved by the Institutional Animal Care and Use Committee of Taipei Medical University, Taipei, Taiwan.

**Analysis.** RBO and soybean oil were analyzed for  $\alpha$ -tocopherol,  $\gamma$ -tocotrienol, and  $\gamma$ -oryzanol by HPLC using a method described previously (16). The HPLC system consisted of a Hitachi L-2000 pump equipped with a Hitachi AS-2000 autosampler and a Hitachi L-7455 diode array detector. A C18 normal phase chromatography column (5 SL-II,  $4.6 \times 250$  mm,  $5 \mu\text{m}$ ; Cosmoscil, Nacalai Tesque) was used. The flow rate was set at 1.0 mL/min, and the wavelength of the detector was set at 295 nm for the detection of  $\alpha$ -tocopherol,  $\gamma$ -tocotrienol, and  $\gamma$ -oryzanol. The mobile phase was hexane:isopropanol (99:1, v:v). There were 35.20 mg  $\gamma$ -oryzanol, 0.6 mg  $\gamma$ -tocotrienol, and 3.02 mg  $\alpha$ -tocopherol/g RBO and 0.45 mg  $\alpha$ -tocopherol per gram of soybean oil. The fatty acid component percentages of RBO and soybean oil were analyzed by GC (17). The saturated fatty acid (SFA), monounsaturated fatty acid (MUFA), and PUFA percentages of RBO were 13.6, 50.7, and

35.5%, respectively. The SFA, MUFA, and PUFA percentages of soybean oil were 18.2, 33.2, and 48.7%, respectively.

The plasma glucose, triglyceride, TC, HDL-C, LDL-C, and nonesterified fatty acid (NEFA) concentrations were determined spectrophotometrically using a glucose kit, triglycerides kit, cholesterol kit, LDL-cholesterol kit and a NEFA kit, respectively (Randox). The plasma insulin concentration was measured by a Rat Insulin ELISA kit (Merckodia). Insulin sensitivity was evaluated using the ratio of the fasting plasma insulin level to the plasma glucose level. The higher the ratio, the more insulin resistant the individual (18).

Triglyceride and cholesterol in liver samples were extracted (19) and levels were measured using a triglycerides kit and a cholesterol kit, (Randox).

Total fatty acid compositions in plasma and hepatic lipids were quantified using a one-step direct transesterification procedure carried out in a 4:1 methanol:hexane solution with acetyl chloride in all steps as previously described (17). The fatty acids were analyzed by GC, with a G-3000 chromatograph (Hitachi) with flame ionization detection. Separations were performed on a Stabilwax-DA capillary column ( $30 \text{ m} \times 0.53 \text{ mm i.d.}$ ; film thickness,  $0.5 \mu\text{m}$ ; RESTEK). Individual fatty acids in the plasma and liver were calculated using 17:0 fatty acid as an internal standard. Identification was based on the retention time. Individual fatty acid levels were expressed as a percentage of the total fatty acids.

Because the amount of the fecal sample for individual rats in the same group was insufficient to analyze neutral sterols and bile acids, fecal samples were pooled in the same group, then divided into 5 equal portions, freeze-dried, and ground. Neutral sterols and bile acids were extracted (19) and determined (20) using cholesterol and bile acids kits (Randox).

**RNA extraction.** The extraction of total RNA from hepatic cells of rats was based on the method of Chomczynski and Sacchi (21). The Trizol reagent was used according to the manufacturer's instructions; the method was modified slightly with the use of isopropyl alcohol for RNA precipitation. The RNA concentration was estimated by measurement with a UV spectrophotometer at 260 nm.

**RNA quantification.** A semiquantitative RT-PCR method adapted from that of Powell and Kroon (22) was used to determine HMG-CoA reductase, CYP7A1, and LDL-receptor mRNA abundances.

A reverse-transcription reaction was performed using 100  $\mu\text{g}$  RNA with 5 units of the MMLV reverse transcriptase (EPICENTRE) in a 50- $\mu\text{L}$  volume containing DEPC water,  $1 \times$  MMLV RT buffer [ $50 \text{ mmol/L Tris-HCl}$  (pH 8.3),  $75 \text{ mmol/L KCl}$  and  $10 \text{ mmol/L MgCl}_2$ ],  $10 \text{ mmol/L dithiothreitol}$ ,  $0.25 \text{ mmol/L dNTP mix}$ , and  $0.5 \mu\text{g oligo(dT)}_{12-18}$ , and then was incubated at  $37^\circ\text{C}$  for 60 min. The PCR protocol consisted of 40 cycles of amplification (30 s of denaturing at  $95^\circ\text{C}$ , 30 s of annealing at  $60^\circ\text{C}$ , and 30 s of extension at  $72^\circ\text{C}$ ). The PCR primer sequences were newly designed and are listed as follows: for the HMG-CoA reductase 5' primer, 5'-TGCTGCTTTGGCTGTATGT-3', and the 3' primer, 5'-TGAGCGTGAACAAGAACCA-3'; for the CYP7A1 5' primer, 5'-CACCATTCTGCAACCTT-3', and the 3' primer, 5'-GTACCGGCAGGTCATTCA-3'; for the LDL-receptor 5' primer, 5'-CAGCTCTGTGTGAACCT-3', and the 3' primer, 5'-TTCTTCAGGTTGGGGATCA-3'; and for the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) 5' primer, 5'-GCAAGTTCA-ACGGCACAGTCAA-3', and the 3' primer, 5'-GAGGGGCCATC-CACAGTCTTCT-3'. The sizes of the reaction products were as follows: for HMG-CoA reductase, 230 bp; for CYP7A1, 170 bp; for the LDL-receptor, 188 bp; and for GAPDH, 415 bp. GAPDH was used as an internal control in all reactions. The PCR products were analyzed by electrophoresis on 1.5% agarose gels, and the gels were photographed under UV light after staining with ethidium bromide. The photographs were scanned with an image scanner, and intensities of the blots were quantified using the software, Image-Pro Plus (Media Cybernetics).

**Statistical analysis.** Values are expressed as means  $\pm$  SEM. Diet and time effects on plasma glucose, insulin, and lipid variables were analyzed by 2-way ANOVA for repeated measures and Fisher's least significant difference test. Treatment-mediated differences in mRNA expressions of hepatic enzymes associated with cholesterol metabolism and fatty acid compositions in plasma and hepatic lipids were identified by one-way ANOVA. When the *F* test indicated a significant effect, differences between means were analyzed by Fisher's least significant difference test using the SAS statistical software (SAS 8.2). Differences were considered significant at  $P < 0.05$ .

## RESULTS

**Weight gain and food intake.** The daily food intake during the experimental period did not differ among groups [ $22.9 \pm 0.1$  g/(rat·d)]. Weight gain was also unaffected by the diet ( $100.9 \pm 9.5$  g/4 wk). No side effects such as diarrhea or death occurred in rats fed the experiment diet, and no rats died as a result of inducing diabetes by the streptozotocin and nicotinamide injections.

**Plasma glucose and insulin concentrations, and insulin sensitivity.** After 4 wk of treatment, the plasma insulin concentration of the control group had increased significantly by 78.5% compared with baseline ( $144.6 \pm 17.2$  pmol/L), but not that of rats fed the RBO diet. The plasma insulin concentrations were 42.6 and 41.2% lower in the RO10 and RO15 groups ( $P = 0.02$ ), respectively, than in the control group after 4 wk of treatment (Table 1). Although the plasma glucose concentration of rats did not differ among the groups, the insulin:glucose ratio, a measure of insulin sensitivity, was lower in rats fed the RBO diets than in rats fed the control diet ( $P = 0.02$ ).

**Plasma lipids levels.** After 4 wk of treatment, the plasma triglyceride concentrations of the RO10 and RO15 groups were 35.4 and 19.3% lower than that of the control group, respectively ( $P < 0.05$ ; Table 1). NEFA, TC, and HDL-C concentrations did not differ significantly among the groups. However, the plasma LDL-C concentration of the RO15 group was lower than that of the control group ( $P = 0.03$ ; Table 1) after 4 wk of treatment.

**Hepatic triglyceride and cholesterol levels.** Liver weight and hepatic cholesterol levels did not differ among the groups (Table 2). However, the hepatic triglyceride levels of the RO10 and RO15 groups were 21.4 and 32.8% ( $P < 0.05$ ) lower than that of the control group, respectively.

**Fatty acid composition of plasma lipids.** After 4 wk of treatment, the total SFA, MUFA, and PUFA of plasma lipids in any group did not differ. However, the palmitic acid level (16:0) of the RO15 group was 19.2% greater than that of the control group; stearic acid (18:0) levels of the RO10 and RO15 groups were 24.9 and 38.8% lower ( $P < 0.05$ , Table 3), and arachidic acid (20:0) levels of the RO10 and RO15 groups were 40.6 and 49.5% ( $P < 0.001$ ) lower than those of the control group, respectively. Moreover, linoleic acid (18:2) levels of the RO10 and RO15 groups were 23.3 and 22.1% lower ( $P < 0.05$ ), and linolenic acid (18:3) levels were 50.7 and 49.3% ( $P < 0.05$ ) lower than those of the control group, respectively. The level of eicosapentaenoic acid (20:5) of the RO15 group was 43.9% ( $P < 0.05$ ) lower than that of the control group.

TABLE 1

*Effects of rice bran oil consumption for 4 wk on plasma glucose, insulin, and lipid concentrations in diabetic rats<sup>1</sup>*

	Control	RO10	RO15
Glucose, mmol/L	$11.01 \pm 0.42$	$10.50 \pm 0.81$	$11.51 \pm 0.54$
Insulin, pmol/L	$258.1 \pm 39.4^a$	$148.2 \pm 17.8^b$	$151.7 \pm 15.4^b$
Insulin/glucose ratio, pmol/mmol	$23.69 \pm 3.72^a$	$14.24 \pm 1.10^b$	$13.59 \pm 1.71^b$
NEFA, <sup>2</sup> mmol/L	$0.72 \pm 0.02$	$0.67 \pm 0.04$	$0.71 \pm 0.05$
Triglyceride, mmol/L	$0.87 \pm 0.06^a$	$0.56 \pm 0.04^b$	$0.70 \pm 0.04^b$
Total cholesterol, mmol/L	$1.84 \pm 0.10$	$1.78 \pm 0.13$	$1.60 \pm 0.06$
LDL-C, mmol/L	$0.58 \pm 0.05^a$	$0.57 \pm 0.06^a$	$0.36 \pm 0.04^b$
HDL-C, mmol/L	$1.00 \pm 0.07$	$1.01 \pm 0.11$	$0.96 \pm 0.06$

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 8$ . Means in a row with superscripts without a common letter differ,  $P < 0.05$ .

TABLE 2

*Effects of rice bran oil consumption for 4 wk on liver weight and hepatic triglyceride and cholesterol levels in diabetic rats<sup>1</sup>*

	Control	RO10	RO15
Liver weight, g	$12.3 \pm 0.4$	$12.2 \pm 0.5$	$12.9 \pm 0.5$
Triglyceride, $\mu$ mol/g	$718.9 \pm 70.4^a$	$565.1 \pm 72.1^{ab}$	$482.5 \pm 79.8^b$
Cholesterol, $\mu$ mol/g	$143.9 \pm 8.6$	$141.2 \pm 5.6$	$143.4 \pm 7.0$

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 8$ . Mean in a row with superscripts without a common letter differ,  $P < 0.05$ .

**Fatty acid composition of hepatic lipids.** After 4 wk of treatment, the total SFA contents of the hepatic lipids of all groups did not differ significantly. But the stearic acid (18:0) levels of the RO10 and RO15 groups were 8.9 and 15.9% lower ( $P < 0.05$ , Table 4), and arachidic acid (20:0) levels of the RO10 and RO15 groups were 30.0 and 40.0% ( $P < 0.05$ ) lower than those of the control group, respectively. MUFA in the RO10 and RO15 groups were 22.5 and 36.3% greater than those of the control group, respectively. Moreover, oleic acid (18:1) levels of the RO10 and RO15 groups were 29.5 and 45.5% ( $P < 0.001$ ) greater than that of the control group. The total PUFA contents of hepatic lipids in the RO10 and RO15 groups were 20.7 and 32.0% ( $P < 0.05$ ) lower than that of the control group. Linoleic acid (18:2) levels of the RO10 and RO15 groups were 13.8 and 24.3% lower ( $P < 0.05$ ), and linolenic acid (18:3) levels were 65.2% and 80.1% ( $P < 0.05$ ) lower than those of the control group, respectively. The eicosapentaenoic acid (20:5) level of the RO15 group was 43.9% ( $P < 0.05$ ) lower than that of the control group.

**Neutral sterol and bile acid levels in feces.** After 4 wk of treatment, the dry weights of feces excreted per day in pooled samples from the control, RO10, and RO15 groups were 3.4, 4.3, and 7.9 g, respectively. Fecal neutral sterol excretions by the RO10 and RO15 groups were 171.3 and 401.3  $\mu$ mol/d greater than that by the control group (39.9  $\mu$ mol/d). Fecal bile acid levels excreted by the control, RO10, and RO15 groups were 159.9, 175.3, and 223.1  $\mu$ mol/d, respectively.

TABLE 3

*Effects of rice bran oil consumed on plasma fatty acid composition in diabetic rats<sup>1</sup>*

	Control	RO10	RO15
<i>g/100 g total fatty acids</i>			
14:0	$0.62 \pm 0.07$	$0.61 \pm 0.07$	$0.74 \pm 0.08$
16:0	$24.44 \pm 0.51^b$	$26.33 \pm 1.27^{ab}$	$29.14 \pm 0.66^a$
18:0	$2.01 \pm 0.10^a$	$1.51 \pm 0.11^b$	$1.23 \pm 0.08^b$
20:0	$1.01 \pm 0.14^a$	$0.60 \pm 0.13^b$	$0.51 \pm 0.11^b$
22:0	$0.55 \pm 0.09$	$0.62 \pm 0.09$	$0.64 \pm 0.18$
$\Sigma$ SFA	$29.34 \pm 1.10$	$29.50 \pm 1.40$	$32.50 \pm 0.57$
16:1	$0.97 \pm 0.10$	$1.21 \pm 0.14$	$1.28 \pm 0.12$
18:1	$16.47 \pm 0.75$	$17.55 \pm 1.06$	$17.16 \pm 0.77$
$\Sigma$ MUFA	$17.52 \pm 0.73$	$18.72 \pm 1.03$	$18.43 \pm 0.73$
18:2	$20.81 \pm 1.01^a$	$15.97 \pm 0.86^b$	$16.22 \pm 0.65^b$
18:3	$0.73 \pm 0.08^a$	$0.36 \pm 0.04^b$	$0.37 \pm 0.09^b$
20:4	$26.7 \pm 0.62$	$27.39 \pm 1.27$	$26.07 \pm 0.91$
20:5	$1.14 \pm 0.20^a$	$0.86 \pm 0.09^{ab}$	$0.64 \pm 0.08^b$
22:6	$4.89 \pm 0.18$	$4.75 \pm 0.28$	$4.40 \pm 0.36$
$\Sigma$ PUFA	$51.62 \pm 1.62$	$51.18 \pm 1.74$	$48.56 \pm 1.12$

<sup>1</sup> Values are means  $\pm$  SEM,  $n = 8$ . Means in a row with superscripts without a common letter differ,  $P < 0.05$ .



TABLE 4

Effects of rice bran oil consumption on hepatic fatty acid compositions in diabetic rats<sup>1</sup>

	Control	RO10	RO15
<i>g/100 g total fatty acids</i>			
14:0	1.23 ± 0.03	1.28 ± 0.01	1.20 ± 0.06
16:0	22.14 ± 0.41	23.34 ± 0.42	23.45 ± 0.77
18:0	3.47 ± 0.07 <sup>a</sup>	3.16 ± 0.08 <sup>b</sup>	2.92 ± 0.07 <sup>b</sup>
20:0	0.20 ± 0.02 <sup>a</sup>	0.14 ± 0.02 <sup>b</sup>	0.12 ± 0.01 <sup>b</sup>
22:0	0.14 ± 0.01 <sup>a</sup>	0.09 ± 0.02 <sup>b</sup>	0.06 ± 0.01 <sup>b</sup>
Σ SFA	27.71 ± 0.52	28.26 ± 0.44	28.04 ± 0.78
16:1	4.99 ± 0.19	4.13 ± 0.66	4.18 ± 0.45
18:1	28.41 ± 0.23 <sup>c</sup>	36.79 ± 0.40 <sup>b</sup>	41.35 ± 0.38 <sup>a</sup>
Σ MUFA	33.40 ± 0.30 <sup>c</sup>	40.92 ± 0.66 <sup>b</sup>	45.53 ± 0.38 <sup>a</sup>
18:2	32.80 ± 1.77 <sup>a</sup>	28.26 ± 0.76 <sup>b</sup>	24.83 ± 0.91 <sup>b</sup>
18:3	4.92 ± 1.43 <sup>a</sup>	1.71 ± 0.07 <sup>b</sup>	0.98 ± 0.05 <sup>b</sup>
20:4	0.46 ± 0.06	0.36 ± 0.06	0.29 ± 0.05
20:5	0.08 ± 0.01 <sup>a</sup>	0.05 ± 0.00 <sup>b</sup>	0.06 ± 0.01 <sup>ab</sup>
22:6	0.44 ± 0.08	0.32 ± 0.12	0.14 ± 0.03
Σ PUFA	38.69 ± 0.60 <sup>a</sup>	30.69 ± 0.94 <sup>b</sup>	26.31 ± 0.99 <sup>c</sup>

<sup>1</sup> Values are means ± SEM, *n* = 8. Means in a row with superscripts without a common letter differ, *P* < 0.05.

**mRNA expression of enzymes associated with cholesterol metabolism.** HMG-CoA reductase mRNA expressions in the RO10 and RO15 groups were 95.4 and 49.4% greater than that in the control group, respectively, and the expression in the RO15 group was significantly lower than that in the RO10 group (Fig. 1). The CYP7A1 mRNA expressions of the RO10 and RO15 groups were 111.1% and 99.6% greater than that of the control group, respectively. The LDL-receptor mRNA expressions of the RO10 and RO15 groups were 142.2 and 88.9% greater than that of the control group, respectively. Consumption of RBO increased hepatic HMG-CoA reductase, CYP7A1, and LDL-receptor expressions (Fig. 1).

## DISCUSSION

The present study shows that a RBO diet containing  $\gamma$ -oryzanol and  $\gamma$ -tocotrienol maintained plasma glucose concentrations, reduced plasma insulin, triglyceride, and LDL cholesterol, and hepatic triglyceride concentrations, and increased the hepatic LDL-receptor, CYP7A1, and HMG-CoA reductase mRNA expressions in diabetic rats fed the RO10 or

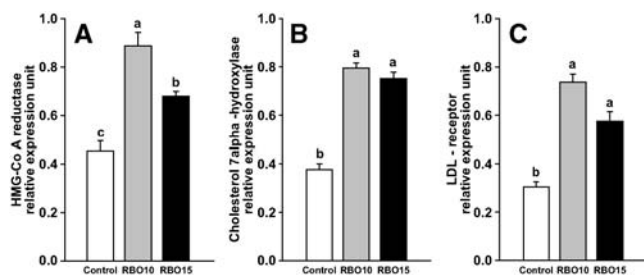
RO15 diet, compared with rats fed a soybean oil diet that served as the control diet without  $\gamma$ -tocotrienol or  $\gamma$ -oryzanol.

The most common lipid abnormalities in diabetics are hypertriglyceridemia and hypercholesterolemia (23). Hypertriglyceridemia is also associated with the metabolic consequences of hyperinsulinemia, i.e., insulin resistance (24). In our study, these variables improved in rats with STZ/nicotinamide-induced type 2 diabetes fed an RBO diet (*P* < 0.05). In addition, diabetic rats fed 15 g/100 g of a soybean oil-containing diet had higher plasma insulin, TC, and triglyceride concentrations; however, consumption of the RBO diet significantly decreased plasma insulin, LDL-C, and triglyceride levels.

In the present study, each rat ingested 88.0 (0.4%, weight percent of the diet), 132.0 mg (0.5%)  $\gamma$ -oryzanol and 1.5 and 2.3 mg  $\gamma$ -tocotrienol/d in the RO10 and RO15 groups, respectively. Therefore, rats had lower plasma LDL-C concentrations when fed the rice bran oil-containing diets for 4 wk. This finding is in agreement with previous studies using rats fed a RBO diet containing 1.6%  $\gamma$ -oryzanol;  $\gamma$ -oryzanol is a mixture of the ferulic acid esters of triperene such as cycloartenol and 24-methylene cycloartanyl (25). In the present study, the soybean oil had higher SFA (18.2%) and PUFA contents (48.7%) than the RBO (13.6% SFA and 35.5% PUFA), but a lower MUFA content (33.2 vs. 50.7%). In addition, soybean oil is rich in  $\alpha$ -tocopherol (0.45 mg/g) but does not contain  $\gamma$ -oryzanol or  $\gamma$ -tocotrienol. RBO contained 3.02 mg/g  $\alpha$ -tocopherol, 35.22 mg/g  $\gamma$ -oryzanol, and 0.60 mg/g  $\gamma$ -tocotrienol. Numerous studies demonstrated the effect of rice bran oil and  $\gamma$ -oryzanol activity on lipid metabolism in animals and humans (26). In previous studies, nondiabetic rats were fed either a cholesterol-containing or a cholesterol-free diet, with the addition of RBO (10%) to the diet for 8 wk. This produced significantly decreased plasma TC, LDL-C, and VLDL-C levels (3,27). Differences between previous studies and this research were probably due to different animal models and fat levels of the diets.

In this study, the PUFA and MUFA proportions of plasma and hepatic fatty acid compositions in diabetic rats fed RBO were altered after the intake of RBO, which is in agreement with previous studies. Dietary fatty acids were shown to alter plasma and hepatic fatty acid compositions (28,29). In previous studies, it was demonstrated that a diet with a high MUFA content decreased plasma triglyceride, cholesterol, and LDL-C levels compared with a high SFA diet (30). However, this differed from previous studies in which the SFA contents of soybean oil and RBO were similar to those in the present study. We speculated that a higher MUFA content and unsaponifiable components in RBO may have a synergistic hypocholesterolemic effect.

The increased fecal neutral sterols and bile acids due to RBO diet consumption can be explained in part by the reduction in cholesterol reabsorption in the intestines. This finding agrees with previous studies in rats that showed a mechanism of inhibiting cholesterol absorption by phytosterol whose chemical structure is very similar to that of cholesterol, thus interfering with cholesterol movement into micelles and reducing cholesterol absorption in the intestines. In addition, phytosterols are able to increase the excretion of bile acids, which results in a lowering of plasma and liver cholesterol levels (31).  $\gamma$ -Oryzanol affected biliary secretion and fecal excretion of cholesterol and bile acids. It significantly increased the fecal excretion of bile acids and neutral sterols. We speculated that the observed hypolipidemic effect of the RBO diet may have been due to increased hepatic LDL-receptor expression, which facilitated the lowering of LDL-C, and to increased CYP7A1 expression, which facilitated cholesterol catabolism, and then upregulation



**FIGURE 1** Effects of RBO consumption for 4 wk on hepatic mRNA expressions of HMG-CoA reductase (A), 7 $\alpha$ -hydroxylase (B), and the LDL-receptor (C) in diabetic rats. The graph represents the mRNA levels from RT-PCR relative to the control gene, GAPDH. Values are means ± SEM, *n* = 8. Means without a common letter differ, *P* < 0.05.

of HMG-CoA reductase expression to synthesize cholesterol for the cholesterol homeostasis in vivo.

In the present study, diabetic rats fed the RO10 or RO15 diets had higher LDL-receptor and HMG-CoA reductase mRNA expressions than the control group; this is similar to a previous study in which phytosterol ester supplementation increased LDL-receptor and HMG-CoA reductase mRNA expressions in mononuclear blood cells and livers of healthy humans (32). HMG-CoA reductase and the LDL-receptor are regulated in a coordinated manner at the level of gene expression (33).

In the present study, none of the experimental diets contained any cholesterol; thus, the neutral sterol in feces would have been catabolized mainly from cholesterol synthesized in vivo. We speculated that the effect of  $\gamma$ -tocotrienol in decreasing the activity of HMG-CoA reductase was probably overshadowed by the effect of  $\gamma$ -oryzanol on HMG-CoA reductase activity, because  $\gamma$ -oryzanol consumption caused higher excretion of fecal neutral sterols and bile acids, which resulted in increased cholesterol synthesis and catabolism in vivo.

We found for the first time that diabetic rats fed an RBO diet exhibited a significantly suppressed hyperinsulinemic response to a high-fat diet. In a previous study, a diet high in monounsaturated fat was shown to decrease the postprandial plasma glucose and insulin levels (34). The reduction in hepatic triglyceride accumulation is associated with decreased insulin resistance (35). In the present study, we speculated that rats fed the RBO diet had a suppressed hyperinsulinemic response that may have resulted from the higher MUFA dietary intake and lower plasma and hepatic triglyceride concentrations than rats fed the control diet. The detailed mechanism of RBO suppression of the hyperinsulinemic response is not clear and requires further study.

In the present study and pretest, the plasma glucose concentrations of diabetic rats without dietary treatment gradually decreased until they were stably hyperglycemic (>10 mmol/L) from >14 mmol/L during the 14-d period after the final STZ injection. The 14-d period should not precede any low-glycemic study to avoid influence of unstable hyperglycemia of diabetic rats on the study results.

In conclusion, the results of this study suggest that consumption of a RBO diet significantly suppresses the hyperlipidemic and hyperinsulinemic responses to a high-fat diet in diabetic rats. The hypocholesterolemic mechanism of RBO which is rich in  $\gamma$ -oryzanol and  $\gamma$ -tocotrienol may occur by increasing fecal neutral sterol and bile acid excretion, via upregulating cholesterol synthesis and catabolism.

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