

## Red Yeast Rice Increases Excretion of Bile Acids in Hamsters

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**Objective** To investigate the hypocholesterolemic activity of red yeast rice (RYR) and its underlying mechanism. **Methods** Three groups of hamsters were fed either the control diet or one of the two experimental diets containing by weight 0.1% RYR (0.1RYR) or 0.3% RYR (0.3RYR). Blood (0.5 mL) was collected from the retro-orbital sinus into a heparinized capillary tube at the end of week 0, 3, and 6. Plasma lipoproteins were measured using enzymatic kits, while fecal neutral and acidic sterols were quantified using a gas-liquid chromatography. **Results** Plasma total cholesterol was reduced by 12% in 0.1RYR group and by 18% in 0.3RYR group compared with the control value. Similarly, plasma triacylglycerol was decreased by 11% in 0.1RYR group and by 24% in 0.3RYR group. Western blotting analysis demonstrated that RYR had no effect on sterol regulatory element binding protein 2, liver X receptor, 3-hydroxy-3-methylglutaryl-CoA reductase, LDL receptor, and cholesterol-7 $\alpha$ -hydroxylase. HPLC analysis confirmed that RYR contained 0.88% monacolin K. It was recently found that RYR supplementation increased excretion of fecal acidic sterols by 3-4 folds compared with the control value. **Conclusion** Hypocholesterolemic activity of RYR is mediated at least partially by enhancement of acidic sterol excretion.

**Key words:** Bile acid; Cholesterol; Monacolin; Red yeast rice; Triacylglycerols

### INTRODUCTION

Red yeast rice (RYR), a variety of fermented rice with mold *Monascus purpureus*, is marketed in China not only as a red food colorant but also as a traditional Chinese medicine for improvement of blood circulation and reduction of plasma cholesterol<sup>[1]</sup>. Cholesterol-lowering activity of RYR has been demonstrated in rabbits<sup>[2]</sup>, quails<sup>[3]</sup>, chicken<sup>[4]</sup>, and humans<sup>[5]</sup>. A meta-analysis of 90 human trials has assessed the effectiveness and safety of three RYR preparations in lowering blood lipids in primary hyperlipidemia, concluding that RYR treatment is capable of reducing plasma total cholesterol (TC), triacylglycerols (TG) and low-density lipoprotein cholesterol (LDL-C) levels with a concomitant increase in plasma high-density lipoprotein cholesterol (HDL-C) level<sup>[5]</sup>. It is believed that monacolin K and its derivatives in RYR are the active compounds responsible for this

hypocholesterolemic activity, because they are inhibitors of 3-hydroxy-3-methylglutaryl reductase (HMGR)<sup>[6]</sup>. In this regard, HepG2 cells treated with RYR has a decrease in cholesterol synthesis by 31%-54% and a decrease in secretion of both free cholesterol and cholesteryl ester by 14%-33%<sup>[7]</sup>. It has been also reported that the hypocholesterolemic activity of RYR preparations is comparable to that of statin drugs and is better than that of nicotinate<sup>[5]</sup>.

Cholesterol homeostasis is governed at the transcriptional level by sterol regulatory element-binding protein 2 (SREBP-2) and liver X receptor (LXR) in a coordinated manner<sup>[8]</sup>. SREBP-2 regulates the transcription of two proteins; namely HMGR and low-density lipoprotein receptor (LDLR), with HMGR acting as a rate-limiting enzyme in cholesterol synthesis and LDLR being responsible for the removal of LDL cholesterol from the circulation. LXR activates transcription of a gene encoding cholesterol-7 $\alpha$ -hydroxylase (CYP7A1), which is a

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rate-limiting enzyme in conversing cholesterol to bile acids in the liver<sup>[8]</sup>.

Despite extensive research on RYR, little is known of how it interacts with the genes and proteins involved in cholesterol metabolism *in vivo*. Using hamsters as an animal model, the present study was undertaken to (i) characterize the interaction of dietary RYR with SREBP-2, LXR, HMGR, LDLR, and ACAT-2; and (ii) investigate the effect of dietary RYR on fecal excretion of individual neutral and acidic sterols.

## MATERIALS AND METHODS

### *RYR and Analysis of Monacolins*

RYR was obtained from Zhejiang Sanhe Bio-Tech, Zhejiang, China. Individual monacolins were identified and analyzed as previously described<sup>[9]</sup>. RYR powder (10 mg) was sonicated in 1 mL of a mixed solvent of methanol: water (70:30, vol/vol) in a screw-cap vial for 10 min. After being cooled to room temperature and filtered by a syringe filter (0.45  $\mu$ m), the supernatant (20  $\mu$ L) was injected into HPLC for assay. A Waters HPLC System (Waters, Milford, MA, USA) equipped with 2695 solvent delivery module and a 996 photodiode-array (PDA) UV detector were used. A C<sub>18</sub> column (Thermo Hypersil-Keystone, 4.6  $\times$  250 mm, 5  $\mu$ m) was used for separation and eluted by a linear gradient solvent system that consisted of solvents A (acetonitrile) and B (0.04% aqueous phosphoric acid) with a flow rate of 1 mL/min. The proportion of solvent A was increased from 20% to 60% in the first 20 min, increased from 60% to 80% in the following 10 min, held 80% for 10 min, and then returned to 20% in the last 10 min for the next injection. The total run time was 50 min. The effluent was monitored at 237 nm. Individual monacolins were quantified using the calibration curve of monacolin K.

### *Diets*

Three diets were formulated. The control was prepared by mixing the following ingredients in proportion (g/kg diet): cornstarch, 500; casein, 200; lard, 100; sucrose, 119; mineral mix AIN-76, 40; vitamin mix AIN-76A, 20; DL-methionine, 1; cholesterol, 1. The other two experimental diets were prepared by adding 0.1% and 0.3% by weight of red yeast rice into the control diet, respectively. The powdered diets were then mixed with gelatin solution (20 g/L) in a ratio of 200 g diet per liter. Once the gelatin had set, the food was cut into 10 g cubed portions and stored in a freezer at -20 °C.

### *Animals*

Male adult Syrian golden hamsters (*Mesocricetus auratus*,  $n=39$ , 113 $\pm$ 3 g) were obtained from the Laboratory Animal Services Centre, the Chinese University of Hong Kong. Experiment was conducted following approval and in accordance with the guidelines set by the Animal Experimental Ethical Committee, the Chinese University of Hong Kong.

Hamsters were randomly divided into three groups ( $n=13$ ) and housed in wire-bottom cages at 23 °C with a 12-h light-dark cycle in the animal room. All animals were stabilized by being fed the control diet for 2 weeks. For the next 6 weeks, hamsters were fed one of three diets, namely the control diet, 0.1% RYR (0.1RYR) and 0.3% RYR (0.3RYR) diets, respectively, according to their groups. Animals were allowed freely to access the food and tap water *ad libitum*. Food intake was measured daily and body weight was recorded weekly. The total fecal output of each hamster was manually separated from other bedding materials and was pooled in each week throughout the experiment.

Blood (0.5 mL) was collected from the retro-orbital sinus into a heparinized capillary tube at the end of weeks 0, 3, and 6 after overnight food deprivation and light anaesthesia, using a mixture of ketamine, xylazine and saline (4:1:5, v/v/v). The blood was centrifuged at 1 500 g for 10 minutes and the plasma was collected and stored at -20 °C until analysis. Following the last collection of blood sample at week 6, all hamsters were kept for 3 days to allow for recovery and then killed by carbon dioxide suffocation after overnight fasting. The liver, kidney, heart, perirenal, and epididymal adipose tissues were removed, washed with ice-cold saline, weighed, flash frozen in liquid nitrogen and stored at -80 °C until analysis.

### *Measurement of Plasma Lipoproteins*

Plasma TC and TG were measured using enzymatic kits from Infinity (Waltham, MA, USA.) and Stanbio Laboratories (Boerne, TX, USA.) respectively. For measurement of plasma HDL-C, non-HDL-C, and very low-density lipoprotein (VLDL) were first precipitated with phosphotungstic acid and magnesium chloride using a commercial kit (Stanbio Laboratories). HDL-C in the supernatant was determined similarly as did TC. Non-HDL-C was calculated by deducting HDL-C from TC.

### *Determination of Liver Cholesterol*

Hepatic cholesterol concentration was determined as previously described<sup>[10]</sup>. In brief, stigmasterol (1 mg as an internal standard) was

added into the liver sample (300 mg). 15 mL of methanol-chloroform mixture (2:1, v/v) were used to extract lipids together with 5 mL of saline. The chloroform-methanol layer was saved and evaporated to dryness under a nitrogen stream. The liver lipids were then mildly saponified and the cholesterol was converted into its trimethylsilyl-ether derivative before the GC analysis.

#### *Determination of Fecal Neutral and Acidic Sterols*

Concentrations of neutral and acidic sterols in the feces were determined as described previously<sup>[10]</sup>. Stigmasterol and hydeoxycholic acid (0.5 mg each) were used as internal standards for quantification of fecal neutral and acidic sterols, respectively. Briefly, dried fecal samples (300 mg) were mildly hydrolyzed and extracted with cyclohexane. The neutral sterols in cyclohexane phase were converted into their trimethylsilyl derivatives. The acid sterols in the bottom aqueous layer were saponified, extracted, and converted similarly into their trimethylsilyl derivatives. Both neutral and acidic sterol trimethylsilyl derivatives were subjected to the GC analysis.

#### *Western Blotting Analysis of Liver SREBP-2, LDL-R, HMGR, LXR, and CYP7A1*

These five proteins were quantified as previously described<sup>[11]</sup>. Total hepatic protein was extracted according to the method described by Vaziri and Liang<sup>[12]</sup>. In brief, the liver was homogenized in buffer A which contained 20 mmol/L Tris-HCl (pH 7.5), 2 mmol/L MgCl<sub>2</sub>, 0.2 mol/L sucrose and Complete® protease inhibitor cocktail (Roche, Mannheim, Germany). The extract was centrifuged at 12 000 g for 15 min at 4 °C and the supernatant was retained (total protein). A portion of total protein was centrifuged at 126 000 g for 60 min at 4 °C. The pellet (membrane protein) was re-suspended in buffer A. Protein concentration of two fractions was determined using a protein concentration assay kit obtained from Bio-Rad.

Membrane protein (100 µg) was size-fractionated on a 7% SDS-PAGE gel to quantify LDLR, CYP7A1, LXR, and HMGR. The proteins bound in the gel were transferred to a Hybond-P PVDF membrane (Amersham Pharmacia Biosciences, Uppsala, Sweden), which was immediately blocked with 5% nonfat milk in 1× TBS containing 0.1% Tween-20 and then incubated overnight at 4 °C with anti-LDLR (Santa Cruz Biotechnology, Inc., California, USA), anti-HMGR (Upstate USA Inc., Lake Placid, NY, USA), 1:400 anti-LXR or anti-CYP7A1 antibodies

(Santa Cruz Biotechnology, Inc., California, USA). The membrane was further incubated with diluted horseradish peroxidase-linked rabbit anti-goat IgG (Zymed Laboratories Inc., San Francisco, USA) or donkey anti-rabbit IgG (Santa Cruz Biotechnology) followed by detection with ECL enhanced chemiluminescence agent (Amersham Life Science) and subjected to autoradiography on SuperRX medical X-ray film (Fuji, Tokyo, Japan). Quantification was made using the computer software Photoshop® (Adobe Systems Inc, CA, USA).

For measurement of SREBP-2, equal amounts of the membrane protein and the total protein aliquots were mixed and run on a 7% SDS-PAGE gel. The primary antibody used was anti-SREBP-2 antibody (Santa Cruz Biotechnology). Data on abundance of SREBP-2, LDLR, HMGR, LXR, and CYP7A1 were normalized with β-tubulin.

#### *Measurement of Liver HMGR Activity*

Liver HMGR activity was measured according to the method described by van Heusden and Wirtz<sup>[13]</sup> with minor modification. Briefly, liver microsomal protein was prepared as previously described<sup>[14]</sup>. The microsomal enzyme was assayed in 250 µL buffer containing 0.378 mol/L NaCl, 0.2 mol/L DTT, 1.6 unit/mL glucose-6 phosphase dehydrogenase, 16.2 mmol/L NADP and 0.2 mol/L glucose-6 phosphate, pH 7.2. The mixture was incubated in at 37 °C for 20 min. The reaction was initiated by adding 10 nmole HMG-CoA (4.0 Ci/mol). After incubation at 37 °C for 1 h, the reaction was stopped by adding 35 µL of 5 N HCl followed by 50 pCi RS-[5-<sup>3</sup>H]-mevalonolactone. The mixture was then incubated for 15 min at 37 °C and the labeled mevalonic acid formed was separated from the reaction mixture onto an AG1-X8 formate column (0.7 × 10 cm). The sample was eluted with water and the fraction corresponding to mevalonic acid was collected and added to 10 mL of OptiPhase HiSafe 3 scintillation fluid (Perkin-Elmer). Radioactivity was measured in a LS 6500 scintillation counter (Beckman) and the data was calculated based on [<sup>3</sup>H] recovery.

#### *Statistical Analysis*

Data were expressed as  $\bar{x} \pm s$ . The group means were statistically analyzed using one-way analysis of variance (ANOVA) and post hoc LSD test on SigmaStat Advisory Statistical Software (SigmaStat version 14.0, SPSS Inc., Chicago, USA). Significance was defined as *P*-value less than 0.05.

## RESULTS

### Composition of Monacolins in RYR

HPLC analysis showed that RYR used in the present study contained six monacolin derivatives (Fig. 1). RYR had monacolin K most (8.81 mg/g) followed by hydroxyl acid form of monacolin K (7.02 mg/g), dehydromonacolin K (1.04 mg/g), hydroxyl acid form of dehydromonacolin K (0.48 mg/g), methyl ester of hydroxyl acid form of monacolin L (0.35 mg/g) and monacolin L (0.06 mg/g).

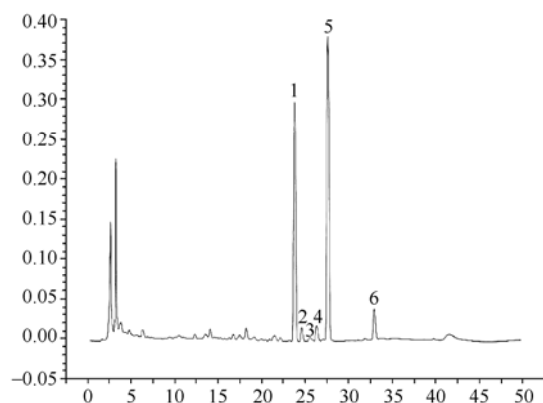


FIG. 1. High-performance liquid chromatogram of monacolins in red yeast rice (RYR). Peak identifications: 1, Hydroxyl acid form of monacolin K; 2, Methyl ester of hydroxyl acid form of monacolin L; 3, Monacolin L; 4, Hydroxyl acid form of dehydromonacolin K; 5, Monacolin K; 6, Dehydromonacolin K.

### Body Weight Gain, Food Intake, and Organ Weights

The data on food intake, body and organ weights are shown in Table 1. No significant differences in food intake were observed among the control,

0.1RYR and 0.3RYR groups. Liver, heart, and kidney weights were not different among the three groups. However, the final body weight of 0.3RYR group was significantly lower than that of the control and 0.1RYR groups. Similarly, perirenal adipose tissue in 0.3RYR was smaller compared with that of the control hamsters.

### Effect of RYR Supplementation on Plasma Lipoproteins

There was no difference in plasma lipoprotein profiles among the three groups at week 0 (Table 2). At the end of week 3, two experimental groups had plasma TC and non-HDL-C levels significantly lower than the control with HDL-C being unchanged. At the end of week 6, hypocholesterolemic activity of RYR appeared to be dose-dependent, although two experimental groups had no significant difference in concentrations of plasma TC, non-HDL-C, and HDL-C. To be specific, RYR decreased plasma TC by 12% in 0.1RYR group and by 18% in 0.3RYR group compared with that of the control value. Similarly, plasma TG was decreased by 11% in 0.1RYR group and 24% in 0.3RYR group compared with the control value.

### Fecal Neutral and Acidic Sterol Output and Cholesterol Balance

Concentrations of individual neutral and acidic sterols in the feces of the hamsters are shown in Table 3. In general, dietary RYR had no effect on fecal excretion of total neutral sterols except for campesterol. In contrast, RYR supplementation increased excretion of total acidic sterols by 3 folds in 0.1RYR group and by 4 folds in 0.3RYR group compared with that in the control group. Two experimental groups excreted greater amount of three acidic sterols including lithocholic acid, chenodeoxycholic acid and cholic acid than the control hamsters.

TABLE 1

Body Weight, Organ Weight, and Food Intake in Hamsters Fed the Control Diet and the Experimental Diets Supplemented with 0.1% Red Yeast Rice (0.1RYR) and 0.3% Red Yeast Rice (0.3RYR)

	Control	0.1RYR	0.3RYR
Final Body Weight (g)	140.9±10.2 <sup>a</sup>	137.7±7.2 <sup>a</sup>	130.5±9.6 <sup>b</sup>
Food Intake (g/day)	10.9±0.8	10.5±0.6	11.7±0.5
Liver (g)	7.03±1.22	7.89±1.70	7.34±0.92
Epididymal Adipose Tissue (g)	2.50±0.70	2.45±0.62	2.36±0.52
Perirenal Adipose Tissue (g)	1.95±0.66 <sup>a</sup>	1.61±0.45 <sup>a,b</sup>	1.43±0.47 <sup>b</sup>
Kidney (g)	1.34±0.15	1.23±0.21	1.22±0.14
Heart (g)	0.54±0.11	0.60±0.17	0.55±0.06

Note. Values are  $\bar{x} \pm s$ ,  $n=13$ . Means in a row with different letters (a,b) differ significantly,  $P<0.05$ .

TABLE 2

Changes in Plasma Total Cholesterol (TC), Total Triacylglycerols (TG), High-density Lipoprotein Cholesterol (HDL-C), Non-HDL Cholesterol (non-HDL-C), Non-HDL-C/HDL-C, Liver Cholesterol, and HMG-CoA Reductase Activity in Hamsters Fed the Control Diet and Two Experimental Diets Supplemented with 0.1% Red Yeast Rice (0.1RYR) and 0.3% Red Yeast Rice (0.3RYR)

	Control	0.1RYR	0.3RYR
Week 0			
TC (mg/mL)	260.55±40.48	262.20±46.88	270.33±49.52
HDL-C (mg/mL)	103.27±14.71	93.75±10.62	96.06±15.90
Non-HDL-C (mg/mL)	157.28±34.58	168.45±48.77	174.27±47.62
Non-HDL-C/HDL-C	1.54±0.38	1.83±0.59	1.87±0.66
TG (mg/mL)	331.78±105.95	343.96±141.20	305.63±100.03
Week 3			
TC (mg/mL)	253.51±22.15 <sup>a</sup>	234.47±25.43 <sup>b</sup>	233.13±26.89 <sup>b</sup>
HDL-C (mg/mL)	92.04±11.09	98.93±20.49	100.94±15.88
Non-HDL-C (mg/mL)	161.47±29.37 <sup>a</sup>	135.54±34.97 <sup>b</sup>	132.18±29.65 <sup>b</sup>
Non-HDL-C/HDL-C	1.80±0.45	1.46±0.51	1.35±0.39
TG (mg/mL)	253.33±67.19 <sup>a</sup>	248.78±53.85 <sup>a,b</sup>	195.19±61.28 <sup>b</sup>
Week 6			
TC (mg/mL)	278.51±42.46 <sup>a</sup>	245.74±22.74 <sup>b</sup>	227.61±33.68 <sup>b</sup>
HDL-C (mg/mL)	95.08±26.42	93.75±10.62	91.94±18.58
Non-HDL-C (mg/mL)	183.44±38.23 <sup>a</sup>	158.79±26.44 <sup>a,b</sup>	143.45±52.49 <sup>b</sup>
Non-HDL-C/HDL-C	2.08±0.75	1.88±0.44	1.68±0.84
TG (mg/mL)	253.14±82.53 <sup>a</sup>	244.81±80.74 <sup>a,b</sup>	191.79±68.33 <sup>b</sup>
Liver Cholesterol (mg/g)	46.55±5.12 <sup>a</sup>	40.98±7.26 <sup>b</sup>	41.03±7.29 <sup>b</sup>
HMG-CoA Reductase Activity (pmole/min/mg protein)	18.86±4.45	18.60±5.03	21.24±6.62

Note. Values are  $\bar{x} \pm s$ ,  $n=13$ . Means in a row with different letters (a, b) differ significantly,  $P<0.05$ .

TABLE 3

Individual Fecal Neutral and Acidic Sterols in Hamsters Fed the Control Diet and Experimental Diets Supplemented with 0.1% Red Yeast (0.1RYR) and 0.3% Red Yeast Rice (0.3RYR)

	Control	0.1RYR	0.3RYR
Coprostanol (mg)	0.81±0.19	0.85±0.20	0.97±0.17
Coprostanone (mg)	0.02±0.01	0.02±0.01	0.03±0.01
Cholesterol (mg)	0.23±0.15	0.13±0.02	0.19±0.03
Dihydrocholesterol (mg)	0.24±0.04	0.22±0.04	0.25±0.04
Campersterol (mg)	0.06±0.01 <sup>b</sup>	0.06±0.01 <sup>b</sup>	0.08±0.02 <sup>a</sup>
Total Neutral Sterol (mg)	1.36±0.13	1.27±0.25	1.51±0.20

(to be continued)

(continued)

	Control	0.1RYR	0.3RYR
Lithocholic Acid (mg)	0.47±0.56 <sup>a</sup>	1.24±0.34 <sup>b</sup>	2.22±0.82 <sup>c</sup>
Deoxycholic Acid (mg)	0.14±0.06	0.89±0.50	0.68±0.52
Chenodeoxycholic Acid (mg)	0.15±0.05 <sup>b</sup>	0.57±0.36 <sup>a</sup>	0.52±0.15 <sup>a</sup>
Cholic Acid (mg)	0.09±0.04 <sup>b</sup>	0.57±0.44 <sup>a</sup>	0.55±0.22 <sup>a</sup>
Ursodeoxycholic Acid (mg)	0.34±0.20	0.37±0.08	0.60±0.14
Total Acidic Sterol (mg)	1.03±0.65 <sup>a</sup>	3.15±0.58 <sup>b</sup>	4.44±2.43 <sup>b</sup>

Note. Values are  $\bar{x} \pm s$ ,  $n=13$ . Means in a row with different letters (a, b, c) differ significantly,  $P<0.05$ .

Total intake of cholesterol was compared with total excretion in neutral and acidic sterols (Table 4). Net cholesterol equivalent retained was calculated by difference between intake and excretion of both neutral and acidic sterols. The apparent cholesterol absorption was calculated in an equation [(cholesterol

intake-excretion of total neutral and acidic sterols)/cholesterol intake]. It was found that net cholesterol retention was the most in the control group followed by 0.1RYR and 0.3RYR groups in a decreasing order. However, no difference in cholesterol retention was seen between 0.1RYR and 0.3RYR groups.

TABLE 4

Cholesterol Balance in Hamsters Fed the Control Diet and Experimental Diets Supplemented with 0.1% Red Yeast Rice (RYR) and 0.3% Red Yeast Rice (RYR). Cholesterol retained/cholesterol intake (%) = [(cholesterol intake - excretion of total neutral and acidic sterols)/cholesterol intake]

	Control	0.1RYR	0.3RYR
Fecal Neutral Sterol (mg)	1.36±0.13	1.27±0.25	1.51±0.20
Fecal Acidic Sterol (mg)	1.03±0.65 <sup>a</sup>	3.15±0.58 <sup>b</sup>	4.44±2.43 <sup>b</sup>
Cholesterol Intake (mg)	11.47±0.94	10.99±0.56	12.04±1.18
Cholesterol Retained (mg)	8.76±1.21 <sup>a</sup>	6.49±0.53 <sup>b</sup>	6.19±1.70 <sup>b</sup>
Cholesterol Retained/Cholesterol Intake (%)	78.09±7.34 <sup>a</sup>	59.25±4.83 <sup>b</sup>	51.93±16.64 <sup>b</sup>

Note. Values are  $\bar{x} \pm s$ ,  $n=13$ . Means at the same row with different letters (a,b) differ significantly,  $P<0.01$ .

#### Effect of RYR Supplementation on Liver Cholesterol and HMGR Activity

Liver cholesterol content in two experimental groups was significantly lower than that in the control group (Table 2). No difference in hepatic cholesterol was seen between 0.1RYR and 0.3RYR groups. The statistical analysis did not find any difference in HMGR activity among the three groups.

#### Western Blot Analysis of SREBP-2, LDLR, HMGR, LXR, and CYP7A1

Western blot analysis of SREBP-2, LDLR, HMGR, LXR, and CYP7A1 is shown in Fig. 2. No significant difference in immunoreactive mass of these five proteins was observed among the three groups.

#### DISCUSSION

The present study has confirmed the hypocholesterolemic activity of RYR using hamsters as an animal model. In this regard, supplementation of RYR into diet has reduced not only plasma TC and non-HDL-C levels but also plasma TG (Table 2). Results are in agreement with those reported in human clinical trials<sup>[5,15]</sup>. The present study has also clearly demonstrated that supplementation of 0.1% and 0.3% RYR can decrease plasma TC by 12% and 18% in hamsters, respectively. Similarly, patients with stable angina have plasma TC reduced by 13% and 22%, respectively, when they are given 1200 mg or 2400 mg of Xuezhikang, which is a commercial RYR extract<sup>[16]</sup>. In rabbits given 0.4 and 1.35 g/kg/day of RYR for 200 days, serum TC is markedly reduced

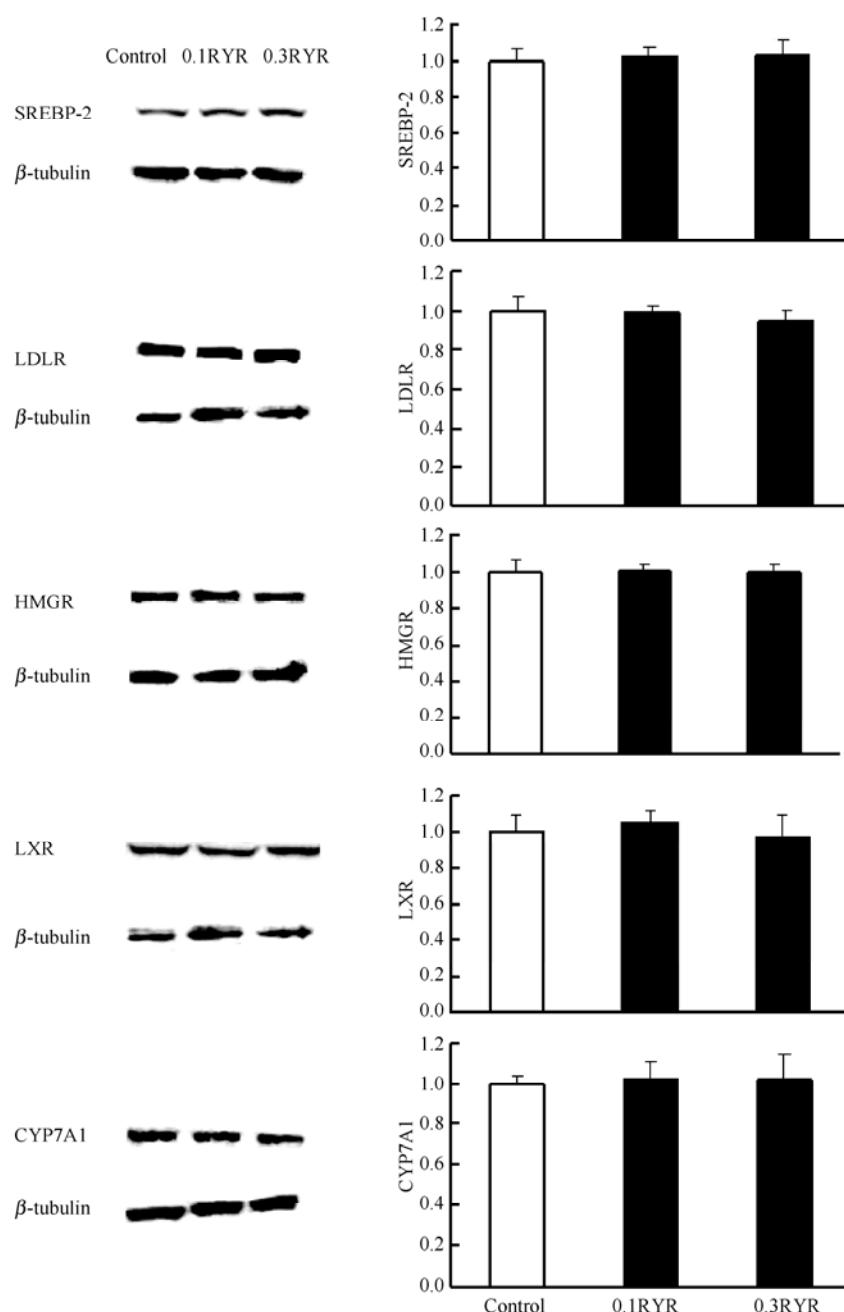


FIG. 2. Effect of 0.1% red yeast rice (0.1R.Y.R.) and 0.3% red yeast rice (0.3 R.Y.R.) on the relative immunoreactive mass of hepatic sterol regulatory element-binding protein 2 (SREBP-2), LDL receptor (LDLR), 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR), liver X receptor (LXR), and cholesterol-7 $\alpha$ -hydroxylase (CYP7A1) as determined by Western blot analysis. Data are normalized with  $\beta$ -tubulin so that value of the control group is regarded as 1.0. Values are expressed as  $\bar{x} \pm s$  ( $n=13$ ).

by 25% and 40%, respectively<sup>[2]</sup>. Similar trend and extent in serum TC reduction are also observed in quails given orally 0.8-1.6 g/kg/day R.Y.R. and maintained on a high fat diet<sup>[17]</sup>. Together with these available reports in the literatures, the present study

reaffirms that R.Y.R. can modify favorably plasma lipoproteins.

Cholesterol-lowering activity of R.Y.R. is believed to be mainly attributable to its active ingredient monacolin K (also known as mevinolin and

lovastatin), which varies from batch to batch in RYR depending the conditions of fermentation. Monacolin K reduces cholesterol biosynthesis by inhibiting HMGR in the liver. HPLC analysis found that the RYR used in the present study contained 8.8 mg/g monacolin K, which was comparable to or higher than the commercial available RYR extracts<sup>[9,18-19]</sup>. Although monacolin K in RYR is effective in lowering blood cholesterol level, one study in humans found that effect of RYR on blood cholesterol could not be fully explained by its monacolin alone, instead, it might be the combined effect of monacolins and other substances in RYR<sup>[6]</sup>.

Excessive cholesterol in body is converted to bile acids and eliminated in bile fluid. The present study is for the first time demonstrating that RYR has markedly increased fecal acidic sterol excretion by 3-4 folds. This study is an important step in establishing that observed cholesterol-lowering activity of RYR is not solely due to monacolin K alone, but also due to other substance(s) in RYR that enhances bile acids excretion. The future study is deemed necessary to identify the substance(s) that are responsible for this activity and so the underlying molecular mechanism.

We have investigated the effect of RYR on the two transcriptional factors, SREBP-2 and LXR. The former governs expression of LDLR and HMGR, whereas the latter promotes expression of CYP7A1. No differences in the protein levels of SREBP-2, HMGR, LDLR, LXR, and CYP7A1 among the three groups have been seen (Fig. 2). No study up to date has examined effect of dietary RYR on protein abundances of these proteins *in vivo* in intact animals. Therefore, nothing is available in the literatures to compare with our data. However, it has been shown that HepG2 cells treated with RYR has a decrease in cholesterol synthesis by 31%-54% because monacolin K inhibits HMGR<sup>[7]</sup>. In contrast, our Western blotting analysis has clearly demonstrated that dietary RYR has no effect on abundance of these proteins in hamsters. We offer the following explanation about this discrepancy on results between hamsters and HepG2 cells. Firstly, feeding a 0.1% cholesterol diet could have regulated the expression of SREBP-2, HMGR, LDLR, LXR, and CYP7A1 to their maximum capacity, so that effect of RYR at the present doses on expression of these genes was too trivial to have an effect. This view is supported by an observation that dietary cholesterol ranging from 0.05% to 0.5% diet suppresses strongly the gene expression of HMGR and LDLR while it has either no effect on the gene expression of CYP7A1 or increases it slightly in hamsters at higher doses<sup>[20]</sup>. Secondly, it is known that hamsters have a diurnal pattern in expression of HMGR with its activity in

midnight being 10 times higher than that in midday<sup>[21]</sup>. Perhaps, the abundance in HMGR and other four proteins was already very low because hamsters in the present study were sacrificed between 9:00-11:00 am, so that cholesterol catabolism rate was nil and no effect of dietary RYR in these proteins could be seen in hamsters after overnight fasting. We are currently investigating the possible effect of dietary RYR on these proteins in hamsters with a full stomach.

Hamsters fed the 0.3RYR diet had a significant smaller body weight ( $P<0.05$ ) and a larger liver (not statistically significant), suggesting that the supplementation of RYR into diet at 0.3% was high and possibly caused the side effect in hamsters. In this regard, RYR has been shown to cause myopathy in some patients as it is a well-known complication of HMGR inhibitors<sup>[22-23]</sup>. It is also possible that RYR may cause rhabdomyolysis in some patients<sup>[24]</sup>. However, toxicity evaluation of RYR in rabbits for as long as four months have shown no toxic symptoms<sup>[3]</sup>. Human trials have not found elevations of liver enzymes or renal impairment associated with use of RYR<sup>[6]</sup>. Nevertheless, safety concern warrants further investigation of RYR and its related supplements.

The present study confirms that supplementation of RYR into diet is hypocholesterolemic in hamsters. We have found that RYR at both 0.1% and 0.3% levels have markedly increased fecal excretion of bile acids, suggesting that enhancement of bile acid excretion is an additional mechanism by which RYR decreases plasma cholesterol.

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