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Scientific Research

Effect of Starvation-Refeeding Status on Cholesterol Metabolism in Rats Fed High-Cholesterol Diet

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ABSTRACT

The present study investigated the effect of starvation-refeeding status on cholesterol metabolism in rats fed a high-cholesterol diet or a cholesterol-free diet. Twenty male and 20 female Donryu rats (age 5 weeks) were fed a cholesterol-free diet for 14 days. Then the male and female rats were each divided into two groups: feeding and starvation-refeeding groups. The feeding groups were fed the experimental diet for 3 days, and the starvation-refeeding groups fasted for 2 days followed by 3 days of feeding. Half of each of groups was fed a cholesterol-free diet and the other half was fed a high-cholesterol diet. Starvation-refeeding significantly increased the plasma free cholesterol and HDL-cholesterol concentrations in both the high-cholesterol-diet-fed rats and the cholesterol-free-diet-fed rats. In the female rats, plasma total cholesterol and cholesteryl ester concentrations were significantly higher in the high-cholesterol groups than in the cholesterol-free groups, whereas TG concentration and total cholesterol/TG ratio were not significantly different among all of the groups. Liver total cholesterol and cholesteryl ester were significantly higher in the high-cholesterol groups than in the cholesterol-free groups in both male and female rats. These results suggest that starvation-refeeding affected cholesterol metabolism at least in part. The reactivity of the cholesterol metabolism may be different between male and female rats.

Keywords*: *Starvation-Refeeding*, *Cholesterol Metabolism*, *High-Cholesterol Diet*, *Rat

1. Introduction

Many studies have suggested that starvation reduces the activities of hepatic enzymes in animals and fishes [1-7]. Some of the largest decreases in activity occur in the NADP-linked dehydrogenases of the cytosol [3] and organelles [1,5]. However, short-term starvation and later refeeding (starvation-refeeding) is a technique widely used to increase hepatic lipogenesis (*de novo* synthesis of long-chain fatty acids). 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 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 lipids of physiological significance, as well as triacylglycerol (TG) or fatty acids, is present in tissues and in 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]. Cholesterol synthesis in the liver may be accelerated under an activated condition of hepatic lipogenesis because cholesterol and fatty acids are closely related in mammalian lipid metabolism [12].

In the present study, we investigated the effect of starvation-refeeding status on cholesterol metabolism in rats fed a diet with or without dietary cholesterol. Furthermore, we examined whether or not a difference was found in the response to starvation-refeeding between male and female rats.

2. Materials and Methods

2.1. Animals, Diets, and Experimental Design

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

Twenty male and 20 female Donryu rats (age 5 weeks) were purchased from Japan SLC, Inc. (Shizuoka, Japan). All rats were housed individually at 22˚C ± 1˚C with light from 08:00 to 20:00 h and free access to water. Rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) *ad libitum* until 6 weeks old. Rats were fed a synthetic diet containing the following ingredients, in grams per kilogram: casein, 250; α-potato starch, 440; corn oil, 200; vitamin mixture [13], 8.5; mineral mixture [13], 50; choline chloride, 1.5; and cellulose 50. This diet also contained per kg: retinyl palmitate, 60,000 IU; ergocalciferol, 600 IU; α-tocopheryl acetate, 1 g. After a 14-day feeding period, the male and female rats were each divided into two groups: feeding and starvation-refeeding groups. The feeding group was fed the experimental diet for 3 days, and the starvation-refeeding group fasted for 2 days followed by 3 days of feeding. Half of each of these groups was fed the experimental diet (cholesterol-free diet), and the other half was fed a diet to which 1% cholesterol and 0.25% gall powder were added (high-cholesterol diet). After the feeding or starvation-refeeding period, rats were killed by heart puncturing under anaesthesia. Blood was collected to obtain plasma, and the liver was quickly removed, weighed, and stored at –40˚C.

2.2. 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). 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.* [14]. Liver total cholesterol, free cholesterol and cholesteryl ester contents were determined by the method previously described [15,16]. Plasma cholesteryl ester was divided using a thin-layer chromatography technique [17]. 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 × 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.

2.3. StatisticalAnalysis

The values are expressed as means ± standard deviation (SD). Data were evaluated by two-way ANOVA and Turkey's test was used to determine specific mean differences. A p value of < 0.05 was considered to show statistical significance. All analyses were performed with a commercially available statistical package (Excel Statistics, SSRI Co., Ltd., Tokyo, Japan).

3. Results

3.1. Food Intake, Body Weight and Liver Weights

Table 1 shows the food intake, body and liver weight. The food intakes of male and female rats were significantly higher in the starvation-refeeding groups than in the feeding groups regardless of the additional dietary cholesterol. No significant differences were found in the final body weight and relative liver weight in either male or female rats. In the female rats, the liver weight was higher in the high-cholesterol groups than in the cholesterol-free groups.

3.2. Plasma Substrates

Table 2 shows the plasma cholesterol and TG concentrations. In the male rats, no significant differences were found in the concentrations of plasma total cholesterol, cholesteryl ester, or TG, nor in the total cholesterol/TG ratio. Starvation-refeeding significantly increased the plasma free cholesterol and HDL-cholesterol concentrations in both the high-cholesterol-diet-fed rats and the cholesterol-free-diet-fed rats. The high-cholesterol diet caused the plasma HDL-cholesterol concentration to decrease significantly regardless of feeding status. In the female rats, plasma total cholesterol and cholesteryl ester concentrations were significantly higher in the high-cholesterol groups than in the cholesterol-free groups, whereas TG concentration and total cholesterol/TG ratio did not differ significantly among any of the groups. The high-cholesterol diet caused a significant decrease in the plasma HDL-cholesterol and a significant increase in the non-HDL cholesterol concentrations. The plasma free cholesterol concentration was increased in the starvation-refeeding groups than in the feeding groups.

Table 1. Effect of starvation-refeeding status on food intake, body and liver weights in high-cholesterol-diet-fed rats.

	Groups		Food intake	Final body weight	Liver weight	
	Status	Chol	g/day	g	g	g/100 g b.w.
Male	F	–	21.7 ± 1.2^{b}	302 ± 6	14.8 ± 0.9	4.9 ± 0.2
	F	+	19.0 ± 0.2^{c}	290 ± 3	14.7 ± 0.6	5.1 ± 0.2
	S-R	–	25.1 ± 2.2^{a}	292 ± 11	14.1 ± 0.9	4.8 ± 0.3
	S-R	+	24.4 ± 2.1^{a}	286 ± 16	14.7 ± 0.6^{b}	5.2 ± 0.3
Female	F	–	13.3 ± 1.0^{b}	213 ± 8	10.2 ± 0.5^{ab}	4.8 ± 0.2
	F	+	14.0 ± 0.9^{b}	223 ± 11	11.3 ± 0.8	5.1 ± 0.2
	S-R	–	16.7 ± 1.2^{a}	214 ± 10	10.2 ± 1.7^{b}	4.8 ± 0.8
	S-R	+	17.7 ± 0.6^{a}	223 ± 10	12.4 ± 0.9^{a}	5.4 ± 0.2

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. F, feeding; S-R, starvation-refeeding; Chol, cholesterol; TG, triacylglycerol; b.w., body weight.

Table 2 Effect of starvation-refeeding status on plasma concentrations of cholesterol and triacylglycerol in high-cholesterol-diet-fed rats.

	Groups		Total chol	Free chol	Chol ester	HDL-Chol	Non HDL-Chol	TG	Total chol/TG
	Status	Chol	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	mg/100 ml	
Male	F	–	119 ± 7	26.8 ± 1.4^{ab}	93 ± 6	40.1 ± 9.3^{b}	79 ± 15	130 ± 25	0.95 ± 0.2
	F	+	111 ± 15	21.4 ± 3.9^{b}	90 ± 12	27.6 ± 8.0^{b}	84 ± 9	150 ± 57	0.81 ± 0.3
	S-R	–	136 ± 14	292 ± 11	104 ± 14	47.5 ± 6.8^{a}	88 ± 17	109 ± 25	1.28 ± 0.2
	S-R	+	150 ± 28	31.6 ± 5.3^{a}	118 ± 24	32.0 ± 4.8^{b}	117 ± 27	119 ± 43	1.35 ± 0.4
Female	F	–	98 ± 6^{b}	21.2 ± 1.3^{b}	77 ± 6^{b}	36.4 ±7.0^{a}	62 ± 11^{b}	93 ± 19	1.10 ± 0.3
	F	+	179 ± 38^{a}	26.8 ± 3.0^{ab}	153 ± 35^{a}	21.1 ± 7.9^{b}	158 ± 41^{a}	107 ± 10	1.69 ± 0.4
	S-R	–	113 ± 10^{b}	29.8 ± 5.1^{a}	84 ± 8^{b}	43.5 ± 9.9^{b}	70 ± 10^{b}	114 ± 49	1.14 ± 0.4
	S-R	+	180 ± 37^{a}	32.7 ± 63	147 ± 32^{a}	15.5 ± 4.3^{b}	164 ± 41^{a}	102 ± 25	1.89 ± 0.7

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. F, feeding; S-R, starvation-refeeding; Chol, cholesterol; TG, triacylglycerol.

3.3. Liver Lipids

Table 3 shows the liver cholesterol and TG contents. Liver total cholesterol and cholesteryl ester were significantly higher in the high-cholesterol groups than in the cholesterol-free groups in both male and female rats. Starvation-refeeding decreased liver TG content, and this difference was especially significant in the male rats. Liver free cholesterol content did not differ significantly among any of the groups.

3.4. Fatty Acid Composition of Plasma Cholesteryl Ester

Table 4 shows the fatty acid composition of plasma cholesteryl ester. The percentage of oleic and linoleic acids were significantly higher whereas that of arachidonic acid was significantly lower in the high-cholesterol groups than in the cholesterol-free groups in either male or female rats. Starvation-refeeding significantly increased the percentage of oleic acid in the high-cholesterol groups. In the female rats, palmitoleic acid was significantly higher and docosahexaenoic acid was significantly lower in the high-cholesterol groups regardless of the feeding status.

4. Discussion

In the present study, we found that starvation-refeeding increased the plasma cholesterol concentration, in particular the free cholesterol concentration (i.e., it decreased the cholesteryl ester/free cholesterol ratio), indicating that starvation-refeeding might influence the synthesis of lecithin cholesterol acyltransferase (LCAT). LCAT is present in higher concentrations in plasma than in other tissues, and mainly transfers fatty acids from the 2-position of phospholipids to cholesterol [18]. The LCAT reaction is a physiologically important source of plasma cholesteryl esters [18]. In our present findings, the high-cholesterol diet increased the plasma cholesteryl ester concentration in female rats, and decreased the plasma cholesteryl ester/free cholesterol ratio. Moreover, the percentages of oleic and linoleic acids were increased by high-cholesterol diet, whereas the percentage of arachidonic acid was decreased. These results suggest that the reaction of LCAT is influenced by the feeding status or by a high-cholesterol diet, especially in female rats. There might be substrate specificity in LCAT.

Table 3. Effect of starvation-refeeding status on live cholesterol and triacylglycerol in high-cholesterol-diet-fed rats.

	Groups		Total chol	Free chol	Chol ester	TG
	Status	Chol	mg/g liver	mg/g liver	mg/g liver	mg/g liver
Male	F	–	11.4 ± 2.9[b]	5.90 ± 0.5	5.45 ± 3.2[b]	65 ± 33[a]
	F	+	27.1 ± 2.4[a]	7.60 ± 1.6	19.5 ± 3.4[a]	73 ±17[a]
	S-R	–	7.39 ± 1.8[b]	5.30 ± 1.7	2.10 ± 0.9[b]	28 ± 12[b]
	S-R	+	24.7 ± 5.6[a]	5.84 ± 2.1	18.8 ± 4.5[a]	36 ± 10[b]
Female	F	–	7.33 ± 0.5[b]	5.46 ± 0.8	1.87 ± 0.8[b]	25 ± 9[ab]
	F	+	23.1 ± 1.7[a]	6.96 ± 1.8	16.2 ± 1.0[a]	51 ± 26[a]
	S-R	–	6.03 ± 1.3[b]	5.15 ± 1.4	0.88 ± 0.3[b]	13 ± 5[b]
	S-R	+	25.0 ± 2.8[a]	6.13 ± 2.5	18.9 ± 2.5[a]	32 ± 14[ab]

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. F, feeding; S-R, starvation-refeeding; Chol, cholesterol; TG, triacylglycerol.

Table 4. Effect of starvation-refeeding status on fatty acid composition of plasma cholesteryl ester in high-cholesterol-diet-fed rats.

	Groups		14:0	16:0	16:1	18:0	18:1	18:2	20:4	22:6
	Status	Chol	%	%	%	%	%	%	%	%
Male	F	–	0.5 ± 0.0	9.6 ± 0.5[a]	1.0 ± 0.2	1.5 ± 0.4	6.8 ± 0.7c	28.3 ± 2.4[b]	49.4 ± 1.9[a]	1.6 ± 0.8
	F	+	0.4 ± 0.1	9.1 ± 0.6[a]	1.3 ± 0.1	1.4 ± 0.2	19.8 ± 1.7[b]	34.4 ± 1.8[a]	30.6 ± 2.3[b]	1.1 ± 0.5
	S-R	–	0.5 ± 0.1	9.9 ± 0.6[a]	1.0 ± 0.2	1.3 ± 0.4	6.9 ± 1.1[c]	27.9 ± 1.8[a]	49.1 ± 1.4[a]	1.7 ± 0.3
	S-R	+	0.1 ± 0.1	9.2 ± 1.7[a]	1.6 ± 0.6	1.0 ± 0.2	24.2 ± 3.5[a]	31.5 ± 1.7[b]	29.5 ± 4.2[b]	1.3 ± 0.6
Female	F	–	0.5 ± 0.1	7.1 ± 0.3[b]	1.1 ± 0.5[c]	2.3 ± 1.4	7.0 ± 0.5[c]	21.4 ± 1.7[b]	54.4 ± 3.9[b]	2.0 ± 0.4[ab]
	F	+	0.2 ± 0.1	7.1 ± 0.7[b]	2.2 ± 0.7[b]	1.2 ± 0.2	35.4 ± 4.2[b]	30.6 ± 2.0[a]	19.4 ± 5.9[c]	0.7 ± 0.2[b]
	S-R	–	0.2 ± 0.1	7.1 ± 0.7[b]	0.7 ± 0.2[c]	1.4 ± 0.4	5.0 ± 0.7[c]	20.7 ± 4.0[b]	60.5 ± 4.1[a]	2.3 ± 0.9[a]
	S-R	+	0.2 ± 0.1	7.2 ± 0.5[a]	2.8 ± 0.6[a]	1.0 ± 0.3	39.3 ± 3.8[a]	31.4 ± 0.9[a]	15.0 ± 4.1[c]	0.4 ± 0.3[b]

Values are means ± SD for 5 rats. Means with different superscripts within a column are significantly different at $p < 0.05$. F, feeding; S-R, starvation-refeeding.

The high-cholesterol diet decreased the plasma HDL-cholesterol concentration and increased the plasma non HDL-cholesterol concentration, indicating that a high-cholesterol diet facilitates the transfer of cholesteryl ester from HDL to other lipoproteins by means of the cholesteryl ester transfer protein [19]. This result supports previous findings.

Ultimately, cholesterol must enter the liver and be excreted in the bile acids [20,21]. Approximately half of the cholesterol fed or synthesized is excreted in the feces after being conversed to bile acids. Much of the cholesterol secreted in the bile is reabsorbed, and it is believed that at least some of the cholesterol that serves as a precursor for the fecal sterols is derived from the intestinal mucosa. A large fraction of the bile salts excreted is reabsorbed into the portal circulation, taken up by the liver, and re-excreted in the bile, a cycle known as enterohepatic circulation [22,23]. Unfortunately, in our experiment we did not determine the fecal excretions of cholesterol and bile salts: therefore, the effects of starvation-refeeding and high-cholesterol diets on fecal excretion or the enterohepatic circulation of cholesterol are not known. However, since the liver total cholesterol content increased 3-4 times as a result of the high-cholesterol diet, it is likely that the liver acts as a buffer for the plasma cholesterol concentration.

Many researchers have reported that starvation-refeeding increased hepatic lipogenesis [8-10]. However, in this experiment, starvation-refeeding decreased the liver TG content regardless of dietary cholesterol. No significant difference was found among the groups in the plasma TG concentration. The cause of these unexpected results is not clear. In our experiment, the rats were fed high-fat (20% w/w corn oil) diets. The process of lipogenesis is concerned with the conversion of surplus glucose and intermediates such as pyruvate, lactate, and acetyl-CoA to fat, which constitutes the anabolic phase of this cycle [24,25]. The nutritional status of the organism is the main factor regulating the rate of lipogenesis. Thus, the rate is higher in well-fed animals whose diet contains a high proportion of carbohydrate. It is depressed under conditions of restricted caloric intake or a high-fat diet, particularly a diet high in unsaturated fatty acids [26]. It may be necessary to consider the quantity and composition of the dietary fat in future studies. The present study suggests that the starvation-refeeding affected the cholesterol metabolism at least in part, but a further detailed study is required to confirm and clarify the mechanism.

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