Plasma activities of lecithin:cholesterol acyltransferase, lipid transfer proteins and post-heparin lipases in inbred strains of rabbits hypo- or hyper-responsive to dietary cholesterol

Gert W. MEIJER,*§ Pierre N. M. DEMACKER,† Arie VAN TOL,‡ Johanna E. M. GROENER,‡||

Johan G. P. VAN DER PALEN,* Anton F. H. STALENHOF,† L. M. F. VAN ZUTPHEN* and Anton C. BEYNEN*

* Department of Laboratory Animal Science, University of Utrecht, 3508 TD Utrecht, † Department of Medicine, Division of General Internal Medicine, University Hospital, 6500 HB Nijmegen, and ‡ Department of Biochemistry, Faculty of Medicine and Health Sciences, Erasmus University, 3000 DR Rotterdam, The Netherlands

Plasma lipoproteins, plasma activities of lecithin:cholesterol acyltransferase (LCAT), phospholipid transfer protein (PLTP), cholesteryl ester transfer protein (CETP) and post-heparin lipases were measured before and after cholesterol challenge in two inbred strains of rabbits with either a high (hyper-responders) or a low (hypo-responders) response of plasma cholesterol to dietary cholesterol. The purpose of this study was to provide clues about the mechanisms underlying the effect of dietary cholesterol on lipoprotein levels and composition, and particularly those underlying the strain difference of this effect. Cholesterol feeding (0.15 g of cholesterol/100 g of diet) caused increased plasma total cholesterol concentrations and an increased ratio of cholesteryl esters: triacylglycerol in all lipoprotein particles in both strains;

these effects were significantly greater in hyper- than hypo-responsive rabbits. Feeding on the high-cholesterol diet lowered plasma triacylglycerols in hyper-responders, but caused increased plasma triacylglycerol levels in hypo-responders. This was accompanied by significantly greater increases in the activities of hepatic triacylglycerol lipase and lipoprotein lipase in hyper- than in hypo-responders. Both strains showed a dietary-cholesterol-induced rise in plasma CETP as well as in PLTP activity. The increase in PLTP activity was greater in the hyper-responders, but that of CETP was less. There was no effect of dietary cholesterol on LCAT activity. It is hypothesized that the lipases are involved in the removal of cholesterol-rich lipoproteins.

INTRODUCTION

Among humans and various animal species, individuals differ in their susceptibility to dietary cholesterol, and hypo-responders showing moderate increases in their plasma cholesterol concentration can be discriminated from hyper-responders, which develop severe hypercholesterolaemia after cholesterol loading [1]. The underlying mechanisms of this individual variation in response to dietary cholesterol are poorly understood. To study these mechanisms, we are using two inbred strains of rabbits with defined but different cholesterolaemic responses [2–5]. In these genetically standardized animals, the response to dietary cholesterol is highly reproducible between experiments.

In the two inbred strains of rabbits, baseline HDL-cholesterol concentrations are lower and VLDL-cholesterol concentrations are higher in hyper- than in hypo-responders [3]. After cholesterol feeding, the hyper-responders, as compared with the hypo-responders, develop severe hypercholesterolaemia, which is associated with high levels of circulating lipoproteins that are enriched with cholesterol [3]. Thus, before and after cholesterol feeding, lipoprotein concentrations and compositions differ between the hypo and hyper-responsive rabbits.

The aim of the present study was to investigate whether hypo- and hyper-responsive rabbits differ with regard to the remodelling of circulating plasma lipoproteins. For this purpose, we measured, before and after cholesterol feeding, plasma lipoprotein levels and composition as well as the following enzyme activities.

(1) LPL, which hydrolyses core triacylglycerols of chylomicrons and VLDL, and is present on the luminal surface of capillary endothelial cells [6]. Surface fragments produced during lipolysis of triacylglycerol-rich lipoproteins are transferred to HDL [7]. (2) HTGL, which may hydrolyse the triacylglycerol component of IDL, LDL and HDL as well as phospholipids in HDL. HTGL action results in conversion of HDL₂ into HDL₃ [8]. HTGL is located on hepatic endothelial cells and, like LPL, can be measured in plasma after heparin injection, which releases the two enzymes from their endothelial binding sites. (3) LCAT, which synthesizes cholesteryl esters in plasma from unesterified cholesterol and phosphatidylcholine. The cholesterol substrate is acquired from various tissues and plasma lipoproteins, in both the pre- and post-prandial state. During the action of LCAT, HDL₂ is converted into HDL₃, whereas HDL₃ surface fragments are metabolized [7]. (4) Plasma lipid transfer proteins: CETP, which transfers the cholesteryl esters between the various lipoprotein classes, and PLTP, a specific phospholipid transfer protein, not transferring cholesteryl esters [7,9,10].

MATERIALS AND METHODS

Animals and diets

The rabbit strains used were IIIVO/JU and AX/JU, which originated from the Jackson Laboratory Colony, Bar Harbor,

Abbreviations used: apo, apolipoprotein; CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; HTGL, hepatic triacylglycerol lipase; IDL, intermediate-density lipoprotein; LCAT, lecithin:cholesterol acyltransferase; LDL, low-density lipoprotein; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids; PLTP, phospholipid transfer protein; VLDL, very-low-density lipoprotein.

§ To whom correspondence should be sent. Present address: Laboratory of Toxicology, National Institute of Public Health and Environmental Protection, P.O. Box 1, 3720 BA Bilthoven, The Netherlands.

|| Present address: Department of Pediatrics, University Hospital, University of Leiden, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

ME, U.S.A. [11]. The IIIVO/JU strain has previously been shown to be hyporesponsive and the AX/JU strain to be hyper-responsive to dietary cholesterol [2–5]. Seven male rabbits of each of the two inbred strains were used; the groups were stratified for age, which ranged from 0.5 to 2.5 years. This stratification was done to eliminate any effect of age on the measures, except for plasma cholesterol response, which is known not to be influenced by age in our rabbit strains. The average age for each group was 1.5 ± 0.7 years ($\pm$ S.D.). All rabbits had been fed on a natural-ingredient diet (LKK-20; Hope Farms, Woerden, The Netherlands) and were housed individually as described [4]; light in the room was on from 07:00 to 19:00 h. According to the manufacturer, the non-purified diet (gross energy 18 200 kJ/kg) consisted of (g/100 g of dietary dry matter): crude protein 18.8; crude fat 3.3; crude fibre 15.8; ash 8.2. In order to eliminate possible time and sequence effects, the feeding trial had an A_1 – B – A_2 design. All rabbits were transferred from the commercial diet to the same diet to which 0.15 g of cholesterol/100 g diet had been added. After consuming the high-cholesterol diet for 8 weeks, all animals were switched back to the commercial diet without added cholesterol for another 14 weeks. Upon analysis, the commercial diets without and with added cholesterol were found to contain 6 and 188 mg of cholesterol/100 g of diet respectively. Each rabbit was given 80 g of feed per day. Tap water was provided *ad libitum*. The rabbits were allowed to practice cecotrophy. Body weights were measured every 1 or 2 weeks.

Blood sampling

Samples of blood were taken from the marginal ear vein. On the day before blood sampling, any remaining food was removed at 16:00 h. Sampling was performed between 8:00 and 10:00 h at 1- or 2-week intervals. Blood was collected on crushed ice in tubes containing either heparin (final blood concentration was about 100 units/ml) or Na_2EDTA (final concentration was about 4 mM). After sampling of blood for determination of plasma lipids and LCAT, PLTP and CETP activities, the rabbits were injected intravenously with 100 units of heparin/kg body wt. to release LPL and HTGL into the circulation. Exactly 15 min after injection, blood was collected in heparinized tubes on crushed ice for measurement of lipolytic activities.

Chemical analyses

Plasma was collected by low-speed centrifugation (10 min, 3000 g, 3 °C) and kept at -20 °C or -80 °C until analysis. Lipoproteins were isolated from heparinized plasma by density-gradient ultracentrifugation [12] at the following relative densities (d): VLDL, $d < 1.006$; IDL, $1.006 < d < 1.019$; LDL, $1.019 < d < 1.063$; HDL, $1.063 < d < 1.210$. Recoveries of total cholesterol and triacylglycerols in the lipoprotein fractions were 89 ± 5 and $91 \pm 7\%$ (means $\pm$ S.D., $n = 56$), respectively. Triacylglycerols [13], total and free cholesterol [14] and phospholipids [15] were measured enzymically in whole plasma and lipoprotein fractions, by using test combinations purchased from Boehringer, Mannheim, Germany. Cholesteryl esters were calculated from the difference between total and unesterified cholesterol. Cholesterol in feed samples was analysed by g.l.c. [16].

CETP activity was assayed in EDTA-treated plasma as described by Groener et al. [17], with the following modifications. Apo B-containing lipoproteins were precipitated with phosphotungstate/ Mg^{2+} before measuring the exchange of cholesteryl esters between excess [^{14}C]cholesteryl esters-labelled

human LDL and excess unlabelled human HDL, as catalysed by the apo B-free supernatant of plasma. Standard preparations of human LDL and HDL were used to compare CETP activities as such in the two rabbit strains, i.e. not influenced by strain differences in lipoprotein composition. After incubation, the tubes were cooled to 4 °C to stop the reaction. LDL was precipitated from the incubation mixture with Mn^{2+} as described previously [10], and the radioactivity was measured in the supernatant containing HDL. CETP activity was linear with time and amount of plasma, independent of the endogenous lipoproteins present in each plasma, and expressed relative (%) to that of a human plasma pool.

LCAT activity levels were determined in EDTA-treated plasma as originally described [18], by using excess exogenous substrate containing [^3H]cholesterol. Samples were incubated for 6 h at 37 °C in a total volume of 0.145 ml. The reaction was stopped by addition of 0.30 ml of cold methanol, and the lipids were extracted with 2×0.4 ml of hexane. Labelled cholesterol was separated from labelled cholesteryl esters by using disposable silica columns, and [^3H]cholesteryl esters were eluted with 3.0 ml of hexane/diethyl ether (6:1, v/v). PLTP activity was measured as described [10]. LCAT and PLTP activities were linear with the amount of plasma used. To check accuracy, a series of reference plasma samples was measured in each run. LCAT and PLTP activities are expressed in arbitrary units, i.e. relative to the activity in a human plasma pool (% of plasma pool). The within-run coefficients of variation were 5.1% (LCAT) and 3.5% (PLTP).

HTGL and LPL activities were selectively assayed in post-heparin plasma samples exactly as described [19]. Using triolein substrate in 0.1 M NaCl, the sum of HTGL and LPL activities was measured. In addition, HTGL was measured selectively in substrate containing 0.1 M NaCl without activating serum. LPL was calculated by subtraction.

Data analyses

The Kolmogorov–Smirnov one-sample test indicated that all data were normally distributed. Comparisons between the hypo- and hyper-responsive strains and between treatments within each strain were evaluated by unpaired and paired Student's t tests, respectively, by using the SPSS/PC+ program. Two-sided P values < 0.05 were pre-set for statistically significant differences. For multiple comparisons with paired Student's t test, P values were pre-set at < 0.017 (Bonferroni adaptation).

RESULTS

Body weight and feed intake

At the beginning of the experiment, body weights of the hypo- and hyper-responsive rabbits were 2.85 ± 0.22 and 2.49 ± 0.11 kg (means $\pm$ S.D., $n = 7$; $P < 0.05$). During the course of the experiment, body weights tended to increase in both strains (weight gain 0.14 ± 0.12 and 0.15 ± 0.10 kg respectively; means $\pm$ S.D.). All rabbits completely consumed the daily restricted amount of feed.

Plasma lipids and lipoproteins

When fed on the diet without added cholesterol, plasma total cholesterol concentration was significantly lower in hyper- than in hypo-responders (Table 1). This was due to low levels of plasma HDL-cholesterol in the hyper-responders. The hyper-responsive rabbits had about 75% of total cholesterol in lipoproteins of $d < 1.063$, whereas in the hyporesponders about 85% of total cholesterol was carried by HDL particles. Chol-

Table 1 Baseline whole plasma and lipoprotein total cholesterol and triacylglycerol concentrations and their changes after cholesterol feeding in hypo- and hyper-responsive rabbits

Results are expressed as means $\pm$ S.D. for 7 rabbits: ^asignificant difference between hyper- and hypo-responders fed on the same diet; ^bsignificant response to dietary cholesterol within hypo-responders; ^csignificant response to dietary cholesterol within hyper-responders; ^dsignificant strain difference in response to dietary cholesterol.

Plasma lipids (mmol/l)	Diet without added cholesterol*		Diet with (0.15%) added cholesterol†		Absolute change after cholesterol feeding‡	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
Total cholesterol						
Whole plasma	0.82 $\pm$ 0.08	0.50 $\pm$ 0.11 ^a	4.75 $\pm$ 0.96 ^b	9.44 $\pm$ 2.31 ^{ac}	3.92 $\pm$ 0.92 ^b	8.95 $\pm$ 2.25 ^{cd}
VLDL	0.04 $\pm$ 0.01	0.22 $\pm$ 0.06 ^a	1.88 $\pm$ 0.46 ^b	3.21 $\pm$ 1.01 ^{ac}	1.84 $\pm$ 0.46 ^b	2.99 $\pm$ 1.01 ^{cd}
IDL	0.03 $\pm$ 0.01	0.03 $\pm$ 0.02	0.29 $\pm$ 0.11 ^b	2.13 $\pm$ 0.79 ^{ac}	0.26 $\pm$ 0.11 ^b	2.10 $\pm$ 0.78 ^{cd}
LDL	0.05 $\pm$ 0.02	0.02 $\pm$ 0.01 ^a	0.53 $\pm$ 0.14 ^b	2.17 $\pm$ 0.59 ^{ac}	0.48 $\pm$ 0.12 ^b	2.15 $\pm$ 0.59 ^{cd}
HDL	0.62 $\pm$ 0.08	0.10 $\pm$ 0.03 ^a	1.37 $\pm$ 0.18 ^b	0.77 $\pm$ 0.10 ^{ac}	0.75 $\pm$ 0.14 ^b	0.67 $\pm$ 0.09 ^c
Triacylglycerol						
Whole plasma	0.47 $\pm$ 0.09	1.02 $\pm$ 0.19 ^a	0.93 $\pm$ 0.41 ^b	0.51 $\pm$ 0.20 ^{ac}	0.46 $\pm$ 0.42 ^b	-0.51 $\pm$ 0.22 ^{cd}

* $\frac{1}{2}$ (final value in period A₁) plus $\frac{1}{2}$ (final value in period A₂).

† Final value in period B.

‡ Final value in period B minus [$\frac{1}{2}$ (final value in period A₁) plus $\frac{1}{2}$ (final value in period A₂)].

esterol feeding increased plasma total cholesterol concentrations in rabbits of both strains, but the increase was significantly greater in the hyper-responders. This difference was reflected mainly in the VLDL, IDL and LDL fractions.

In hypo- as well as in hyper-responders, the increase in total cholesterol concentration after cholesterol feeding was located in all lipoprotein fractions (Table 1). After cholesterol feeding for 8 weeks (final value in period B), 66 and 90% of plasma total cholesterol was carried by lipoproteins of $d < 1.063$ in the hypo- and hyper-responders, respectively. Cholesterol concentrations in all lipoproteins of hypo- and hyper-responders had fallen to baseline values (final value in period A₁) at the end of the experiment (final value in period A₂) (results not shown). Figure 1(a) shows the time course of plasma total cholesterol. About 12 weeks after being switched back to the diet without added cholesterol, plasma total cholesterol levels had returned to baseline values in both hypo- and hyper-responsive rabbits.

After feeding with the diet without added cholesterol, plasma triacylglycerol levels were significantly higher in hyper- than in hypo-responders (Table 1). Cholesterol feeding produced a significant increase in plasma triacylglycerols in the hypo-responsive rabbits, but caused a significant decrease in the hyper-responders. Upon cessation of cholesterol consumption, plasma triacylglycerol concentration returned to baseline values in both hypo- and hyper-responders (Figure 1b). There was a marked inter-individual variation and time-dependent fluctuation in plasma triacylglycerol levels within each strain.

In animals fed on the diet without added cholesterol, VLDL, IDL and LDL of hyper-responders had a composition rather similar to that of hypo-responders (Table 2). HDL particles of hyper-responders fed on the diet without added cholesterol had lower molar ratios of cholesteryl esters:triacylglycerols and of free cholesterol:phospholipids.

After cholesterol feeding, all lipoprotein fractions of hyper-responders, compared with those of hypo-responders, showed larger increases in the molar ratios of cholesteryl esters:triacylglycerols (Table 2). Dietary cholesterol significantly increased the molar cholesteryl ester:free cholesterol ratio in VLDL of both strains. The molar ratio of free cholesterol:phospholipid in all lipoproteins increased in both strains, but the increase was most pronounced in hyper-responders.

Figure 1 (a) Mean plasma total cholesterol and (b) triacylglycerol concentrations of hyper-responsive (■) and hypo-responsive rabbits (□) fed on a low-cholesterol (LCD) and high-cholesterol diet (HCD) according to the A₁–B–A₂ design outlined in the Materials and methods section

Results presented as means $\pm$ S.E.M. ($n = 7$).

Lipolytic activities

Activities of LPL and HTGL in post-heparin plasma were similar in hyper- and hypo-responders when fed on the diet without added cholesterol (Table 3). Dietary cholesterol produced a 2–3-fold increase in LPL and HTGL activities in hyper-responders, but caused smaller increases in hypo-responders. The effect of dietary cholesterol on LPL in hypo-responders was not statistically significant. Figure 2 shows that

Table 2 Baseline lipid components of plasma lipoproteins and their response to cholesterol feeding in hypo- and hyper-responsive rabbits

Results are expressed as means $\pm$ S.D. for 7 rabbits. For superscripts *, †, ‡, ^{a,b,c,d}, see legend and footnotes to Table 1. §Lipids: cholesteryl esters (CE), free cholesterol (FC), triacylglycerols (TG), phospholipids (PL) in mmol/l of whole plasma; ratios in mol/mol.

Lipo-protein	Lipid or lipid ratio§	Diet without added cholesterol*		Diet with (0.15%) added cholesterol†		Absolute change after cholesterol feeding‡	
		Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
VLDL	CE	0.02 $\pm$ 0.01	0.12 $\pm$ 0.04 ^a	1.40 $\pm$ 0.33 ^b	2.36 $\pm$ 0.70 ^{ac}	1.38 $\pm$ 0.32 ^b	2.24 $\pm$ 0.69 ^{cd}
	FC	0.01 $\pm$ 0.01	0.10 $\pm$ 0.03 ^a	0.48 $\pm$ 0.15 ^b	0.85 $\pm$ 0.36 ^{ac}	0.46 $\pm$ 0.15 ^b	0.75 $\pm$ 0.37 ^c
	TG	0.09 $\pm$ 0.04	0.62 $\pm$ 0.13 ^a	0.47 $\pm$ 0.20 ^b	0.26 $\pm$ 0.12 ^{ac}	0.37 $\pm$ 0.20 ^b	-0.37 $\pm$ 0.15 ^{cd}
	PL	0.03 $\pm$ 0.01	0.17 $\pm$ 0.04 ^a	0.44 $\pm$ 0.13 ^b	0.63 $\pm$ 0.26 ^c	0.41 $\pm$ 0.13 ^b	0.46 $\pm$ 0.28 ^c
	CE:FC	1.7 $\pm$ 0.5	1.2 $\pm$ 0.2 ^a	3.0 $\pm$ 0.4 ^b	3.0 $\pm$ 0.7 ^c	1.3 $\pm$ 0.6 ^b	1.8 $\pm$ 0.9 ^c
	CE:TG	0.26 $\pm$ 0.05	0.20 $\pm$ 0.04 ^a	3.3 $\pm$ 1.3 ^b	11 $\pm$ 6 ^{ac}	3.1 $\pm$ 1.3 ^b	11 $\pm$ 6 ^{cd}
	FC:PL	0.54 $\pm$ 0.08	0.59 $\pm$ 0.03	1.08 $\pm$ 0.06 ^b	1.33 $\pm$ 0.11 ^{ac}	0.53 $\pm$ 0.12 ^b	0.75 $\pm$ 0.10 ^{cd}
IDL	CE	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	1.20 $\pm$ 0.08 ^b	1.55 $\pm$ 0.58 ^{ac}	0.18 $\pm$ 0.07 ^b	1.63 $\pm$ 0.56 ^{cd}
	FC	0.01 $\pm$ 0.01	0.01 $\pm$ 0.01	0.08 $\pm$ 0.03 ^b	0.57 $\pm$ 0.21 ^{ac}	0.07 $\pm$ 0.03 ^b	0.60 $\pm$ 0.20 ^{cd}
	TG	0.02 $\pm$ 0.01	0.03 $\pm$ 0.01	0.04 $\pm$ 0.02 ^b	0.07 $\pm$ 0.04 ^c	0.02 $\pm$ 0.01 ^b	0.04 $\pm$ 0.02 ^{cd}
	PL	0.01 $\pm$ 0.01	0.02 $\pm$ 0.01	0.08 $\pm$ 0.03 ^b	0.44 $\pm$ 0.16 ^{ac}	0.06 $\pm$ 0.03 ^b	0.42 $\pm$ 0.15 ^{cd}
	CE:FC	2.0 $\pm$ 0.6	2.1 $\pm$ 0.5	2.5 $\pm$ 0.2	2.7 $\pm$ 0.1 ^{ac}	0.5 $\pm$ 0.7	0.6 $\pm$ 0.6 ^c
	CE:TG	0.94 $\pm$ 0.42	0.67 $\pm$ 0.16	4.9 $\pm$ 1.3 ^b	25 $\pm$ 11 ^{ac}	4.0 $\pm$ 1.3 ^b	23 $\pm$ 11 ^{cd}
	FC:PL	0.75 $\pm$ 0.10	0.62 $\pm$ 0.10 ^a	1.06 $\pm$ 0.07 ^b	1.31 $\pm$ 0.08 ^{ac}	0.31 $\pm$ 0.09 ^b	0.68 $\pm$ 0.16 ^{cd}
LDL	CE	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01 ^a	0.37 $\pm$ 0.09 ^b	1.57 $\pm$ 0.44 ^{ac}	0.34 $\pm$ 0.08 ^b	1.56 $\pm$ 0.44 ^{cd}
	FC	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01 ^a	0.16 $\pm$ 0.05 ^b	0.60 $\pm$ 0.15 ^{ac}	0.14 $\pm$ 0.05 ^b	0.59 $\pm$ 0.15 ^{cd}
	TG	0.02 $\pm$ 0.01	0.02 $\pm$ 0.01	0.08 $\pm$ 0.03 ^b	0.08 $\pm$ 0.04 ^c	0.06 $\pm$ 0.03 ^b	0.06 $\pm$ 0.04 ^c
	PL	0.03 $\pm$ 0.01	0.01 $\pm$ 0.01 ^a	0.26 $\pm$ 0.07 ^b	0.51 $\pm$ 0.12 ^{ac}	0.23 $\pm$ 0.07 ^b	0.50 $\pm$ 0.12 ^{cd}
	CE:FC	1.8 $\pm$ 0.3	2.9 $\pm$ 2.2	2.4 $\pm$ 0.2 ^b	2.6 $\pm$ 0.2 ^a	0.5 $\pm$ 0.3 ^b	-0.3 $\pm$ 2.2
	CE:TG	1.5 $\pm$ 0.6	0.6 $\pm$ 0.4 ^a	4.9 $\pm$ 1.4 ^b	23 $\pm$ 10 ^{ac}	3.4 $\pm$ 1.5 ^b	23 $\pm$ 10 ^{cd}
	FC:PL	0.60 $\pm$ 0.12	0.68 $\pm$ 0.44	0.62 $\pm$ 0.07 ^b	1.17 $\pm$ 0.05 ^{ac}	0.02 $\pm$ 0.10 ^b	0.49 $\pm$ 0.44 ^{cd}
HDL	CE	0.46 $\pm$ 0.06	0.08 $\pm$ 0.02 ^a	0.99 $\pm$ 0.12 ^b	0.58 $\pm$ 0.07 ^{ac}	0.53 $\pm$ 0.09 ^b	0.50 $\pm$ 0.06 ^c
	FC	0.16 $\pm$ 0.03	0.03 $\pm$ 0.01 ^a	0.39 $\pm$ 0.07 ^b	0.19 $\pm$ 0.03 ^{ac}	0.22 $\pm$ 0.06 ^b	0.17 $\pm$ 0.03 ^{cd}
	TG	0.21 $\pm$ 0.06	0.10 $\pm$ 0.02 ^a	0.23 $\pm$ 0.05	0.06 $\pm$ 0.01 ^{ac}	0.02 $\pm$ 0.08	-0.04 $\pm$ 0.02 ^c
	PL	0.60 $\pm$ 0.08	0.17 $\pm$ 0.03 ^a	1.01 $\pm$ 0.10 ^b	0.37 $\pm$ 0.05 ^{ac}	0.41 $\pm$ 0.12 ^b	0.20 $\pm$ 0.07 ^{cd}
	CE:FC	2.8 $\pm$ 0.4	3.4 $\pm$ 0.5	2.6 $\pm$ 0.3	3.1 $\pm$ 0.4 ^a	-0.3 $\pm$ 0.4	-0.3 $\pm$ 0.7
	CE:TG	2.4 $\pm$ 1.0	0.8 $\pm$ 0.2 ^a	4.5 $\pm$ 1.0 ^b	9.9 $\pm$ 2.8 ^{ac}	2.1 $\pm$ 1.3 ^b	9.1 $\pm$ 2.7 ^{cd}
	FC:PL	0.28 $\pm$ 0.02	0.16 $\pm$ 0.02 ^a	1.38 $\pm$ 0.04 ^b	0.51 $\pm$ 0.06 ^{ac}	0.11 $\pm$ 0.03 ^b	0.36 $\pm$ 0.06 ^{cd}

Table 3 Baseline activities of plasma lipolytic enzymes and lipid-transfer proteins and their changes after cholesterol feeding in hypo- and hyper-responsive rabbits

Results are expressed as means $\pm$ S.D. for 7 rabbits. For superscripts *, †, ‡, ^{a,b,c,d}, see legend and footnotes to Table 1. Activities are expressed in μ mol of NEFA/h per ml of plasma for LPL and HTGL, and in arbitrary units (% relative to the activity in a human pool plasma) for LCAT, CETP and PLTP.

Plasma activity	Diet without added cholesterol*		Diet with (0.15%) added cholesterol†		Absolute change after cholesterol feeding‡	
	Hypo	Hyper	Hypo	Hyper	Hypo	Hyper
LPL	6.4 $\pm$ 1.6	5.2 $\pm$ 1.0	8.5 $\pm$ 3.4	12.0 $\pm$ 4.3 ^c	2.1 $\pm$ 3.0	6.8 $\pm$ 3.5 ^{cd}
HTGL	1.34 $\pm$ 0.21	1.46 $\pm$ 0.14	2.01 $\pm$ 0.44 ^b	4.50 $\pm$ 0.75 ^{ac}	0.67 $\pm$ 0.49 ^b	3.04 $\pm$ 0.74 ^{cd}
LCAT	29 $\pm$ 7	14 $\pm$ 5 ^a	36 $\pm$ 24	26 $\pm$ 19	7 $\pm$ 31	13 $\pm$ 24
CETP	89 $\pm$ 6	77 $\pm$ 15	287 $\pm$ 30 ^b	209 $\pm$ 35 ^{ac}	199 $\pm$ 28 ^b	132 $\pm$ 28 ^{cd}
PLTP	65 $\pm$ 4	46 $\pm$ 4 ^a	73 $\pm$ 7 ^b	70 $\pm$ 8 ^c	8 $\pm$ 9 ^b	25 $\pm$ 8 ^{cd}

the effects of dietary cholesterol on LPL and HTGL were completely reversible in both rabbit strains.

Plasma LCAT, PLTP and CETP activity levels

Plasma LCAT activities in hyper-responders fed on the diet without added cholesterol were significantly lower than in hypo-responders (Table 3). Dietary cholesterol tended to increase LCAT activity in both rabbit strains, but this effect did not reach statistical significance; the LCAT response to dietary cholesterol was extremely variable between individual rabbits. After removal of cholesterol from the diet, LCAT activity fell in both rabbit

strains, but remained very variable; the activities in the hypo- and hyper-responders were 28 $\pm$ 8 and 16 $\pm$ 3 % of the activity of the human plasma pool (means $\pm$ S.D., n = 7). No strain difference in the response of LCAT to cholesterol feeding could be detected.

CETP activity in plasma was similar in rabbits of both strains when fed on the diet without added cholesterol (Table 3 and Figure 3a). Addition of cholesterol to the diet caused a large increase in plasma CETP activity in both strains. However, this increase was significantly lesser in the hyper-responders. The increase in CETP activity seen after cholesterol feeding completely disappeared after omitting cholesterol from the diet.

Figure 2 (a) Post-heparin plasma LPL and (b) HTGL activities in hyper- and hypo-responsive rabbits

For explanation of experimental design and symbols, see legend to Figure 1.

After feeding on the diet without added cholesterol, plasma PLTP activity was higher in hypo- than in hyper-responders (Table 3 and Figure 3b). PLTP activity was increased by dietary cholesterol in hyper-, but not in hypo-responders, this increase being reversible.

DISCUSSION

The composition and concentration of plasma lipoproteins are determined by the rates of secretion of lipoprotein components into plasma, by the remodelling of lipoproteins in plasma, and by the rates of removal from plasma and degradation. Thus a strain difference in the lipase system or in plasma activities of LCAT and lipid transfer proteins cannot fully explain all strain differences in plasma lipid concentrations and lipoprotein compositions.

When fed on the diet without added cholesterol, LCAT activity was markedly lower in hyper- than in hypo-responders. One possible function of this enzyme is the conversion of disc-shaped surface fragments, formed from chylomicron or VLDL degradation, into spherical HDL [20]. The low plasma LCAT activity in hyper-responders may result in slow synthesis of HDL cholesteryl esters, leading to the observed low HDL-cholesterol levels in these rabbits. Indeed, when fed on the diet without added cholesterol, HDL in hyper-responders had significantly lower concentrations of cholesteryl esters. After cholesterol challenge, total HDL-cholesterol concentration and LCAT activity increased, so that the difference in these parameters between the two strains had decreased. After cholesterol feeding, the molar ratios of cholesteryl esters:free cholesterol of all lipoprotein fractions in hyper- and hypo-responders were similar.

Figure 3 (a) Plasma CETP and (b) PLTP activities in hyper- and hypo-responsive rabbits

For explanation of experimental design and symbols, see the legend to Figure 1. Activities are given as percentages of the activity in a human plasma pool.

Possibly, intravascular cholesterol esterification is not a contributing factor in determining hypo- or hyper-responsiveness to dietary cholesterol.

VLDL, IDL and LDL were enriched with cholesteryl esters in both strains after cholesterol feeding. Cholesterol feeding causes an increased hepatic secretion of cholesteryl esters in VLDL, IDL and LDL, this effect being greater in hyper- than in hypo-responsive rabbits [21], which in turn may be caused by the higher efficiency of intestinal cholesterol absorption in hyper-responders [5]. The nascent hepatic lipoproteins of hyper-responders have higher total cholesterol:triacylglycerol ratios than do those of hypo-responders [21]. Thus the observed enrichment of VLDL, IDL and LDL from hyper-responders with cholesteryl esters at the expense of triacylglycerols can be explained by hepatic production of such lipoproteins. The dietary-cholesterol-induced increase in plasma activity levels of CETP may be an additional factor, although after cholesterol feeding the increase in CETP activity was significantly lesser in hyper- than in hypo-responders. The dietary-cholesterol-induced increase in plasma CETP activity corroborates earlier cholesterol-challenge trials with rabbits [22,23]. In hyperlipidaemic and normolipidaemic humans, plasma cholesterol concentration is positively associated with CETP activity [24,25]. Diet-induced alterations of CETP activity coincide with alterations of VLDL + IDL cholesterol levels [26]. Plasma CETP activity in our hyper-responders may have been sub-optimal during cholesterol challenge, contributing to the greater increase in plasma cholesterol in these rabbits, because low CETP levels are associated with a low capacity for plasma cholesteryl ester turnover [27].

The significant strain difference in plasma PLTP activities,

when fed on the diet without added cholesterol, disappeared after addition of cholesterol to the diet. This was due to an increase in PLTP activity in the hyper-responders. This observation cannot be interpreted, because the physiological function of PLTP is still unsettled [10]. Putative roles of PLTP involve the provision of substrate for LCAT and/or transferring phospholipid-rich surface fragments from triacylglycerol-rich lipoproteins to HDL during catalysed lipolysis.

Feeding on the high-cholesterol diet decreased plasma triacylglycerols in hyper-responders, but produced increased concentrations in hypo-responders. In cholesterol-fed hypo- and hyper-responders, triacylglycerol secretion by the liver is decreased to the same extent [21]. Thus the strain difference in plasma triacylglycerol response likely relates to a difference in plasma triacylglycerol degradation. Indeed, after cholesterol feeding only in hyper-responders was a significant increase in LPL activity found. Therefore the observed strain difference in the response of plasma triacylglycerols to dietary cholesterol may be explained by a different response of LPL activity.

The accumulation of VLDL-, IDL- and LDL-cholesterol in hyper-responders fed with cholesterol points to impaired removal of these lipoproteins. Cholesterol feeding is usually accompanied by a decrease in LDL-receptor activity [28]. However, absolute clearance of LDL through the LDL-receptor pathway is similar in cholesterol-fed hypo- and hyper-responsive rabbits [29], and binding of β -VLDL to hepatocyte membrane receptors is not over-depressed in cholesterol-fed hyper-responders [30]. Thus in cholesterol-fed hyper-responders, as compared with hypo-responders, more LDL is cleared through the receptor-independent route, but this increase appears secondary to the raised levels of LDL, and its precursors, VLDL and IDL [29].

After addition of cholesterol to the diet, plasma cholesterol and the activity of LPL, and especially that of HTGL, increased in a parallel fashion. This was most evident in the hyper-responders. A similar parallelism was seen for the initial responses of plasma cholesterol levels and plasma CETP activities. The cholesterolaemic response might trigger synthesis and secretion of LPL, HTGL and CETP. Hepatic CETP mRNA levels are increased in rabbit liver after cholesterol feeding, and heparin-releasable HTGL activity is increased after perfusion of rabbit livers *in vitro* with cholesterol-rich β -VLDL [31]. Studies using hamsters revealed that high-cholesterol diets increased CETP mRNA in adipose tissue [32].

In sum, cholesterol feeding of hypo- and hyper-responsive rabbits produced marked changes in lipases and lipid-transfer protein activities. The observed strain differences may in part explain the strain difference in plasma lipoprotein metabolism after cholesterol challenge. Additional regulation must take place at the levels of secretion and degradation of lipoproteins. Since LDL receptors are down-regulated after cholesterol feeding to the same extent in both rabbit strains [29,30], alternative pathways may operate. In WHHL rabbits, which are characterized by a defective LDL receptor, treatment with ethinyloestradiol causes striking decreases in VLDL + IDL lipids and an increased activity of HTGL [19]. It was speculated that the increase in HTGL activity partly overcomes the defect in the LDL receptor, at least with respect to the clearance of VLDL + IDL. The high activities of LPL and HTGL seen after cholesterol feeding in our hyper-responders may point to extra lipase molecules at the surface of the circulating cholesterol-rich lipoproteins, which may activate the binding between lipoproteins and lipoprotein receptors [33,34]. In this way, LDL-receptor-

independent lipoprotein removal mechanisms may be triggered in cholesterol-fed hyper-responders.

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