ISSN : 0974 - 7427 Volume 5 Issue 5

Trade Science Inc.

BIOCHEMISTRY
An Indian Journal

Regular Paper

BCAIJ, 5(5), 2011 [270-274]

Effects of starvation-refeeding conditions on cholesterol metabolism in rats fed high fat diet containing different fats

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Received: 27th June, 2011 ; Accepted: 27th August, 2011

ABSTRACT

The present study was performed to investigate the effects of starvation-refeeding conditions on cholesterol metabolism in rats fed high cholesterol/high fat diets containing different fats. Forty female Donryu rats were divided into two groups and then fed a high fat diet containing beef tallow or corn oil without cholesterol for 14 days. After 14 days, the rats were divided into two sub-groups, cholesterol-free diet and high cholesterol diet subgroups. Half of the animals in all groups were fed the experimental diets for 3 days (feeding). The remaining half animals were fasted for 2 days followed by 3 days of refeeding (starvation-refeeding). Among the corn oil diet groups, no significantly differences were observed in the concentrations of plasma total cholesterol, cholesteryl ester, and non-HDL cholesterol. Starvation-refeeding significantly increased the plasma total cholesterol, cholesteryl ester, and non-HDL cholesterol concentrations in the high cholesterol, beef tallow diet-fed rats. The high cholesterol diet caused the plasma HDL cholesterol concentration to decrease significantly regardless of feeding conditions. Total hepatic cholesterol and cholesteryl ester were significantly higher in the high cholesterol groups than in the cholesterol-free groups in both beef tallow- and corn oil-fed rats. These results suggest that starvation-refeeding partially affects cholesterol metabolism in rats fed high cholesterol/high fat diets containing either saturated or polyunsaturated fats.

KEYWORDS

Starvation-refeeding;
Cholesterol metabolism;
Beef tallow;
Corn oil;
Rat.

INTRODUCTION

Many researchers have reported that starvation followed by refeeding (starvation-refeeding) increases mammalian hepatic lipogenesis compared with that on feeding the same diet fed ad libitum[1-7]. Hepatic lipogenesis is dependent on diet composition and the species, age, and sex of the animals[8-10]. An alteration in the rate of enzymatic protein synthesis is believed to cause alterations in lipogenic enzymatic activity[11-13].

Cholesterol, a physiologically significant lipid, as well as triacylglycerol (TG) and fatty acids are present

Regular Paper

in tissues and plasma lipoprotein either as free cholesterol or, in combination with long-chain fatty acids, as cholesteryl ester[14]. It is synthesized in several tissues from acetyl-CoA and 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[14]. Cholesterol synthesis in the liver may be accelerated under conditions of activated hepatic lipogenesis because cholesterol and fatty acids are closely related to mammalian lipid metabolism[15].

Recently, we suggested that starvation-refeeding activates cholesterol metabolism in rats fed a high cholesterol, unsaturated fat diet[16]. However, several studies have investigated the effects of diet containing dietary fats having varying fatty acid composition on cholesterol metabolism[17-21]. Saturated fatty acids have been suggested to cause hypercholesterolemia both in humans and animal models[17-21]. However, whether starvation-refeeding increases serum and hepatic cholesterol levels in saturated and polyunsaturated fat diet-fed rats in the same manner remains unclear. In this study, we investigated the effects of starvation-refeeding conditions on cholesterol metabolism in rats fed high fat diets containing saturated or polyunsaturated fats.

Materials and methods

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

Animals, diets, and experimental design

Forty female Donryu rats (age 4 weeks) were purchased from Japan SLC, Inc. (Shizuoka, Japan). Half of the animals were fed a beef tallow diet, and the other half were fed a corn oil diet. All rats were housed individually at 22 ± 1 °C under illumination from 08:00 to 20:00 h and water ad libitum. The rats were fed CE-2, a commercial rodent diet (CLEA, Tokyo, Japan) *ad libitum* until 5 weeks of age. The fatty acid compositions of test lipids are shown in TABLE 1. The rats were fed a synthetic high fat diet containing different fats without cholesterol (TABLE 2). In addition, the diet for both groups contained the following components (per kg): retinyl palmitate, 60,000IU; ergocalciferol, 600IU; α-tocopheryl acetate, 1 g. Vitamin and mineral mixtures[22] were purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). After a 14-day feeding period, the rats were divided into two subgroups. Half of each subgroup was fed the experimental diet (cholesterol-free diet, Con), and the other half was fed containing 1% cholesterol and 0.25% bile powder (high cholesterol diet, Chol). Half of the animals in all groups were fed the experimental diets for 3 days (feeding groups). The other half were fasted for 2 days, followed by 3 days of refeeding (starvation-refeeding groups). After the feeding or starvation-refeeding period, the rats were killed by cardiac puncture under anesthesia. Blood was collected to obtain plasma, and the liver was quickly removed, weighed, and stored at -40°C.

TABLE 1 : Fatty acid composition of experimental fats

Fatty acid*	Beef tallow	Corn oil
	g/100g total fatty acid	
14:0	3.0	Trace
16:0	26.5	11.5
16:1	3.7	0.2
18:0	18.3	2.0
18:1	43.9	25.9
18:2	3.1	58.7
18:3	Trace	0.5
20:0	0.1	0.6
20:1	0.5	0.4
22:6	0.3	Trace

***Number of C atoms : number of double bonds.**

TABLE 2 : Composition of experimental diets

Ingredients	Beef tallow		Corn oil	
	Chol	Con	Chol	Con
	g/kg diet			
Casein	250.0	250.0	250.0	250.0
α-Starch	427.5	440.0	427.5	440.0
Beef tallow	200.0	200.0	-	-
Corn oil	-	-	200.0	200.0
Mineral mixture	50.0	50.0	50.0	50.0
Vitamin Mixture	8.5	8.5	8.5	8.5
Cellulose	50.0	50.0	50.0	50.0
Choline chloride	1.5	1.5	1.5	1.5
Cholesterol	10.0	-	10.0	-
Gall powder	2.5	-	2.5	-

These diets contained per kg: retinyl palmitate, 60,000IU; ergocalciferol, 600IU; α-tocopheryl acetate, 1 gram. Con, control; Chol, cholesterol.

Regular Paper

Analysis

Plasma total cholesterol, free cholesterol, HDL cholesterol, and TG concentrations were determined using commercial 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 hepatic and plasma lipids were extracted by the method of Folch et al.[23] Total hepatic cholesterol, free cholesterol, and cholesteryl ester contents were determined by the method described previously[24, 25].

Statistical analysis

All data values are expressed as means ± standard deviation (SD). Data were evaluated by three-way ANOVA, and Tukey's test was used to determine specific mean differences. For all analyses, $p < 0.05$ was considered to be statistically significant. All analyses were performed using a commercially available statistics package (Excel Statistics; SSRI Co., Ltd., Tokyo, Japan).

TABLE 3 : Effect of starvation-refeeding conditions on food intake, body and liver weights in higth fat/cholesterol diet fed rats

	Groups		Food intake	Final body weight	Liver weight	
	Status	Chol	mg/g tissue	mg/g tissue	g	g/100g b.w.
Beef tallow	F	-	13.4±0.9^b	200±10	10.1±0.2	5.1±0.2
	F	+	13.2±1.3^b	193±10	10.2±1.1	5.3±0.4
	S-R	-	17.4±1.2^a	196±10	10.9±1.3	5.6±0.6
	S-R	+	16.9±0.2^a	194±4	10.7±0.5	5.6±0.3
Corn oil	F	-	12.8±1.1^b	205±6	10.2±1.0	5.0±0.5
	F	+	11.8±0.8^b	195±10	10.3±0.4	5.3±0.1
	S-R	-	17.3±1.2^a	202±8	10.9±0.7	5.4±0.2
	S-R	+	16.7±1.2^a	196±8	11.2±1.5	5.7±0.5

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.

RESULTS AND DISCUSSION

Food intake, body weight, and liver weight

TABLE 3 shows the food intake, body weight, and liver weight. The food intakes of rats fed beef tallow diet or corn oil diet was significantly higher in the starvation-refeeding groups than in the feeding groups, regardless of the additional dietary cholesterol. No significant differences were observed in the final body weight or liver weight in either beef tallow or corn oil diet-fed rats.

Plasma substrates

TABLE 4 shows the rat plasma cholesterol and TG concentrations. In the corn oil diet groups, no significant differences were observed in the concentrations of plasma total cholesterol, cholesteryl ester, non-HDL cholesterol, and TG or in the total cholesterol/TG ratio. Starvation-refeeding significantly increased the plasma total cholesterol, cholesteryl ester and non-HDL cholesterol concentrations in the high cholesterol, beef tallow diet-fed rats. The high cholesterol diet significantly decreased the plasma HDL cholesterol concentration regardless of the feeding conditions. In beef tallow diet-fed rats, plasma total cholesterol, free cholesterol, cholesteryl ester and non-HDL cholesterol concentrations were significantly higher in the high cholesterol diet groups compared with those in the cholesterol-free groups under starvation-refeeding conditions, whereas TG concentrations did not differ among any of the groups. The high cholesterol diet significantly decreased the plasma HDL cholesterol and significantly increased non-HDL cholesterol concentrations in the beef tallow diet groups.

Hepatic lipids

TABLE 5 shows the hepatic cholesterol and TG contents. Total hepatic cholesterol and cholesteryl ester were significantly higher in the high cholesterol groups than in the cholesterol-free groups in both beef tallow and corn oil diet-fed rats. Starvation-refeeding significantly increased hepatic cholesteryl ester in the high cholesterol corn oil diet group. Hepatic TG content did not differ among any of the groups.

We had previously suggested that starvation-refeeding increased the plasma cholesterol concentration in high fat/high cholesterol diet-fed rats[16]. As starvation-refeeding may enhance the response to dietary fats, we decided to adopt this method. In this study, we found that supplemental 1% cholesterol increased plasma

TABLE 4 : Effect of starvation-refeeding conditions on plasma components in higth fat/cholesterol diet fed rats

	Groups		Total Chol	Free Chol	Chol ester	HDL Chol	Non-HDL Chol	TG	Total Chol/TG
	Status	Chol	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	mg/100ml	
Beef tallow	F	-	136 ± 15^{b}	34.7 ± 7.7^{b}	101 ± 21^{b}	43.5 ± 11.0^{ab}	93 ± 14^{c}	200 ± 47	0.71 ± 0.2^{b}
	F	+	223 ± 42^{b}	46.5 ± 12.0^{ab}	177 ± 51^{b}	23.1 ± 7.2^{c}	200 ± 41^{b}	182 ± 63	1.28 ± 0.3^{b}
	S-R	-	128 ± 17^{b}	28.3 ± 5.2^{b}	100 ± 22^{b}	46.2 ± 9.4^{ab}	82 ± 16^{c}	157 ± 56	0.89 ± 0.3^{b}
	S-R	+	478 ± 142^{a}	54.4 ± 12.0^{a}	424 ± 130^{a}	20.7 ± 6.9^{c}	458 ± 148^{a}	136 ± 68	4.30 ± 2.4^{a}
Corn oil	F	-	112 ± 37^{b}	26.2 ± 7.6^{b}	86 ± 35^{b}	36.0 ± 15.0^{bc}	76 ± 27^{c}	104 ± 36	1.09 ± 0.2^{b}
	F	+	179 ± 42^{b}	39.4 ± 18.0^{ab}	139 ± 40^{b}	24.4 ± 6.8^{c}	154 ± 48^{bc}	108 ± 7	1.66 ± 0.4^{b}
	S-R	-	125 ± 23^{b}	24.9 ± 7.7^{b}	100 ± 23^{b}	53.5 ± 9.9^{a}	72 ± 15^{c}	105 ± 16	1.20 ± 0.2^{b}
	S-R	+	186 ± 23^{b}	30.7 ± 11.0^{b}	154 ± 22^{b}	20.5 ± 5.4^{c}	164 ± 20^{bc}	152 ± 46	1.31 ± 0.5^{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; Chol, cholesterol; TG, triacylglycerol.

TABLE 5 : Effect of starvation-refeeding conditions on liver components in higth fat/cholesterol diet fed rats

	Groups		Total Chol	Free Chol	Chol ester	TG
	Status	Chol	mg/g tissue	mg/g tissue	mg/g tissue	mg/g tissue
Beef tallow	F	-	7.0±1.0^{c}	5.5±0.7^{b}	1.5±0.3^{c}	36 ± 14
	F	+	21.5±0.6^{b}	4.6±0.7^{b}	16.9±1.3^{b}	63 ± 10
	S-R	-	5.4±0.4^{c}	4.6±0.3^{b}	0.8±0.1^{c}	19 ± 6
	S-R	+	22.6±2.7^{b}	5.4±1.2^{b}	17.3±2.6^{b}	31 ± 5
Corn oil	F	-	8.2±1.7^{c}	5.8±0.8^{b}	2.8±1.7^{c}	37 ± 14
	F	+	23.4±3.1^{b}	7.6±1.6^{a}	15.7±2.6^{b}	58 ± 13
	S-R	-	6.4±0.5^{c}	5.3±0.5^{b}	1.1±0.3^{c}	27 ± 24
	S-R	+	28.0±3.5^{a}	6.5±1.9^{ab}	21.5±3.3^{a}	51 ± 11

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.

cholesterol concentration in rats fed beef tallow diet compared with rats fed corn oil diet under starvation-refeeding conditions. In the corn oil diet groups, starvation-refeeding did not affect the plasma concentrations of total, free, and non-HDL cholesterols or cholesteryl ester in either the high cholesterol or cholesterol-free diet-fed rats. These results support our previous findings[16]. However, in the beef tallow diet groups, starvation-refeeding significantly increased plasma total cholesterol, non-HDL cholesterol, and cholesteryl ester concentrations in rats fed high cholesterol diet. These results suggest that the beef tallow and corn oil diet-fed rats differ in response to high cholesterol diet under starvation-refeeding conditions.

In this study, plasma total cholesterol/TG ratio and non-HDL cholesterol concentration considerably increased in the high cholesterol, beef tallow diet-fed rats under the starvation-refeeding conditions. The increase in the plasma total cholesterol/TG ratio may reflect the increases of plasma LDL because the plasma total cholesterol/TG ratio of VLDL was lower than that of LDL[26]. Therefore, most of the plasma non-HDL cholesterol is speculated to be plasma LDL cholesterol. We observed a correlation between the plasma total cholesterol/TG ratio and plasma non-HDL cholesterol concentration in the beef tallow diet groups ($r = 0.94$, $p < 0.05$), whereas no correlation was observed in the corn oil diet groups ($r = 0.62$). These results suggested that the high cholesterol, beef tallow diet might increase the plasma non-HDL cholesterol cholesterol concentration under the starvation-refeeding conditions because of the increase of plasma LDL-cholesterol concentration.

Grundy[27] reported that monounsaturated fatty acids can favorable effects when substituted for saturated fatty acids in the diet. On substitution monounsaturated fatty acids reduced plasma LDL cholesterol levels without lowering HDL cholesterol level. Brousseau et al.[28] suggested that monounsaturated fatty acid (oleic acid) lowers plasma LDL, and VLDV cholesterols and apolipoprotein B concentrations by specific mechanisms with polyunsaturated fatty acids affecting LDL apolipoprotein B catabolism as well as production. These previous studies did not agree with our present findings. The difference in the results obtained by Grundy[27] and Brousseau et al.[28] and those of our present study may be because of the differences in the composition of diets. However, these studies did not adopt the high cholesterol diets and starvation-refeeding conditions. In this study, beef tallow contained approxi-

Regular Paper

mately 50% saturated fatty acids; therefore, the hypercholesteroleic effects of saturated fatty acids may have nullified the hypocholesteroleic effects of monounsaturated fatty acids.

Evidence suggests that dietary fatty acids affect LDL cholesterol levels through various mechanisms, including effects on whole animal sterol balance and LDL production or turnover.[29] However, the modulation of LDL receptor-mediated uptake of LDL is believed to be the primary pathway that regulates plasma cholesterol levels. Studies on humans and animal models[30, 31] have shown that changes in plasma LDL cholesterol because of dietary fatty acid modification are associated with changes in its fractional catabolic rate. Alterations in the fractional catabolic rate are presumably mediated through changes in the receptor-mediated uptake of LDL particles by the liver. Consequently, fatty acid regulation of the LDL receptor has become a subject of interest in scientific studies. Mustad et al.[32] reported that dietary linoleic acid increases and palmitic acid decreases hepatic LDL receptor protein and mRNA abundance, respectively, in young pigs. In this study, linoleic and palmitic acids were the main components of corn oil and beef tallow, respectively. In the high cholesterol diet groups, plasma non-HDL cholesterol was significantly higher in beef tallow diet-fed rats than in corn oil diet-fed rats under both starvation-refeeding and feeding conditions. Our results at least partially support previous findings.

The results of this study suggest that starvation-refeeding partially affect cholesterol metabolism in rats fed high cholesterol/high fat diets containing saturated or polyunsaturated fats. However, further detailed studies are required to confirm and clarify the mechanism.

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