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## NUTRIBIOTIC RICE PROTEIN LEVEL AFFECTS CHOLESTEROL METABOLISM

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### ABSTRACT

To examine the cholesterol-lowering potential of rice protein at different dietary levels, 20-week-old male Wistar rats were fed cholesterol-free diets containing 14% rice protein (RP) or 28% rice protein (RP-H) and 14% casein (CAS) for 2 weeks. Compared with CAS, RP and RP-H exhibited the significant hypocholesterolemic action in adult rats, reflected mainly the significantly diminished contents of non-HDL cholesterol. Hepatic total cholesterol, particularly esterified cholesterol accumulation, was significantly lowered in adult rats fed RP and RP-H than those fed CAS. Fecal neutral sterol excretions were stimulated with the increasing RP level. As a result, the apparent cholesterol absorption was effectively inhibited with the increasing RP level. These results demonstrate that dietary level of RP can regulate cholesterol metabolism and plays a major role in the modulation of cholesterol absorption.

**KEYWORDS** – Rice protein, level, cholesterol, rats

### I. INTRODUCTION

Rice is a staple cereal and widely consumed in the world. The association of rice protein consumption with modulation of plasma cholesterol level has been demonstrated in some studies [1, 2], but evidence on how rice protein level affects cholesterol metabolism is lacking.

Dietary protein level is suggested to influence cholesterol metabolism through the modification of dietary supply of amino acids. The hypercholesterolemia produced by casein or casein amino acids was found to increase with levels in the diet [3], whereas the plasma cholesterol level in rats tended to decrease with the increasing dietary protein level (10, 20, and 30%) of soybean protein [4]. However, contradictory results were found in some studies, which suggested that dietary protein level did not affect plasma cholesterol concentrations, whatever the type of protein consumed [5, 6]. Taken together, there is not yet a comprehensive understanding for such a link of dietary protein level and plasma cholesterol concentration.

In the present study, a simple procedure for industrial production of rice protein by the alkaline extraction method was developed in order to evaluate the

physiological function of rice protein. The key questions addressed are: (1) whether does rice protein possess a vital function in improving cholesterol metabolism by alteration of dietary protein level in rats and (2) is rice protein level able to affect cholesterol absorption?

### II. METHODOLOGY

1) *Protein sources.* Casein (CAS) and rice protein (RP) were used as the dietary protein sources. RP was prepared by the alkaline extraction method and CAS was obtained from Hualing (Gansu, China). Amino acid analysis of these proteins was performed using a Biochrom 30 amino acid analyzer according to Yang et al. [1]

2) *Animals and diets.* Twenty-week-old male Wistar rats were purchased from Harbin Medical University and individually housed in metabolic cages in a room maintained at  $22 \pm 2$  °C under a 12-h light-dark cycle (06:00 – 18:00 for light). Rats were allowed free access to commercial pellets for 3 d. After acclimatization, rats were randomly divided into three groups of similar body weight. Each group consisted of six rats.

All animals were fed *ad libitum* with experimental diets according to the formula recommended by American

Institute of Nutrition. For 2 weeks, adult rats were fed cholesterol-free diets with normal or high dietary level (as crude protein, CP) of RP (normal, 14%, RP; high, 28%, RP-H) and 14% CAS. Diets were completed to 100% with starch.

3) *Samples collection.* During the feeding period, food consumption and body weight were recorded daily in the morning before replenishing the diet. Feces were collected for the final 3 d of the experimental period and dried to a constant weight and ground to a fine powder for fecal steroids determination according to Yang et al. [1].

At the end of the feeding period, the rats were deprived for 18 h and then sacrificed. Blood was withdrawn from abdominal vein into a heparinized syringe under anesthesia with sodium pentobarbital (50mg/kg body weight), immediately cooled on ice and separated by centrifugation at 12,000 x g for 5 min. The plasma obtained was frozen at -20°C until analysis. After blood collection, the liver was excised immediately, rinsed in saline and weighed after blotted on a filter paper. The whole liver was cut into three portions and quickly freeze-clamped in liquid nitrogen and stored at -80°C until analysis.

4) *Isolation of very-low-density lipoprotein.* Very-low-density lipoprotein (VLDL) ( $d < 1.006$  g/ml fractions) in the plasma was isolated by the ultracentrifugation method of Yang and Kadowaki [7]. Samples of plasma (1.6 ml) were added to ultracentrifuge tubes, and 0.8 ml of  $d 1.006$  g/ml solution was layered over the surface. The 0.8 ml at the top of the centrifuge tube contained the VLDL fraction and was recovered quantitatively.

5) *Plasma lipid analysis.* Plasma concentrations of total cholesterol (TC), VLDL cholesterol (VLDL-C) and high-density lipoprotein cholesterol (HDL-C) were measured colorimetrically with commercial kits (Shanghai Fosun Long March Medical Science Co., Ltd, Shanghai, China). Plasma non-HDL-C and low-density lipoprotein cholesterol (LDL-C) was calculated as: non-HDL-C = TC - HDL-C, LDL-C = TC - HDL-C - VLDL-C.

6) *Liver lipid analysis.* The lipids in the liver were extracted and purified according to the method of Folch et al. [8], and were analyzed as described by Yang et al. [1]. Samples of liver were extracted with chloroform/methanol (2:1, v/v). Total- and free-cholesterol were measured with a commercial kit (Shanghai Fosun Long March Medical Science Co., Ltd, Shanghai, China). The concentration of esterified cholesterol was calculated as: esterified cholesterol = total cholesterol - free cholesterol. Cholesterol esterification ratio was described as: cholesterol esterification ratio = (total cholesterol - free cholesterol) × 100/total cholesterol.

7) *Determination of fecal excretion.* Fecal bile acid and neutral sterol concentrations were measured as described in detail previously [1]. Total bile acids were determined by 3-hydroxysteroid dehydrogenase (Sigma, St.Louis, Mo., USA) with sodium taurocholate as the standard. Fecal neutral sterols were analyzed according to Yang et al. [1]. Apparent cholesterol absorption was calculated as: apparent cholesterol absorption = cholesterol intake - neutral sterol excretion.

*Statistical Analysis.* Data are presented as means ± SEM. Differences between groups were examined for statistical significance using the one-way analysis of variance (ANOVA), and then determined with the least significant difference test. The criterion for significance was  $p < 0.05$ .

### III.RESULTS

#### A.Food Intake and Body Weight

Food intake, final body weight and body weight gain were not significantly different among groups. Gains in body weight were slightly improved with the increasing RP level. RP-H exhibited a 11.22% higher body weight gain than RP (Fig. 1). The dietary levels of RP did not affect food intake

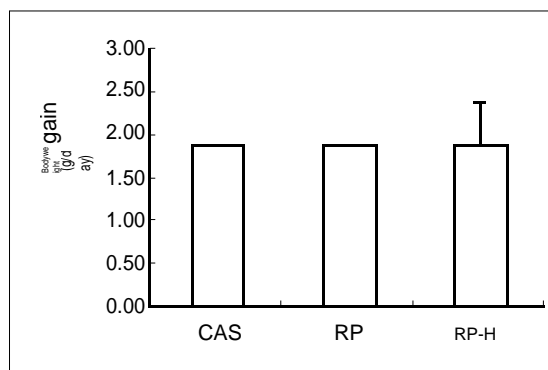


Fig. 1. Body weight gain of rats fed experimental diets.

#### B. Plasma Lipids and Lipoprotein Profiles

As shown in Fig 2, plasma TC and non-HDL-C concentrations were significantly lower in adult rats fed RP and RP-H as compared with CAS ( $p < 0.05$ ). The hypocholesterolemic effects of RP (TC: -21.25%; non-HDL-C: -40.32%) were lower than those of RP-H (TC: -24.38%; non-HDL-C: -45.16%).

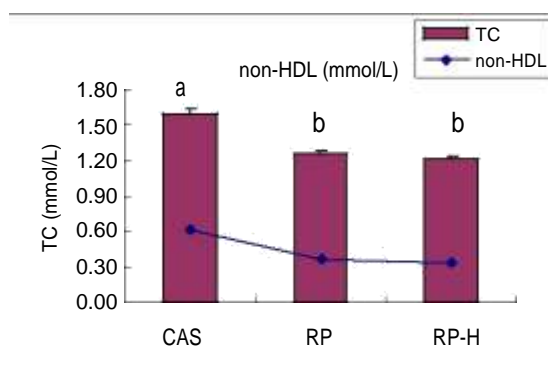
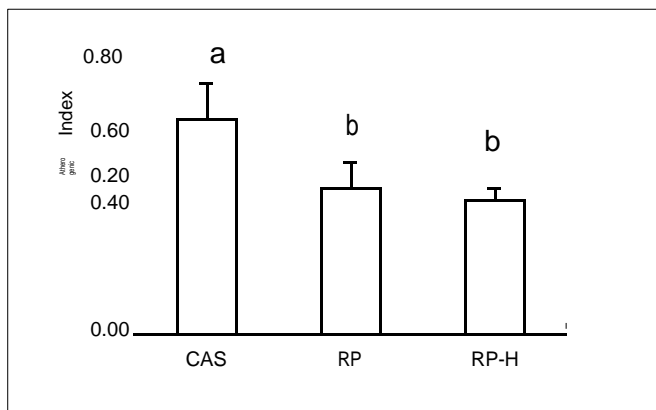


Fig. 2. Plasma TC and non-HDL-C of rats fed experimental diets.

Plasma VLDL-C and LDL-C concentrations were distinctly lower in RP-fed adult rats compared with CAS,



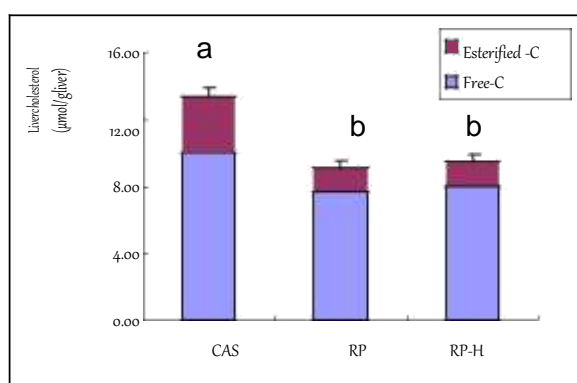
**Fig. 3. Atherogenic index of rats fed experimental diets.**

whereas HDL-C level was not significantly different among groups. As a result, the atherogenic index was significantly lower by -33.33% and -38.10% in RP and RP-H, respectively (Fig. 3).

**C. Hepatic Lipids**

The lowest liver weights were observed in adult rats fed RP, and larger liver swellings were induced by increasing dietary level of RP-H. The liver weight of rats fed RP-H did not differ from those fed CAS.

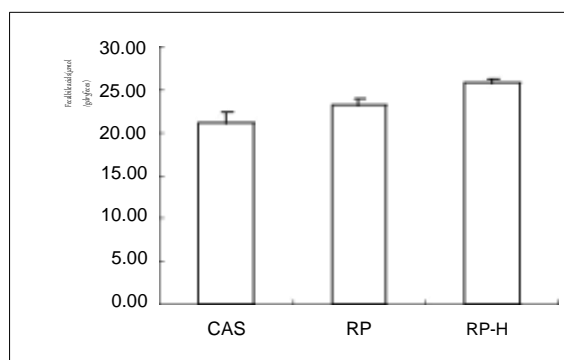
Hepatic accumulations of total cholesterol in RP-feeding groups were significantly lower than those in the CAS group ( $p < 0.05$ ) (Fig. 4). The hepatic cholesterol-lowering actions induced by RP-feeding were mainly reflected by diminished concentrations of free- and esterified cholesterol. RP and RP-H significantly reduced hepatic esterified cholesterol levels by 56.89% and 56.30%, respectively. These changes resulted in the significant reduction in hepatic cholesterol esterification ratio, which fell by 37.06% in RP and 38.24% in RP-H, as compared with CAS.



**Fig. 4. Liver cholesterol concentrations of rats fed experimental diets**

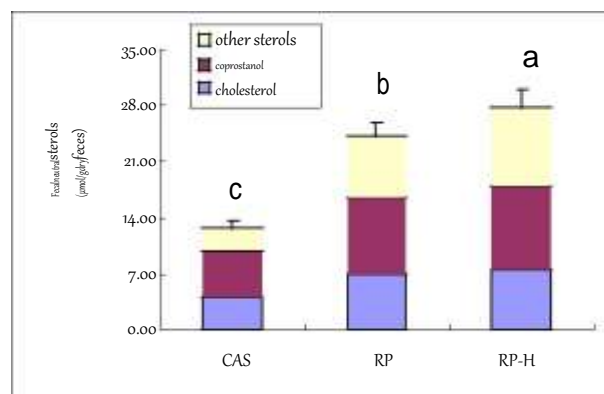
**D. Fecal Sterol Extraction**

The 3-d fecal output tended to be 5-9% lower in RP-feeding groups than those fed CAS. Fecal total bile acid concentrations slightly increased by RP level (Fig. 5).



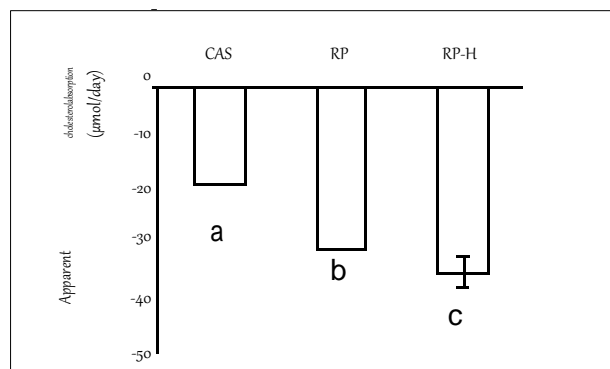
**Fig. 5. Fecal bile acids extraction of rats fed experimental diets.**

As shown Fig. 6, fecal neutral sterol concentrations were significantly stimulated with RP-feeding. Compared with CAS, fecal neutral sterols excretion was 91.80% higher in adult rats fed RP, while RP-H caused a 2-fold increase.



**Fig. 6. Fecal neutral sterol extraction of rats fed experimental diets.**

As a result, apparent cholesterol absorption was effectively inhibited with the increasing RP level (Fig. 7). Apparent cholesterol absorption in adult rats was significantly depressed from -76.13 to -108.84% in RP-feeding as compared with CAS-feeding.



**Fig. 7. Cholesterol absorption of rats fed experimental diets.**

**IV. DISCUSSION**

We examined the lipid-lowering potential of RP

at different dietary levels and especially the effect of RP on cholesterol metabolism. In this study, the plasma total cholesterol and non-HDL-cholesterol concentration in the RP-fed groups were significantly lower than those fed CAS. In agreement with our previous findings [1], the factor lowering the cholesterol concentration in adult rats fed RP was the lowering of the non-HDL-cholesterol level.

Of interest was the finding that fecal excretion of total neutral sterols was significantly increased by RP and RP- H. Thus, data obtained here support our hypothesis that RP level can affect cholesterol metabolism and hypocholesterolemic activity of RP is at least in part due to an inhibition of cholesterol absorption.

The liver plays a central role in maintaining cholesterol homeostasis through regulatory hepatic outputs of cholesterol into either bile or plasma [7]. It has been demonstrated that hepatic cholesterol ester induced by acylCoA: cholesterol acyltransferase (ACAT) availability can regulate hepatic VLDL secretion [9]. There have been evidences from both *in vitro* and *in vivo* studies indicating a correlation between inhibitions of ACAT activity, reduced hepatic cholesterol ester content and reduced VLDL secretion [10]. These considerations, together with our experimental results obtained, support the view that inhibition of newly synthesized cholesterol ester catalyzed by ACAT may result in a reduction in hepatic VLDL secretion into plasma. Thus, the fact that hepatic cholesterol concentration was sufficiently decreased and cholesterol accumulation almost completely suppressed by RP-feeding suggests that hepatic cholesterol output was effectively inhibited in adult rats. Therefore, hepatic cholesterol ester storage and cholesterol esterification ratio were markedly suppressed with the increasing RP level, leading to the hypocholesterolemic action.

## V. CONCLUSION

In conclusion, the present study demonstrates that dietary level of RP can regulate cholesterol metabolism and plays a major role in the modulation of cholesterol absorption. The hypocholesterolemic action induced by different RP levels is attributed to the significant reduction in hepatic cholesterol accumulations involved with the hepatic cholesterol outputs. In addition to an inhibition of

cholesterol absorption, the decrease in VLDL secretion by liver may be the main modulator responsible for the cholesterol-lowering action of RP.

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