

Effects of Cholestyramine and Phytosterol on Cholesterol Metabolism in the Starvation-refeeding Rats*

Reiko INAI* and Tatsuhiro MATSUO

Abstract

The present study investigated the effects of cholestyramine and phytosterol on cholesterol metabolism in the starvation-refeeding rats. Twenty female Donryu rats (age 5 weeks) were fed a cholesterol-free diet for 14 days and then divided into four groups, a control (Con), high-cholesterol (Chol), Chol-cholestyramine (Chol-Cm), and Cm (Experiment 1) or Chol-phytosterol (Chol-PS) (Experiment 2) diet group. All groups were fasted for 2 days followed by 3 days feeding. Food intake, final body weight and liver weight were not different among the four groups. The plasma total cholesterol, free cholesterol, cholesteryl ester, and non HDL-cholesterol concentrations were significantly higher and plasma HDL-cholesterol concentration was significantly lower in the Chol group than in the other groups. Dietary cholestyramine inhibited the change in the plasma cholesterol concentrations induced by a high-cholesterol diet (Experiment 1). However, no effect on the plasma cholesterol concentrations by dietary phytosterol was found (Experiment 2). Dietary cholestyramine and phytosterol significantly inhibited liver cholesterol accumulation but the inhibitory effect of phytosterol was lower than that of cholestyramine. These results suggest that the dietary cholestyramine and phytosterol affected cholesterol metabolism in rats fed a high-cholesterol diet under starvation-refeeding status.

Key words : cholestyramine, phytosterol, cholesterol, starvation-refeeding, rat

INTRODUCTION

Starvation has been recognized to reduce the activities of hepatic enzymes in animals⁽¹⁻⁷⁾. Some of the largest decreases in activity occur with the nicotinamide adenine dinucleotide phosphate (NADP)-linked dehydrogenases of the cytosol⁽³⁾ and organelles^(1,5). However, it is well established that starvation followed by refeeding (starvation-refeeding) causes an increase in rat liver lipogenic enzyme activity in comparison to the levels obtained with the same diet fed *ad libitum*. Szepesi and Berdanier⁽⁸⁾ reported that the response to a 2-day period of starvation followed by a 2-day period of refeeding typically include an increase in liver lipid content and an increase or overshoot in the activities of the hepatic enzymes concerned with lipogenesis. Moreover, Wurdeman *et al.*⁽⁹⁾ and Berdanier and Shubeck⁽¹⁰⁾ demonstrated that glucocorticoid and insulin are involved in the genesis of the enzyme overshoot response to starvation-refeeding, perhaps through an effect on *de novo* RNA synthesis. Cholesterol, one of the

physiologically significant lipids along with triacylglycerol (TG) or fatty acids, is present in tissues and plasma lipoprotein either as free cholesterol, or in combination with long-chain fatty acids as cholesteryl ester⁽¹¹⁾. It is synthesized in many tissues from acetyl-CoA and is ultimately eliminated from the body in the bile as cholesterol or bile salts. Cholesterol is the precursor of all other steroids in the body such as glucocorticoid and sex hormones⁽¹¹⁾. We previously confirmed that cholesterol synthesis in the liver was accelerated under the activated condition of hepatic lipogenesis because the enzymes related to cholesterol synthesis occur with the NADP-linked dehydrogenases (data not shown).

Cholestyramine, a resin which strongly binds bile acids, is used as a hypolipidemic drug. It is considered to lower serum cholesterol levels by inhibiting the enterohepatic circulation of bile acids⁽¹²⁾. Phytosterols are cholesterol-like compounds that occur naturally in plant foods and reduce cholesterol absorption⁽¹³⁾. In the present study, we investigated the effect of cholestyramine and phytosterol on cholesterol metabolism in

* Department of Health and Nutritional Science, Nagoya Keizai University, 61-1 Uchikubo, Inuyama, Aichi 484-8504

the starvation-refeeding rats.

Materials and Methods

All procedures involving the rats were approved by the Experimental Animal Care Committee of Kagawa University.

Experiment 1

Animals, diets, and experimental design

Twenty female Donryu rats (age 5 weeks) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All rats were housed individually at 22 ± 1 °C with light from 08:00 to 20:00 h and free access to water. The rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) *ad libitum* until 6 weeks of age. The rats were fed synthetic high-fat diets (Table 1). This diet also contained per kilogram: retinyl palmitate, 60,000IU; ergocalciferol, 600IU; α -tocopheryl acetate, 1 gram. The vitamin and mineral mixtures based on Harper's mixture were used⁽¹⁴⁾. After a 14 day feeding period, the rats were divided into four groups, a control (Con), high-cholesterol (Chol), cholestyramine (Cm), and high-cholesterol and cholestyramine (Chol-Cm) diet groups. All groups were fasted for 2 days followed by 3 days refeeding. After the starvation-refeeding period, the rats were killed by heart puncturing under anesthesia. Blood was collected to obtain plasma, and the liver was quickly removed, weighed, and stored at -40°C.

Analysis

The plasma total cholesterol, free cholesterol, HDL cholesterol and TG concentrations were determined using kits (Cholesterol E-Test, Free Cholesterol E-Test, HDL-Cholesterol E-Test and Triglyceride E-Test, Wako Pure Chemical Industries, Osaka, Japan). The plasma cholesteryl ester concentration was calculated from the plasma total cholesterol and free cholesterol concentrations. Total liver lipid and plasma lipid were extracted by the method of Folch *et al.*⁽¹⁵⁾. The liver total cholesterol, free cholesterol and cholesteryl ester contents were determined by the method previously^(16, 17). Plasma cholesteryl ester was divided using a thin-layer chromatography technique⁽¹⁸⁾. The fatty acid composition of plasma cholesteryl ester was determined using gas chromatography. The TG extract liquid was vaporized by nitrogen gas and then transmethylated using methanol-sulfuric acid (230:2, v/v). The fatty acid methyl esters were extracted with hexane and separated in a gas chromatograph (ModelG-163, Hitachi Co., Tokyo, Japan) equipped with a 3 mm x 2 m glass column which filled up packing material (EGSS-Y, Shinwa Chemical Industries, Ltd., Tokyo, Japan). The column temperature was set at 187°C. The carrier gas was helium at a flow rate of 40 ml·min⁻¹. Methyl esters of individual fatty acids were identified in the chromatograms by comparing their retention times to those of pure methyl esters, and were quantified by comparing the areas under their peaks.

Table 1 Composition of experimental diets.

Groups	Experiment 1				Experiment 2			
	Con	Chol	Cm	Chol-Cm	Con	Chol	Chol-Cm	Chol-PS
<i>Ingredients</i>	<i>g/kg diet</i>							
Casein	250.0	250.0	250.0	250.0	250.0	250.0	250.0	250.0
α -Starch	440.0	427.5	420.0	407.5	440.0	427.5	407.5	417.5
Beef tallow	200.0	200.0	200.0	200.0	200.0	200.0	200.0	200.0
Mineral mixture*	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin Mixture*	8.5	8.5	8.5	8.5	8.5	8.5	8.5	8.5
Cellulose	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Choline chloride	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cholesterol	-	10.0	-	10.0	-	10.0	10.0	10.0
Gall powder	-	2.5	-	2.5	-	2.5	2.5	2.5
Cholestyramine	-	-	20.0	20.0	-	-	20.0	-
Stigmasterol	-	-	-	-	-	-	-	10.0

These diets contained per kilogram: retinyl palmitate, 60,000IU; ergocalciferol, 600IU; α -tocopheryl acetate, 1 gram. Con, Control; Chol, Cholesterol; Cm, Cholestyramine; PS, Phytosterol. *Harper's mixture.

Experiment 2

Animals, diets, and experimental design

Twenty female Donryu rats (age 5 weeks) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All rats were housed individually at 22 ± 1 °C with light from 08:00 to 20:00 h and free access to water. The rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) *ad libitum* until 6 weeks of age. The rats were fed synthetic high fat diets (Table 1). This diet also contained per kilogram: retinyl palmitate, 60,000IU; ergocalciferol, 600IU; α -tocopheryl acetate, 1 gram. The vitamin and mineral mixtures based on Harper's mixture were used⁽¹⁵⁾. After a 14 day of feeding period, the rats were divided into four groups, a control (Con), high-cholesterol (Chol), high-cholesterol and cholestyramine (Chol-Cm), high-cholesterol and phytosterol (Chol-PS) diet diets. All groups were then fasted for 2 days followed by 3 days feeding. After the starvation-refeeding period, the rats were killed by heart puncturing under anesthesia. Blood was collected to obtain plasma, and liver was quickly removed, weighed, and stored at -40°C.

Analysis

The plasma substrates, liver lipids and fatty acid composition of plasma cholesteryl ester were assayed as in Experiment 1.

Statistical analysis

The values are expressed as means \pm standard deviation (SD). Data were evaluated by one-way ANOVA and Turkey's test was used to determine specific mean differences. A *p* val-

ue of < 0.05 was considered statistically significant. All analyses were performed with a commercially available statistical package (Excel Statistics, SSRI Co., Ltd., Tokyo, Japan).

Results and Discussion

Experiment 1

Food intake, body weight and liver weights

Food intake, final body weight and liver weight were not different among the four groups (mean values: food intake, 17 g; final body weight 204 g; liver weight, 10 g [all dietary groups]).

Plasma substrates and liver lipids

Table 2 shows the concentrations of plasma cholesterol and TG and the content of liver lipids. The plasma total cholesterol, free cholesterol, cholesteryl ester, non HDL-cholesterol concentrations, and total cholesterol/TG ratio were significantly higher and the plasma HDL-cholesterol concentration was significantly lower in the Chol group than in the Con, Cm, and Chol-Cm groups. Dietary cholestyramine inhibited the changes in the plasma cholesterol concentrations induced by a high-cholesterol diet. The plasma TG concentration was not different among the four groups.

The liver total cholesterol, cholesteryl ester and TG content were significantly higher and the liver free cholesterol content was significantly lower in the Chol group than in the other groups. Dietary cholestyramine inhibited the accumulation of liver cholesterol and TG induced by high-cholesterol diet.

Table 2 Effect of dietary cholesterol and cholestyramine on plasma and liver components in rats under starvation-refeeding status (Experiment 1).

Plasma	Total Chol	Free Chol	Chol ester	HDL-Chol	Non HDL-Chol	TG	Total Chol/TG
Groups	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	
Con	82.8 \pm 6.6 ^b	21.3 \pm 3.9 ^b	61.6 \pm 9.1 ^b	55.9 \pm 7.8 ^a	22.9 \pm 6.2 ^b	127.1 \pm 32.6	0.69 \pm 0.21 ^b
Chol	226.1 \pm 55.0 ^a	38.6 \pm 10.3 ^a	187.4 \pm 44.9 ^a	17.8 \pm 6.4 ^c	208.4 \pm 60.6 ^a	102.1 \pm 22.6	2.42 \pm 1.28 ^a
Cm	80.5 \pm 10.4 ^b	19.0 \pm 3.6 ^b	61.5 \pm 8.0 ^b	36.7 \pm 5.1 ^b	43.9 \pm 5.9 ^b	139.1 \pm 36.4	0.60 \pm 0.14 ^b
Chol-Cm	93.3 \pm 12.7 ^b	20.6 \pm 2.2 ^b	72.6 \pm 10.6 ^b	41.2 \pm 7.2 ^b	52.1 \pm 7.0 ^b	139.8 \pm 27.6	0.69 \pm 0.18 ^b
Liver	Total Chol	Free Chol	Chol ester	TG			
Groups	mg/g tissue	mg/g tissue	mg/g tissue	mg/g tissue			
Con	7.0 \pm 1.4 ^b	5.4 \pm 1.0 ^a	1.9 \pm 0.4 ^c	21.4 \pm 5.7 ^b			
Chol	21.5 \pm 0.9 ^a	1.4 \pm 0.3 ^b	20.1 \pm 0.9 ^a	33.5 \pm 4.7 ^a			
Cm	4.7 \pm 0.2 ^c	2.4 \pm 0.3 ^c	2.3 \pm 0.3 ^{bc}	10.9 \pm 1.5 ^c			
Chol-Cm	8.2 \pm 1.4 ^b	1.2 \pm 0.6 ^c	7.3 \pm 0.8 ^b	17.9 \pm 4.4 ^b			

Values are means \pm SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. Con, Control; Chol, Cholesterol; Cm, Cholestyramine; TG, triacylglycerol.

Fatty acid composition of plasma cholesteryl ester

Table 4 shows the fatty acid composition of plasma cholesteryl ester. The percentage of palmitic, palmitoleic, stearic, and oleic acids were significantly higher whereas linoleic and arachidonic acids were significantly lower in the Chol group than in the other groups. Dietary cholestyramine inhibited the changes in the percentage of fatty acids in plasma cholesteryl ester induced by a high-cholesterol diet.

Experiment 2

Food intake, body weight and liver weights

Food intake, final body weight and liver weight were not different among the four groups (mean values: food intake, 16 g; final body weight 211 g; liver weight, 11 g [all dietary groups]).

Plasma substrates and liver lipids

Table 3 shows the concentrations of plasma cholesterol and TG and the content of liver lipids. The plasma total cholesterol, free cholesterol, cholesteryl ester, and non HDL-cholesterol concentrations were significantly higher and the plasma HDL-cholesterol concentration was significantly lower in the Chol group than in the Con group. Dietary cholestyramine inhibited the changes in the plasma cholesterol concentrations induced by high-cholesterol diet. However, no effect by dietary phytosterol was found.

The liver total cholesterol and cholesteryl ester content were significantly higher and the liver free cholesterol content was significantly lower in the Chol group than in the Con

group. Dietary cholestyramine inhibited the accumulation of liver cholesterol induced by a high-cholesterol diet. Dietary phytosterol significantly inhibited liver cholesterol accumulation but the inhibitory effect of phytosterol was lower than that of cholestyramine.

Fatty acid composition of plasma cholesteryl ester

Table 4 shows the fatty acid composition of plasma cholesteryl ester. The percentage of palmitoleic, palmitoleic, stearic, and oleic acids were significantly higher whereas those of linoleic and arachidonic acids were significantly lower in the Chol group than in the Con and Cm groups. Dietary cholestyramine inhibited the changes in the percentage of fatty acids in plasma cholesteryl ester induced by a high-cholesterol diet.

Many researchers reported that cholestyramine and phytosterol in experiments on animals⁽¹⁹⁻²²⁾ and humans^(23, 24). Both cholestyramine and phytosterol exhibited a hypocholesterolemic effect, and increased the fecal excretion of sterols. In the present study, we found that 1% cholestyramine in the diet dramatically decreased the plasma total and non-HDL cholesterol concentrations and increased HDL-cholesterol concentration in rats fed a high-cholesterol diet, results which support those previous findings.

In Experiment 2, however, phytosterol did not have a very strong cholesterol-lowering effect in rats a fed high-cholesterol diet. Ling and Jones⁽²³⁾ suggested that phytosterol (1% sitosterol) effectively modify circulating lipoprotein cholesterol concentrations at the level of the intestine, rather than internally at the level of cholesterologenesis in rats. Chien *et al.*⁽²¹⁾

Table 3 Effect of dietary fats and cholestyramine on plasma and liver components in rats under starvation-refeeding status (Experiment 2).

Plasma	Total Chol	Free Chol	Chol ester	HDL-Chol	Non HDL-Chol	TG	Total Chol/TG
Groups	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	
Con	95.5 ± 29.8	18.7 ± 2.6	76.8 ± 29.6	47.1 ± 18.4 ^a	48.4 ± 12.8 ^b	148.5 ± 60.4	0.67 ± 0.2
Chol	223.1 ± 136.4	38.1 ± 20.7	185.0 ± 116.0	20.4 ± 4.6 ^b	202.7 ± 140.2 ^{ab}	93.9 ± 32.1	2.56 ± 1.9
Chol-Cm	108.7 ± 32.2	21.7 ± 4.5	87.0 ± 28.6	33.7 ± 5.0 ^{ab}	75.0 ± 31.4 ^{ab}	138.1 ± 44.7	0.89 ± 0.4
Chol-PS	229.5 ± 105.3	36.9 ± 12.7	192.6 ± 92.8	19.3 ± 4.8 ^b	210.2 ± 109.2 ^a	129.3 ± 53.2	2.09 ± 1.1
Liver	Total Chol	Free Chol	Chol ester	TG			
Groups	mg/g tissue	mg/g tissue	mg/g tissue	mg/g tissue			
Con	4.9 ± 0.6 ^d	3.9 ± 0.7	0.9 ± 0.3 ^d	15.1 ± 10.3			
Chol	22.4 ± 3.6 ^a	4.1 ± 2.7	18.3 ± 3.3 ^a	31.5 ± 18.2			
Chol-Cm	8.6 ± 1.9 ^c	4.5 ± 1.1	4.2 ± 0.9 ^c	14.4 ± 5.9			
Chol-PS	13.2 ± 1.9 ^b	3.1 ± 1.4	10.2 ± 2.6 ^b	15.9 ± 5.9			

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. Con, Control; Chol, Cholesterol; Cm, Cholestyramine; PS, Phytosterol; TG, triacylglycerol.

demonstrated that phytosterol-containing lactic-fermented milk powder (0.74-1.85% phytosterol) could be used as a potential cholesterol-lowering ingredient in the management of hypercholesterolemia in hamsters. The differences between our results and previous findings might be due to differences in experimental design or the nutritional status of the rats.

The previous findings demonstrated that diets supplemented with cholestyramine or phytosterol significantly increased the fecal cholesterol concentration, and the increase was proportional to the supplemental contents of the diets^(20, 22). In the present study (Experiment 2), fecal dry weight was higher in the Chol-Cm and Chol-PS group than in the Chol group (4.2 ± 0.8, 2.4 ± 0.7 vs. 1.7 ± 0.5 g/2 days). Fecal cholesterol excre-

tion was higher in the Chol-Cm and Chol-PS group than in the Chol group (data not shown). It has been reported that the net effect of dietary cholesterol absorption, endogenous cholesterol synthesis and biliary cholesterol excretion regulates body cholesterol balance^(25, 26). The cholesterol-lowering effect of diets containing cholestyramine and phytosterol might be due to the inhibition of intestinal dietary cholesterol absorption, but also to the interference of biliary cholesterol re-absorption.

The results of the present study suggest that the dietary cholestyramine and phytosterol affected cholesterol metabolism in starved-refed rats fed a high cholesterol diet, but a further detailed study is needed to confirm and clarify this mechanism.

Table 4 Fatty acid composition of plasma cholesteryl ester in rats under starvation-refeeding status (Experiments 1 and 2).

Groups	g/100g total fatty acid							
	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:6
Experiment 1								
Con	0.4 ± 0.2	6.5 ± 1.7 ^b	3.6 ± 1.0 ^b	0.7 ± 0.6 ^b	18.6 ± 2.6 ^b	11.9 ± 2.2 ^a	55.6 ± 4.1 ^a	2.2 ± 1.0
Chol	0.4 ± 0.3	14.3 ± 4.2 ^a	7.7 ± 1.4 ^a	3.8 ± 1.4 ^a	60.3 ± 8.2 ^a	4.3 ± 1.3 ^b	9.6 ± 4.9 ^b	1.2 ± 0.8
Cm	0.4 ± 0.2	6.8 ± 1.4 ^b	3.5 ± 0.6 ^b	0.3 ± 0.3 ^b	19.9 ± 2.6 ^b	11.6 ± 1.0 ^a	52.5 ± 5.0 ^a	1.8 ± 1.6
Chol-Cm	0.5 ± 0.3	6.7 ± 2.1 ^b	3.3 ± 0.8 ^b	0.8 ± 0.8 ^b	19.4 ± 3.5 ^b	11.2 ± 1.6 ^a	51.5 ± 9.9 ^a	2.6 ± 1.2
Experiment 2								
Con	0.5 ± 0.1	6.1 ± 0.8 ^c	3.7 ± 0.4 ^c	0.8 ± 0.4 ^c	18.7 ± 3.3 ^c	10.8 ± 0.6 ^a	50.5 ± 6.4 ^a	0.5 ± 1.0 ^b
Chol	0.4 ± 0.1	13.3 ± 2.4 ^a	7.1 ± 1.9 ^a	3.7 ± 0.5 ^a	55.6 ± 6.2 ^a	4.9 ± 2.3 ^c	11.2 ± 6.3 ^c	0.2 ± 0.4 ^b
Chol-Cm	0.5 ± 0.1	8.6 ± 0.9 ^b	5.4 ± 0.9 ^b	2.0 ± 0.7 ^b	35.7 ± 9.2 ^b	8.2 ± 1.6 ^b	33.0 ± 8.4 ^b	0.4 ± 0.6 ^b
Chol-PS	0.4 ± 0.0	13.2 ± 2.6 ^a	8.0 ± 1.7 ^a	2.6 ± 0.5 ^b	55.1 ± 7.7 ^a	4.7 ± 1.5 ^c	11.5 ± 7.7 ^c	1.3 ± 1.3 ^a

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at p<0.05.

Con, Control; Chol, Cholesterol; Cm, Cholestyramine; PS, Phytosterol.

References

1. SICULELLA, L., SABETTA, S., di SUMMA, R., LEO, M., GIUDETTI, A.M., PALMIERI, F., and GNONI, G.V. Starvation-induced posttranscriptional control of rat liver mitochondrial citrate carrier expression. *Biochem. Biophys. Res. Commun.*, **299**, 418-423 (2002).
2. BARROSO, J.B., PERAGÓN, J., CONTRERAS-JURADO, C., GARCÍA-SALGUERO, L., CORPAS, F.J., ESTEBAN, F.J., PEÑADO, M.A., De La HIGUERA, M., and LUPIÁÑEZ, J.A. Impact of starvation-refeeding on kinetics and protein expression of trout liver NADPH-production systems. *Am. J. Physiol.*, **274**, R1578-R1587 (1998).
3. FREEDLAND, R.A. Effect of progressive starvation on rat liver enzyme activities. *J. Nutr.*, **91**, 489-495 (1967).
4. GUDERLEY, H.G., LAPOINTE, D., BEDARD, M., and DUTIL, J. Metabolic priorities during starvation: enzyme sparing in liver and white muscle of Atlantic cod, *Gadus morhua* L. *Comp. Biochem. Physiol.*, **135**, 347-356 (2003).
5. AWAD, S., CONSTANTIN-TEODOSIU, D., MACDONALD, I.A., and LOBO, D.N. Short-term starvation and mitochondrial dysfunction – A possible mechanism leading to postoperative insulin resistance. *Clin. Nutr.*, **28**, 497-509 (2009).
6. LONGO, V., INGELMAN-SUNDBERG, M., AMATO, G., SALVETTI, A., and GERVASI, P.G. Effect of starvation and chlormethiazole on cytochrome P450s of rat nasal mucosa. *Biochem. Pharmacol.*, **59**, 1425-1432 (2000).
7. SCHIMKE, R.T. Differential effect of fasting and protein-free diets on levels of urea cycle enzymes in rat liver. *J. Biol. Chem.*, **237**, 1921-1927 (1966).

8. SZEPSI, B., and BENDER, C.D. Time course of the starve-refeed response in rats; the possible role of insulin. *J. Nutr.*, **101**, 1563–1574 (1971).
9. WURDEMAN, R., BERDANIER, C.D., and TOBIN, R.B. Enzyme overshoot in starved-refed rats: role of the adrenal glucocorticoid. *J. Nutr.*, **108**, 1457–1461 (1978).
10. BERDANIER, C.D., and SHUBECK, D. Interaction of glucocorticoid and insulin in the responses of rats to starvation-refeeding. *J. Nutr.*, **109**, 1766–1771 (1979).
11. ROTHBLAT, G.H., and KRITCHEVSKY, D. The metabolism of free and esterified cholesterol in tissue culture cells: A review. *Exp. Mol. Pathol.*, **8**, 314–329 (1968).
12. SHIBATA, S., HAYAKAWA, K., EGASHIRA, Y., and SANADA, H. Role of nuclear receptors in the upregulation of hepatic cholesterol 7 α -hydroxylase by cholestyramine in rats. *Life Sci.*, **80**, 546–553 (2007).
13. NESTEL, P., CEHUM, M., POMEROY, S., ABBEY, M., and WELDON, G. Cholesterol-lowering effects of plant sterol esters and non-esterified stanols in margarine, butter and low-fat foods. *Eur. J. Clin. Nutr.*, **55**, 1084–1090 (2001).
14. ROGERS, Q.R., and HARPER, A.E. Amino acid diets and maximal growth in the rat. *J. Nutr.*, **87**, 267–273 (1965).
15. FOLCH, J., LEES, M., and SLOANE-STANDLEY, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497–509 (1957).
16. ZAK, B. Simple rapid microtechnic for serum total cholesterol. *Am. J. Clin. Pathol.*, **27**, 583–588 (1957).
17. CHAM, B.E., HURWOOD, J.J., KNOWLES, B.R., and POWELL, L.W. Rapid, sensitive method for the separation of free cholesterol from ester cholesterol. *Clin. Chim. Acta.*, **49**, 109–113 (1973).
18. ALLING, C., SVENNERHOLM, L., and TICHY, J. Fatty acid composition of serum cholesteryl ester fractions is isolated by thin-layer chromatography. *J. Chromatogr.*, **34**, 413–415 (1968).
19. MAROUNEK, M., VOLEK, Z., SKRIVANOVA, E., and TUMA, L. Effects of amidated pectin alone and combined with cholestyramine on cholesterol homeostasis in rats fed a cholesterol-containing diet. *Carbohydr. Res.*, **80**, 989–992 (2010).
20. MATSUMOTO, K., and YOKOYAMA, S., Gene expression analysis on the liver of cholestyramine-treated type 2 diabetic model mice. *Biomed. Pharm.*, **64**, 373–378 (2010).
21. CHIEN, Y., WU, L., LEE, T., and HWANG, L. Cholesterol-lowering effect of phytosterol-containing lactic fermented mill powder in hamsters. *Food Chem.*, **119**, 1121–1126 (2010).
22. LING W.H., JONES, P.J.H. Enhanced efficacy of sitostanol-containing versus sitostanol-free phytosterol mixtures in altering lipoprotein cholesterol levels and synthesis in rats. *Atherosclerosis*, **118**, 319–331 (1995).
23. ANDREWS, T.C., WHITNEY, E.J., GREEN, G., KALENIAN, R., and PERSONIUS, B.E. Effect of gemfibrozil \pm niacin \pm cholestyramine on endothelial function in patients with serum low-density lipoprotein cholesterol levels < 160 mg/dl and high-density lipoprotein cholesterol levels < 40 mg/dl. *Am. J. Cardiol.*, **80**, 831–835 (1997).
24. LIN, X., MA, L., RACETTE, S.B., SPEARIE, C.L.A., and OSTLUND, R.E. Phytosterol glycosides reduce cholesterol

(Accepted November 8, 2010)

コレステラミンと植物ステロールが絶食-再摂食ラットのコレステロール代謝に及ぼす影響

稲井玲子、松尾達博

要 約

コレステラミンと植物ステロールが絶食-再摂食ラットのコレステロール代謝に及ぼす影響について検討した。20匹のDonryu系雌ラットに14日間、コレステロールを含まない高脂肪食で飼育した後、以下の4群に分けた。対照群(Con)、高コレステロール食群(Chol)、高コレステロール+コレステラミン添加群(Chol-Cm)、およびコレステラミン添加群(Cm)(実験1)あるいは高コレステロール+植物ステロール添加群(Chol-PS)(実験2)。全てのラットを2日間絶食させ、次の3日間再摂食させた。食餌摂取量、屠殺時体重、および肝臓重量には各群間に差は見られなかった。血漿総コレステロール、遊離コレステロール、コレステロールエステル、非HDLコレステロール濃度はChol群が他群に比べて有意に高値であり、血漿HDLコレステロール濃度はChol群で有意に低値であった。コレステラミン添加は高コレステロール食による血漿コレステロール濃度上昇を抑制した(実験1)。しかしながら、植物ステロールの血漿コレステロール上昇抑制効果は見られなかった(実験2)。コレステラミンおよび植物ステロール添加のいずれも肝臓コレステロール蓄積を抑制したが、その効果はコレステラミンに比べて植物ステロールで小さかった。以上の結果から、コレステラミンと植物ステロールが絶食-再摂食ラットのコレステロール代謝に影響を与えることが示唆された。

