ISSN : 0974 - 7427 Volume 5 Issue 5

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 5(5), 2011 [275-280]

Effects of cholestyramine and phytosterol on cholesterol metabolism in the starvation-refeeding rats

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Received: 29th June, 2011 ; Accepted: 29th September, 2011

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 phytoseterol affected cholesterol metabolism in rats fed a high-cholesterol diet under starvation-refeeding status.

KEYWORDS

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

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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 cholesterol 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[12].

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[13]. Phytosterols are cholesterol-like compounds that occur naturally in plant foods and reduce cholesterol absorption[14]. In the present study, we investigated the effect of cholestyramine and phytosterol on cholesterol metabolism in 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[15] were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). 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). Thelasma 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.[16]. The liver total cholesterol, free cholesterol and cholesteryl ester contents were determined by the method previously[17, 18]. Plasma cholesteryl ester was divided using a thin-layer chromatography technique[19]. 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

	Experiment 1				Experiment 2			
Groups	**Con**	**Chol**	**Cm**	**Chol-Cm**	**Con**	**Chol**	**Chol-Cm**	**Chol-PS**
Ingredients				**g/kg diet**				
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 ixture*	50.0	50.0	50.0	50.0	50.0	50.0	50.0	50.0
Vitamin ixture*	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[15] were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). 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 ± standard deviation (SD). Data were evaluated by one-way ANOVA and Turkey's test was used to determine specific mean differences. A p value 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-

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

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; TG, triacylglycerol.

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.

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.

Fatty acid composition of plasma cholesteryl ester

TABLE 3 shows the fatty acid composition of plasma chlesteryl 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 chlesteryl 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 cho-

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

Groups	14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:6
	g/100g total fatty acid							
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.

lesterol 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 3 shows the fatty acid composition of plasma chlesteryl 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 chlesteryl ester induced by a high-cholesterol diet.

Many researchers reported that cholestyramine and phytosterol in experiments on animals[20-23] and humans[24, 25]. 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[24] suggested that phytosterol (1% sitosterol) effectively modify circulating lipoprotein cholesterol concentrations at the level of the intestine, rather than internally at the level of chlesterogenesis in rats. Chien et al.[22] 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[21, 23]. 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 excretion was higher in the Chol-Cm and Chol-PS group than in the Chol group (data not shown). It has been

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reported that the net effect of dietary cholesterol absorption, endogenous cholesterol synthesis and biliary cholesterol excretion regulates body cholesterol balance[26, 27]. 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.

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