Prev. Nutr. Food Sci. 2013;18(3):210-213
http://dx.doi.org/10.3746/pnf.2013.18.3.210
pISSN 2287-1098 · eISSN 2287-8602

Research Note

Cholesterol-lowering Effect of Rice Protein by Enhancing Fecal Excretion of Lipids in Rats

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ABSTRACT: The aim of this study was to investigate the effects of isolated protein from white rice on lipid metabolism in a hypercholesterolemic animal model. Male Sprague-Dawley rats were divided into three groups and fed either a normal diet or a high-cholesterol diet (HCD) containing either casein or isolated rice protein for 4 weeks. Compared with rats fed a HCD with casein, the total cholesterol (TC) level in the plasma was significantly reduced in the rats fed rice protein. However, no significant differences were observed in the triglycerides, high-density lipoprotein (HDL), and glucose levels among the experimental groups. Hepatic total lipids and TC levels were significantly decreased by supplementation with rice protein. In addition, rice protein significantly increased the levels of TC and bile acids in the feces. These results suggest that rice protein may improve HCD-induced hypercholesterolemia by enhancing fecal excretion of cholesterol.

Keywords: rice protein, cholesterol, fecal fat

INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality in the world and hypercholesterolemia is recognized as a major independent risk factor in CVD (1). Maintaining cholesterol levels in the normal range can help prevent the initiation and progression of CVD. Although several effective drugs have been developed to treat hypercholesterolemia, the risk factors for CVD can be modulated by diet (2).

Dietary protein is the source of essential amino acids required for growth and/or maintenance. In recent years, antioxidant, antitumor, and lowering-cholesterol effects have been identified in dietary proteins derived from either plant or animal sources (3,4). In particular, soy protein exerts a cholesterol-lowering effect compared with milk protein in humans and animals (5,6). However, little information is available about the effects of rice protein despite the important consumption of rice products among low-risk CVD populations. Therefore, in the current study, we focused on the effects of rice protein on lipid metabolism in a high-cholesterol diet (HCD)-fed rat.

MATERIALS AND METHODS

Isolation of rice protein

Rice protein was isolated from white rice (*Oryza sativa* L. *Japonica*) according to the modified method described by Morita and Kiriyama (7). The defatted rice flour was suspended in 0.6% termamyl α-amylase solution (v/v) and boiled for 30 min. After boiling, the hydrolysate was filtered, and the residue was washed several times with boiling water and absolute ethanol. Finally, the residue was dried at room temperature. The purity of the rice protein was approximately 87%.

Animals and diets

All animal procedures were approved by the Institutional Animal Care and Use Committee of the Korea Food Research Institute (IACUC No. 2012-0045). Six-week-old Sprague-Dawley male rats were purchased from DBL (Eumseong, South Korea). After a week of adaptation, the rats were randomly divided into three groups (each group, n=10) and fed different experimental diets for four weeks (8): normal diet, HCD, and HCD incorporated with rice protein (HCD+R). The HCD (in % weight) contained 20% casein as the protein source and 5% lard

Received May 23, 2013; Accepted July 8, 2013

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supplemented with 0.3% cholesterol. The HCD+R included rice protein in place of casein. These experimental diets were based on the American institute of nutrition-76 (AIN-76) diet formula. The rats were allowed *ad libitum* access to food and water. The composition of experimental diets is listed in Table 1. At the end of the four-week period, the rats were sacrificed in a fasting state. The liver was weighed and immediately frozen in liquid nitrogen.

Plasma lipids and glucose levels

Plasma levels of the total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), and glucose were determined by enzymatic methods using commercial kits (Shinyang Chemical Co., Seoul, South Korea).

Hepatic and fecal lipids levels

Before the rats were sacrificed, feces were collected for three consecutive days, then dried, milled, and stored at −70°C. Hepatic and fecal lipids were extracted according to the method described by Folch et al. (9). TG and TC levels in the liver and feces were determined by a colorimetric method using commercial kits (Shinyang Chemical Co). The fecal bile acid levels were measured using a total bile acid assay kit (Bioquant, Heidelberg, Germany).

Table 1. Compositions of the experimental diets (unit: g/kg diet)

Group	ND	HCD	HCD+R
Casein	200.0	200.0	−
Rice protein	−	−	200.0
Corn starch	500.0	497.0	497.0
Sucrose	150.0	150.0	150.0
Cellulose	50.0	50.0	50.0
Corn oil	50.0	−	−
Lard	−	50.0	50.0
Mineral mixture (AIN-76)	35.0	35.0	35.0
Vitamin mixture (AIN-76)	10.0	10.0	10.0
Choline chloride	2.0	2.0	2.0
Cholesterol	−	3.0	3.0
DL-Methionine	3.0	3.0	3.0

ND, normal diet; HCD, high cholesterol diet+casein; HCD+R, high cholesterol diet+rice protein.

Statistical analysis

Results were expressed as means±SEM (standard error of mean) and were analyzed by one-way analysis of variance (ANOVA). Significance of the differences between groups was determined by Duncan's multiple range test at $P<0.05$. Statistical analysis was performed using the SPSS 14.0 package (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

In this study, we evaluated the effects of rice protein on lipid metabolism in a hypercholesterolemic animal model. Our study showed that changes in body weight, food intake, food efficacy ratio, and protein efficacy ratio did not differ among experimental groups (Table 2). Compared with the rats fed casein, the TC level in the plasma of rats fed rice protein significantly decreased by 18.9% ($P<0.05$, Table 2). However, plasma TG, HDL-C, and glucose levels did not differ significantly among the experimental groups. These results are in agreement with other studies suggesting consumption of rice protein has a positive impact in animals (5,10). In addition, hepatic total lipids and TC levels in rats fed rice protein were significantly decreased by 37.5% and 40% ($P<0.05$), respectively, compared with the HCD group. Similarly, hepatic TG levels in the HCD+R group tended to be lower than the HCD group, but these differences were not significant (Fig. 1). One of the interesting findings of this study was that rats fed rice protein had a significantly higher fecal excretion of lipids and cholesterol (Table 3). Therefore, the reduction of TC in the plasma

Table 2. Body weight, food intake, food efficacy ratio, and plasma components of rats fed experimental diets for four weeks

Group	ND	HCD	HCD+R
Initial body weight (g)	160.7±11.1	168.4±5.5	164.1±3.2
Final body weight (g)	299.4±12.6	305.6±11.8	281.8±10.5
Body weight gain (g)	138.7±8.1	137.2±7.7	117.6±8.2
Food intake (g/day)	20.5±1.1	20.2±0.7	20.0±0.9
Food efficiency ratio	0.227±0.007	0.226±0.003	0.195±0.008
Protein efficiency ratio	1.135±0.038	1.135±0.016	0.973±0.021
TG (mg/dL)	49.7±4.3	62.3±4.7	54.3±2.3
TC (mg/dL)	60.3±5.5[a]	87.3±5.8[b]	70.8±2.9[a]
HDL-C (mg/dL)	36.1±1.6	31.0±2.9	35.4±2.6
Fasting glucose (mg/dL)	87.7±5.8	103.4±7.4	107.4±2.5

Results are expressed as mean±SEM (each group, n=10).
[a,b]Means in a row with superscripts without a common letter differ, $P<0.05$.
Food efficiency ratio=body weight gain/food intake.
Protein efficiency ratio=body weight gain/protein intake.
ND, normal diet; HCD, high cholesterol diet+casein; HCD+R, high cholesterol diet+rice protein; TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol.

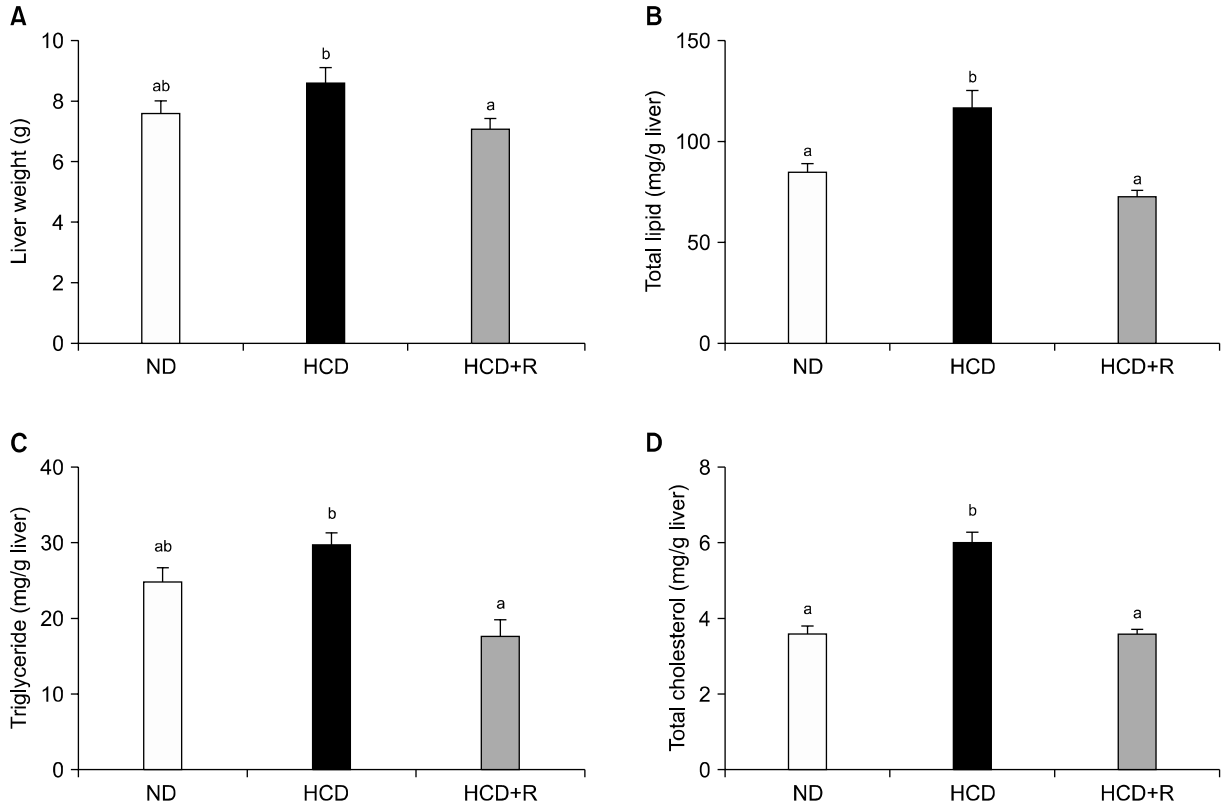

Fig. 1. Effects of rice protein isolate on liver weight and hepatic lipid levels of rats fed experimental diets for four weeks. (A) Liver weight; (B) Total lipid; (C) Triglyceride; (D) Total cholesterol. Results are expressed as mean±SEM (each group, n=10). Different letters indicate significant differences at $P<0.05$, as determined by Duncan's multiple range test.
ND, normal diet; HCD, high cholesterol diet+casein; HCD+R, high cholesterol diet+rice protein.

Table 3. Fecal lipids and bile acid of rats fed experimental diets for four weeks

Group	ND	HCD	HCD+R
Weight (g/day)	1.6±0.3[a]	2.1±0.1[b]	2.6±0.6[b]
Total lipid (mg/g dried feces)	48.8±2.0[a]	72.9±1.3[b]	80.0±2.7[b]
TG (mg/g dried feces)	2.2±1.0[a]	4.4±0.6[b]	5.1±1.6[b]
TC (mg/g dried feces)	6.9±2.1[a]	31.3±3.5[b]	48.5±8.8[c]
Bile acids (mg/g dried feces)	1.9±0.5[a]	4.2±0.6[b]	5.4±0.9[c]

Results are expressed as mean±SEM (each group, n=10).
[a-c]Means in a row with superscripts without a common letter differ, $P<0.05$.
ND, normal diet; HCD, high cholesterol diet+casein; HCD+R, high cholesterol diet+rice protein; TG, triglycerides; TC, total cholesterol.

appears to be associated with an increase in the fecal excretion of lipids and cholesterol as well as a decrease in the total lipids and cholesterol in the liver.

Rice protein likely exerts its effect on cholesterol metabolism in rats as a result of its lower digestibility. Yang et al. (11) reported a significant positive correlation between the apparent protein digestibility and the fecal excretion of total fat. Hydrophobic peptides can reduce the micellar lipid-carrying capacity by binding bile acids and reducing them below the critical micellar concentration (4). Additionally, the amino acid composition of rice protein can influence digestibility. Yang et al. (11) reported that rice protein contains more arginine (88.0 μg/mg) but less lysine (27.9 μg/mg) than casein. The higher ratio of arginine/lysine found in rice protein may regulate the digestibility of the rice protein (12). Thus, this study suggests that the cholesterol-lowering response to rice protein may be in part attributable to lower digestibility of rice protein and to the fecal excretion of TC. Some studies reported that the high arginine to lysine ratios can result in an elevation of 7-alpha-hydoxylase activity, which is a rate-limiting enzyme for the conversion of cholesterol to bile acids (13). Indigestible protein and residual peptides formed by rice protein during digestion may indeed possess the capability to bind bile acids and to inhibit the micellular solubility of cholesterol, thereby suppressing their absorption in the small intestine and increasing their fecal excretion; however, the precise mechanism remains to be clarified in further studies. In conclusion, the present study demonstrated that the cholesterol-lowering action of rice protein was associated with increased fecal excretion of cholesterol and bile acids. This finding suggests that rice protein might possess a hypocholesterolemic effect.

ACKNOWLEDGMENTS

This work was supported by the Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry and Fisheries, South Korea.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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