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CETP Activity in Liver Perfusates and Plasma from Rabbits Hypo- or Hyperresponsive to Dietary Cholesterol

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ABSTRACT. We investigated the relationship between the development of hypercholesterolemia in rabbits and cholesteryl ester transfer protein (CETP) activity secretion by their perfused livers. Two inbred strains of rabbits were compared which differ markedly in their hypercholesterolemic response to dietary cholesterol. Feeding a high-cholesterol (0.3%) diet, increased plasma and liver cholesterol levels in the two strains, the increments being 15 mM and 30 μmol/g greater in the hyperresponders, respectively. The high-cholesterol diet caused an about 2-fold increased hepatic secretion of CETP activity, but there was no difference between the two rabbit strains. Feeding a lower amount of dietary cholesterol (0.08%) also caused higher cholesterolemic (2 mM) and hepatocholesterolic (28 μmol/g) responses in hyper- than in hyporesponsive rabbits. The activity of hepatic CETP secretion was not increased by the low-cholesterol diet, and there was no difference between hypo- and hyperresponsive rabbits. Cholesterol feeding increased plasma CETP activity by 90% in both rabbit strains, but there was no difference between the strains. Our combined data suggest that with increasing plasma cholesterol levels, hepatic CETP secretion may be increased in a parabolic manner, reaching its maximum rate far before plasma cholesterol concentrations are maximal. There were no differences in hepatic CETP activity secretion or plasma CETP activity levels between the genetically different strains of hypo- and hyperresponsive rabbits. COMP BIOCHEM PHYSIOL 114B;4:403–407, 1996.

KEY WORDS. Lipid transfer protein, dietary cholesterol, hypercholesterolemia, hyporesponders, hyperresponders, liver cholesterol, liver perfusion, rabbit

INTRODUCTION

The cholesteryl ester transfer protein (CETP) has been identified in plasma from man, rabbit, and various other species (1,2). This protein catalyses the transfer/exchange of cholesteryl esters (CE) and triglycerides between lipoproteins in plasma, thus altering their lipid composition and metabolism (3–5).

The mechanism(s) for the metabolic regulation of CETP are not fully understood (6). In rabbits, diet-induced hypercholesterolemia is positively (7,8), whereas pregnancy- and drug-induced hypercholesterolemia is negatively associated (9,10) with plasma CETP activity levels. An increase in plasma CETP mass in cholesterol-fed rabbits corresponds with an increase in CETP mRNA in their livers, suggesting that the CETP gene is subject to dietary regulation (11). CETP activity is present in recirculating perfusates of isolated rabbit livers (12,13) and diet-induced hypercholesterolemia in rabbits is associated with a marked increase in the hepatic secretion of lipoprotein cholesterol (14,15). The objective of the current study was to investigate whether the degree of hypercholesterolemia in rabbits and the rate of CETP activity secretion by their perfused livers are proportionally related. We used two inbred strains of rabbits which differ markedly in their hypercholesterolemic response to dietary cholesterol (16–18) and hypothesized that any effect of dietary cholesterol on plasma CETP activity and on hepatic secretion of CETP would be more pronounced in the hyperresponsive rabbits than in their hyporesponsive counterparts.

METHODS

The experimental protocols were approved by the ethical committee on animal experimentation of the University of Utrecht.

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Animals and Housing

Rabbits (*Oryctolagus cuniculus*) of two inbred strains were used. The IIIVO/JU strain has previously been shown to be hyporesponsive and the AX/JU strain to be hyperresponsive to dietary cholesterol (16–18). The animals were kept individually as described (17). They had been fed a commercial diet without added cholesterol (LKK-20®, Hope Farms, Woerden, The Netherlands).

Experimental Diets and Design

Experiment A: CETP activity in liver perfusate from hypo- and hyperresponsive rabbits. In the first part of experiment A, we used hypo- and hyperresponsive male rabbits aged 6–30 months (mean age 13 and 19 months, respectively). The animals were fed 100 g/day of the commercial rabbit pellets without or with added cholesterol (0.3%, w/w) for 28 days. Cholesterol was added to the grounded diet prior to pelleting, by the manufacturer (Hope Farms). Upon analysis, the diets without and with added cholesterol were found to contain 2 and 288 mg cholesterol/100 g, respectively. Each dietary group consisted of 4 hypo- and 8 hyperresponders.

The amount of cholesterol ingested by the two strains, while on the 0.3% cholesterol diet, was much higher than that produced by whole body cholesterol synthesis while on the low-cholesterol diet (18). Thus, down-regulation of cholesterol synthesis after cholesterol feeding could not compensate for the increased cholesterol intake. This situation may be considered extreme and, by overwhelming compensatory mechanisms, might reduce any strain difference in cholesterol-induced hepatic secretion of CETP activity. Therefore, the second part of experiment A was performed with the two strains to which the amount of cholesterol given with the diet was of the same order of magnitude as the baseline, daily amount of cholesterol synthesized (18). In order to increase dietary control (19), a purified diet was used instead of the commercial, natural ingredient diet. Each diet was fed to 3 hyper- and 4 hyporesponsive female rabbits and two male animals of either strain. The rabbits, aged between 24–54 months (average 35 and 39 months for the hypo- and the hyperresponders), were fed 75 g of a purified pelleted diet without or with 0.08% (w/w) added cholesterol per day for 42 days as described (20).

Within each dietary group, hypo- and hyperresponsive rabbits were paired according to sex, age, and body weight. Livers were isolated from each pair and perfused in random order on the same day. Procedures were exactly as described (21). In short, after nonrecirculating pre-perfusion with 1 L of oxygenated Krebs-Henseleit bicarbonate buffer, livers were perfused simultaneously in a recirculating fashion through the hepatic artery and portal vein with oxygenated perfusate and with a mixture of oxygenated and deoxygenated perfusate, respectively. Each liver was perfused at constant pressure with 250 mL Krebs-Henseleit bicarbonate buffer containing bovine erythrocytes at a hematocrit of 0.22, 1.5 g/L D-glucose, 30 g/L bovine serum albumin, 0.5 g/L amino acids, 100 kU/L penicillin G and 0.05 g/L streptomycin sulphate. The perfusion buffer was equilibrated with an atmosphere of O_2:CO_2 = 95:5 (v/v). The livers were viable for at least 2 h as assessed with the use of physiological, biochemical and histological measurements (21). After 2 h of perfusion, 10 mL samples were taken from the perfusate for CETP activity determination and a part of the liver was frozen (−20°C) for cholesterol analysis. Supernatants were prepared by centrifugation (15 min; 3,000 ***g***) and stored at −70°C. The hepatic secretion of lipoproteins, and plasma and liver cholesterol values of this experiment have been reported earlier (20).

Experiment B: CETP activity in plasma from hypo- and hyperresponsive rabbits. In experiment B, the relation between plasma CETP activity and the differential cholesterolemic response in the two rabbit strains was evaluated. For this purpose three male and three female rabbits of each of the two strains were fed the commercial diet, to which 0.1% (w/w) of cholesterol was added by the manufacturer (Hope Farms), during 35 days. The amount of cholesterol in the diet was kept low to prevent overwhelming of compensatory mechanisms. Upon analysis, the diet was found to contain 141 mg of cholesterol/100 g of diet. The rabbits were fed 75 g of diet per day. Tap water was provided ad libitum.

Chemical Analyses

Samples of blood were taken in the fasted state from the marginal ear vein. Sampling was performed in heparinized tubes between 8.00 and 10.00 h. Plasma was collected by low-speed centrifugation and kept at −20 or −70°C until analysis. Total cholesterol in plasma was measured enzymatically with a test-combination purchased from Boehringer, Mannheim, Germany. Liver cholesterol was extracted and analyzed colorimetrically as described (22); cholesterol in feed samples was analyzed by gas-liquid chromatography (23).

Assay of CETP Activity in Serum or Liver Perfusate

CETP activity levels were measured in the supernatant fraction of each serum after precipitation of endogenous apo B-containing lipoproteins with phosphotungstate/Mg^{2+} (24), according to the method developed by Groener *et al.* (25). The exchange of CE between excess exogenous [^{14}C]CE-labelled low-density lipoprotein (LDL) and unlabelled high-density lipoprotein (HDL) was measured during a 16 h incubation. The measured values reflect the activity of CETP, independent of endogenous serum lipoproteins. CETP activity in liver perfusates was measured as described (25), except that apoB containing lipoproteins were not removed from the perfusate prior to the assay. Lipoprotein levels in liver perfusate are very low compared to serum and

TABLE 1. Experiment A: Plasma and liver cholesterol concentrations and hepatic secretion rate of CETP activity by perfused livers from hypo- and hyperresponsive rabbits fed diets without or with added cholesterol (0.3% by weight) for 28 days

	Diet without cholesterol		Diet with 0.3% cholesterol		
Measure	**Hypo (n = 4)**	**Hyper (n = 8)**	**Hypo (n = 4)**	**Hyper (n = 8)**	**ANOVA***
			Plasma total cholesterol (mM)		
Initial	0.65 ± 0.10	0.39 ± 0.08	0.66 ± 0.10	0.42 ± 0.09	S
Final	0.55 ± 0.12	0.37 ± 0.09	7.5 ± 3.0	22.2 ± 5.2	C,S,C*S
Change	−0.09 ± 0.17	−0.03 ± 0.09	6.9 ± 2.9	21.8 ± 5.1	C,S,C*S
			Liver total cholesterol (μmol/g)		
	4.5 ± 0.4	6.3 ± 1.2	41.8 ± 9.1	73.4 ± 7.5	C,S,C*S
			Hepatic CETP activity secretion (μmol CE/h.100 g) in 2 h		
	0.69 ± 0.17	0.41 ± 0.13	1.05 ± 0.36	1.02 ± 0.27	C

Values represent means ±SD.
*Analysis of variance: C = significant effect of dietary cholesterol; S = significant effect of rabbit strain; C*S = significant interaction.

do not interfere with the high concentrations of added exogenous LDL and HDL.

Data Analyses

Results are expressed as means ± SD, unless indicated otherwise. The Kolmogorov-Smirnov 1-sample test was used to check for the normal distribution of data. The data from experiment A were evaluated by two-factor analysis of variance with rabbit strain and dietary cholesterol as main effects. Comparisons between the hypo- and hyperresponsive strain in experiment B were evaluated by Student's *t*-test. Two-sided *P* values <0.05 were considered statistically significant.

RESULTS

CETP Activity in Liver Perfusate from Hypo- and Hyperresponsive Rabbits

Throughout both parts of experiment A, mean body weights of the inbred rabbit strains did not change. In the rabbits fed the commercial diets (first part), feed intake did not differ between the strains. In the second part of the experiments, mean body weight and feed intake were about 10% lower in hyper- than in hyporesponders (data not shown).

As reported earlier (20), feeding the high-cholesterol (0.3%) diet, dramatically increased plasma cholesterol levels in the two strains, the increment being significantly greater in the hyperresponders (Table 1). On the diet without added cholesterol, the hyperresponders had higher liver cholesterol levels than the hyporesponders. The addition of cholesterol to the diet had a marked hepatocholesterolic effect in both strains, the hyperresponders being more sensitive (Table 1). In both rabbit strains, feeding of the 0.3% cholesterol diet caused a significant increase in secretion of CETP activity into the perfusate. There was no significant difference between hypo- and hyperresponsive rabbits in CETP secretion (Table 1).

The addition of a low amount of cholesterol (0.08%) to the diet also caused a higher cholesterolemic response in the hyper- than in the hyporesponsive rabbits (Table 2). On the purified diet with added cholesterol, liver cholesterol concentrations were increased in both hypo- and hyperresponders, the increase being significantly greater in the latter. In this experiment, the total activity of CETP secreted by livers from cholesterol-fed rabbits did not differ significantly from that secreted by livers from their counterparts fed the purified diet without added cholesterol (Table 2). Again there was no significant difference between hypo-

TABLE 2. Experiment A: Plasma and liver cholesterol concentrations and hepatic secretion rate of CETP activity by perfused livers from hypo- and hyperresponsive rabbits fed diets without or with added cholesterol (0.08%) for 42 days

	Diet without cholesterol		Diet with 0.08% cholesterol		
Measure	**Hypo (n = 6)**	**Hyper (n = 5)**	**Hypo (n = 6)**	**Hyper (n = 5)**	**ANOVA**
			Plasma total cholesterol (mM)		
Initial	1.33 ± 0.45	0.98 ± 0.42	1.49 ± 0.55	1.00 ± 0.23	S
Final	1.24 ± 0.34	0.79 ± 0.22	2.56 ± 0.75	4.03 ± 1.47	C,C*S
Change	−0.10 ± 0.24	−0.19 ± 0.25	1.08 ± 0.43	3.03 ± 1.66	C,C*S
			Liver total cholesterol (μmol/g)		
	7.7 ± 1.0	12.1 ± 3.4	15.1 ± 3.4	48.0 ± 7.3	C,S,C*S
			Hepatic CETP activity secretion (μmol CE/h.100 g) in 2 h		
	0.88 ± 0.25	1.04 ± 0.44	0.93 ± 0.24	0.89 ± 0.21	

For explanation of ANOVA, see legend to Table 1.

TABLE 3. Experiment B: Plasma and liver cholesterol concentrations and CETP activity in plasma of hypo- and hyperresponsive rabbits fed a 0.1% cholesterol diet for 35 days

Measure	Diet with 0.1% cholesterol	
	Hypo (n = 6)	Hyper (n = 6)
	Plasma total cholesterol (mM)	
Initial	1.17 ± 0.15	0.51 ± 0.18*
Final	2.77 ± 0.52	4.45 ± 0.93*
Change	1.61 ± 0.44	3.94 ± 0.91*
	Liver total cholesterol (μmol/g)	
	8.1 ± 1.7	14.7 ± 2.8*
	Plasma CETP activity (nmol CE/h.ml)	
Initial	*69 ± 11*	*56 ± 19*
Final	*127 ± 17*	*108 ± 24*
Change	*57 ± 10*	*52 ± 12*

*Significant difference between hypo- and hyperresponsive rabbit strain (2-sided Student's *t*-test).

and hyperresponsive rabbits in CETP secretion. Also no differences were observed between female and male animals (not shown).

CETP Activity in Plasma from Hypo- and Hyperresponsive Rabbits

All animals consumed their rations completely and retained their body weight during the course of experiment B. Feeding the commercial diet with 0.1% added cholesterol caused a more pronounced increase of plasma total cholesterol in the hyperresponsive rabbits than in the hyporesponders. Mean hepatic cholesterol concentration was highest in the hyperresponders (Table 3). Despite similar plasma total cholesterol levels, liver total cholesterol concentrations were lower in experiment B than in the second part of experiment A. This discrepancy is most likely explained by the different dietary background in the two experiments (chow versus semi-purified). Cholesterol loading increased plasma CETP activity in both rabbit strains, but initially as well as finally there were no differences between hypo- and hyperresponders (Table 3). There were no significant differences between the sexes (not shown).

DISCUSSION

The objective of these studies was to test the hypothesis that high CETP activities in perfusate of isolated livers are associated with high degrees of hypercholesterolemia in rabbits. Using two inbred strains of rabbits with either a low (hyporesponders) or a high (hyperresponders) response of plasma cholesterol to dietary cholesterol, it was shown that the addition of a relatively high amount of cholesterol (0.3%) to the diet for 4 weeks caused an increased hepatic CETP activity secretion (Table 1). However, despite marked differences in plasma and liver cholesterol levels between the two rabbit strains, the hepatic CETP activity secretion was similar in both strains, both on diets with and without cholesterol. The feeding of a diet with a low amount of cholesterol (0.08%) for 6 weeks also increased plasma and liver cholesterol concentrations to a greater extent in hyper- than in hyporesponders, but again did not influence hepatic CETP activity secretion in either rabbit strain (Table 2). In a further experiment, with the two inbred strains fed a 0.1% cholesterol diet for 5 weeks, plasma CETP activity was measured and found to be elevated to a similar extent in both strains, whereas plasma total cholesterol was again significantly higher in the hyperresponders (Table 3). The increases of plasma and liver cholesterol in both strains in this experiment were comparable with those seen in the experiment using the 0.08% cholesterol diet (see Table 2), in which no increase in hepatic CETP activity secretion by cholesterol feeding was observed in either rabbit strain.

Published data show that plasma CETP activity is unrelated with hepatic cholesterol secretion rate in rabbits, as determined with short-term blockade of very-low-density lipoprotein (VLDL) clearance (10). The present results on plasma CEPT responses to cholesterol feeding in the different rabbit strains differ from earlier data showing a greater increase of plasma CETP activity after 8 weeks of cholesterol feeding in hyporesponders versus hyperresponders (26). The reason for the discrepancy is unknown, but may be related to the longer time on the cholesterol diet and/or the higher cholesterol content (0.15%). The present results suggest that the concerted increase of plasma cholesterol and CETP activity in rabbits during high-cholesterol feeding (7,8,26) may be partly due to an increase in the hepatic secretion of CETP activity. With high dietary cholesterol levels (0.3% by weight), we found increased hepatic CETP secretion and extremely high plasma cholesterol levels. Plasma cholesterol was especially elevated in the hyperresponders (>20 mM). Lower plasma cholesterol concentrations were seen in plasma from rabbits on diets with 0.08% or 0.1% cholesterol but again hyporesponders (average values 2–3 mM) had lower levels than hyperresponders (average values 4.0–4.5 mM). On these diets with a relatively low cholesterol content, hepatic CETP activity secretion was not increased. Our combined data (see Fig. 1) suggest that with increasing plasma cholesterol levels, hepatic CETP secretion may be increased in a parabolic manner, reaching its maximum rate far before plasma cholesterol concentrations are maximal. In human plasma, CETP activity levels measured with exogenous substrates as in the present study, correlate well with CETP protein concentrations (27). CETP activity, as measured in the present experiments, is therefore likely to reflect the amount of transfer protein. However, activation of CETP protein or changes in the amount of CETP inhibitor present cannot be excluded (7). The results presented in this report show no differences in hepatic CETP activity secretion or plasma CETP activity levels between the genetically different strains of hypo- and hyperresponsive rabbits.

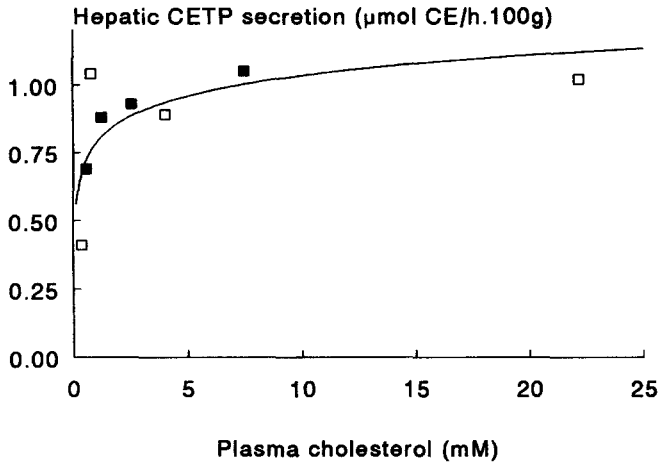

FIG. 1. Activity of CETP activity secreted by the perfused liver in 2 h as function of plasma total cholesterol concentration in hypo- (■) and hyperresponsive (□) rabbits. In this figure, based on Tables 1 and 2, the results of the two parts of experiment A are combined.

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