Lipoprotein lipase and hepatic lipase activities in a hypercholesterolaemic (RICO) strain of rat

Effect of dietary cholesterol

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Hepatic lipase (HL) and lipoprotein lipase (LPL) were assayed in heparinized plasma from male normocholesterolaemic (SW) and genetically hypercholesterolaemic (RICO) rats. Both strains were fed on either a semi-purified control diet or the same diet enriched with 0.5% or 1% cholesterol. HL activity was similar in both groups of rats fed on the control diet. LPL activity was found to be significantly lower in RICO rats (35% decrease, $P < 0.05$). Feeding with a high-cholesterol diet led to a decrease in HL activity (15–23%) in both groups of rats but no change was detected in LPL activity, which remained consistently lower in the RICO rats. Thus, with the control diet, LPL activity is lower in RICO rats but presumably is not rate-limiting for their triacylglycerol clearance, given the normal triacylglycerol levels present. After cholesterol feeding, however, the lower LPL activity may become rate-limiting together with the decrease in HL activity, as in these circumstances hypertriacylglycerolaemia was evident and the hypercholesterolaemia of this strain was further increased.

INTRODUCTION

The main characteristics of the RICO strain of rat (rats with inherited hypercholesterolaemia) are enhanced hepatic and intestinal cholesterologenesis [1,2] together with an increased mobile cholesterol pool [2] as compared with the normocholesterolaemic control strain (SW). When fed on a semi-purified control diet, the hypercholesterolaemia of the RICO rat stems mainly from an increased cholesterol content in the $d > 1.006$ lipoprotein fractions [low-density lipoproteins (LDL) 1 and 2 and high-density lipoprotein (HDL) 3] [3]. When 0.5% cholesterol was added to the diet, hypercholesterolaemia was induced in both strains of rats, but cholesterol enrichment was located in the lighter lipoproteins [very-low-density lipoprotein (VLDL) and chylomicrons] which contained respectively 60% (SW) and 45% (RICO) of the plasma cholesterol [3]. Under the latter conditions, cholesterol enrichment of VLDL (9-fold) was comparable for both strains of rats, but that of chylomicrons was significantly higher in the RICO rats (12-fold) than in the SW rats (7-fold), suggesting a greater delay in chylomicron removal in the former. Concerning triacylglycerol-rich-lipoprotein catabolism, little is known at present about plasma lipolytic enzymes in both strains of rats. *In vivo*, muscle and adipose tissue lipoprotein lipases (LPL) are involved in the hydrolysis of chylomicron and VLDL triacylglycerols, whereas chylomicron remnants, small VLDL and intermediate-density lipoprotein (IDL) triacylglycerols [4] and LDL and HDL phospholipids [5] are hydrolysed by hepatic lipase (HL; EC 3.1.1.3). The injection of anti-HL antibodies into rats

leads to accumulation of different lipoprotein fractions and components [6–10]. These studies thus suggest a role for HL in the metabolism of chylomicron remnants, IDL and HDL.

In the present work, the activities of LPL and HL in post-heparin plasma were assayed, as well as the activity of HL secreted by hepatocytes isolated from both strains of rats. Uptake of chylomicron remnants (obtained from Wistar rats) by isolated hepatocytes of SW and RICO rats was also assessed as an indication of the receptor-mediated processing of these particles. Two cholesterol-rich diets, known to increase the less dense lipoproteins in the plasma [3,11] were given to both RICO and SW rats in order to ascertain the effect of exogenous cholesterol on post-heparin lipolytic activities.

MATERIALS AND METHODS

Animals and diets

Male RICO and SW rats weighing 140–150 g were provided by the Ciba-Geigy breeding unit (Basel, Switzerland). They were fed on a semi-purified (control) diet containing 53% saccharose, 23% casein, 9.2% lard, 5% mineral mix, 4% skimmed milk, 2.5% yeast, 2.5% vitamins, 0.8% walnut oil, 0.2% cystine and 0.05% cholesterol until they were 4 months old. At that age, one group continued on this control diet, while two other groups were fed on the same diet enriched with either 0.5% or 1% cholesterol for 4 weeks. Cholesterol was dissolved in hot methanol and recrystallized before addition to the diets. The animals were housed in wire-

Abbreviations used: LPL, lipoprotein lipase; HL, hepatic lipase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; VLDL, very-low-density lipoprotein; IDL, intermediate-density lipoprotein.

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bottomed cages in a temperature-controlled room (21–22 °C) artificially lit between 07:00 and 19:00 h. They had free access to food and water. At the end of the experiment, SW and RICO rat mean body weights ($\pm$ S.E.M.) were 487 ± 22 g and 540 ± 10 g ($n = 11$) respectively. Blood sampling or hepatocyte preparations were started at 09:00 h. Animals were treated in randomized blocks.

Determination of HL activity

RICO and SW rats fed *ad libitum* were injected in the jugular vein with 20 units of heparin/100 g body wt. (Choay, Paris, France), and 0.5 ml of blood was withdrawn from the same vein 2 min later. Plasma samples were stored immediately at -20 °C. HL activity assays were performed 1–2 days later. For this purpose, 5 μ l of plasma was incubated for 10 min in a shaking bath at 30 °C, with 0.2 ml of an emulsion of 1.33 mM-[14 C]triolelylglycerol (10^6 d.p.m./ μ mol) and 0.1 mM-lysophosphatidylcholine [12], made in 0.2 M-Tris/HCl buffer, pH 9, containing 0.4% BSA and 0.5 M-NaCl. LPL activity was inhibited completely by 0.5 M-NaCl instead of the 1 M concn. usually used. This lower concentration of salt prevented phospholipid aggregation. The reaction was stopped with 3.25 ml of methanol/chloroform/heptane (1.41:1.25:1, by vol.), and then 1.05 ml of 0.1 M-borate buffer, pH 10.5, was added according to [13]. The released fatty acids were recovered in the upper extraction phase and counted for radioactivity using a liquid scintillation counter (Beckman). Activity was expressed in munits (nmol of free fatty acids released/min) per ml of plasma. It was expressed per 10^6 cells when testing the activity released into the medium by isolated hepatocytes.

Determination of LPL activity

LPL (EC 3.1.1.34) was assayed in post-heparin plasma obtained as described above using the same emulsion as for HL assay, but with the following differences: no NaCl was added, and BSA and rat serum (previously inactivated at 56 °C for 30 min) were added only after emulsion of the substrate [12]. All assays were carried out at 37 °C and the reaction was stopped with the same extraction system as for the HL assay. Activity was expressed in munits/ml of plasma.

Chylomicron remnant preparation and labelling

Chylomicrons were isolated from lymph obtained from the thoracic duct of 400 g Wistar rats as described [14]. The chylomicrons were injected into functionally eviscerated rats (50 mg of chylomicron triacylglycerol/rat). Rats were bled 30 min later and remnants were isolated at $d = 1.019$ g/ml by ultracentrifugation at 10 °C. Labelling with [14 C]cholesterol oleyl ether was as described in [15].

Hepatocyte preparation and incubation procedures

Cells from one RICO and one SW rat were prepared on the same day between 9:00 and 11:00 h according to a modification of Seglen's procedure [16] as described in [14]. They were purified further by Percoll density-gradient centrifugation [17], and cell viability was greater than 90%. Hepatocytes [$(10-12) \times 10^6$ cells] were added to plastic Nalgen flasks in a final volume of 3 ml of Krebs' bicarbonate medium (pH 7.45) as described previously [14]. To measure the HL activity secreted into the

medium, cells were incubated for up to 150 min at 37 °C in the presence of heparin, as indicated in Fig. 1. At the end of the incubation, the medium was separated from the cell pellet by a brief centrifugation (1 min at 500 g and 4 °C) and stored at -20 °C. HL activity was determined on the next day. To measure chylomicron-remnant uptake, hepatocytes were first preincubated for 150 min to restore their lysosomal degradation capacity [14]. Labelled remnants (30 or 60 μ g of protein/flask) were added thereafter for 60 and 120 min. At the end of the incubation, the cell pellets were washed several times with 0.15 M-NaCl/20 mM-Tricine, pH 7.4, and extracted by the Folch procedure [18]. Cell-associated radioactivity was then counted.

Total blood cholesterol and free cholesterol were determined with Boehringer kits (no. 709891 and no. 290319 respectively). Esterified cholesterol was deduced from the difference between total and free cholesterol.

Chemicals

Collagenase and fat-free BSA (fraction V) were from Boehringer (Meylan, France); heparin grade II, phosphatidylcholine and lysophosphatidylcholine were from Sigma Chemical Co. (St. Louis, MO, U.S.A.); and glyceryl [14 C]trioleate and [14 C]cholesteryl oleyl ether were from CEN (Gif-sur-Yvette, France). All other chemicals were of reagent grade.

Statistics

Results were expressed as means $\pm$ S.E.M., with the numbers of experiments in parentheses. Statistical significance of differences was determined with Student's *t*-test.

RESULTS

Rats fed on the control diet

As shown in Table 1, plasma levels of total cholesterol were significantly higher in RICO rats compared with SW rats (60% and 74% increases for free and esterified cholesterol respectively). Plasma post-heparin LPL activity (Table 2) was significantly lower (35% decrease) in RICO rats, whereas no differences in post-heparin HL could be detected (Table 2).

In vitro, activity of HL secreted by hepatocytes and stabilized by increasing doses of heparin (Fig. 1a) was

Table 1. Plasma total cholesterol in SW and RICO rats fed on the control, 0.5% or 1% cholesterol diets

Rats were fed on a control diet until they were 4 months old. Thereafter, two groups of rats received either a 0.5% or a 1% cholesterol diet for 1 month. Results are means $\pm$ S.E.M. with numbers of rats in parentheses. Values for RICO versus SW rats were significantly different at * $P < 0.001$. Effects of cholesterol diets were significant at † $P < 0.05$ and †† $P < 0.001$.

Diet	Total cholesterol (mg/ml)	
	SW	RICO
Control	1.02 ± 0.05 (11)	1.72 ± 0.04 (11)*
0.5% Cholesterol	1.14 ± 0.06 (6)	1.92 ± 0.04 (6)††
1% Cholesterol	1.21 ± 0.06 (6)†	2.09 ± 0.04 (5)††

Table 2. Plasma post-heparin LPL and HL activities in SW and RICO rats fed on control and high-cholesterol diets

The same nutritional conditions as in Table 1 were used. After blood samples were taken for cholesterol determination, heparin (20 units/100 g) was injected intravenously and blood samples were withdrawn 2 min later. LPL and HL activities were assayed as described in the Materials and methods section. Results are means $\pm$ S.E.M. with numbers of rats in parentheses. Values for RICO versus SW rats were significantly different at * $P < 0.05$ and ** $P < 0.01$. Effects of cholesterol diets were significant at † $P < 0.05$ and †† $P < 0.01$.

	Enzyme activity (munits/ml)	
	SW	RICO
LPL:		
Control diet	485 $\pm$ 71 (8)	316 $\pm$ 43 (10)*
+0.5% Cholesterol	488 $\pm$ 30 (6)	287 $\pm$ 18 (6)**
+1% Cholesterol	517 $\pm$ 27 (4)	297 $\pm$ 45 (3)**
HL:		
Control diet	1306 $\pm$ 89 (5)	1369 $\pm$ 57 (6)
+0.5% Cholesterol	1108 $\pm$ 32 (6)†	1161 $\pm$ 86 (5)
+1% Cholesterol	1058 $\pm$ 37 (6)†	1057 $\pm$ 71 (5)††

comparable for SW and RICO rats after 150 min of incubation. The time courses of this activity (Fig. 1b) were also similar in the two strains, although with a slight trend towards a lesser activity in RICO hepatocytes. HL activity measured in liver homogenates was identical for SW and RICO rats [1.56 ± 0.13 ($n = 5$) and 1.65 ± 0.12 ($n = 4$) units/g respectively].

Chylomicron remnant uptake, measured with the same cell preparation as for the HL secretion study, increased

Table 3. Uptake of chylomicron remnants by isolated hepatocytes from SW and RICO rats fed on the standard semi-purified diet

Labelled chylomicron remnants obtained from Wistar rats were added to hepatocytes (30 or 60 μ g of remnant protein per 10^7 cells) after preincubation of the cells for 2.5 h at 37 °C. Results, expressed as nmol of [14 C]cholesteryl oleyl ether/ 10^6 cells, are means $\pm$ S.E.M. with numbers of rats in parentheses. No significant differences were found between hepatocytes from SW and RICO rats.

Incubation time (min)	Amount (μ g)	Uptake (nmol/ 10^6 cells)	
		SW	RICO
60	30	0.18 $\pm$ 0.02 (5)	0.18 $\pm$ 0.04 (6)
60	60	0.31 $\pm$ 0.04 (5)	0.34 $\pm$ 0.08 (6)
120	30	0.46 $\pm$ 0.09 (6)	0.41 $\pm$ 0.09 (6)
120	60	0.87 $\pm$ 0.17 (6)	0.71 $\pm$ 0.20 (6)

with time and with dose (Table 3). No differences in the uptake of the heterologous chylomicron remnants could be detected between SW and RICO hepatocytes.

Rats fed on the 0.5% and 1% cholesterol diets

Cholesterol-enriched diets led to an increase in cholesterolaemia. However, this effect was significant in the SW rats only with the 1% cholesterol diet, whereas in the RICO rats it was significant with the 0.5% cholesterol diet as well.

Post-heparin LPL activity was not modified by the 0.5% cholesterol diet and thus its values remained significantly lower in RICO rats than in controls (Table

Fig. 1. HL activity secreted into the medium during incubation of isolated hepatocytes from SW and RICO rats fed on the standard diet

Cells (10^7 per flask) were incubated at 37 °C in Krebs' medium for 150 min with increasing concentrations of heparin (a) or with 5 units of heparin/ml for different times (b). At the indicated times, medium was separated from the cell pellet by brief centrifugation and stored at -20 °C until HL activity was determined, as described in the Materials and methods section. Values are means $\pm$ S.E.M. for five independent experiments. No significant difference was found between hepatocytes from SW (—) and RICO (----) rats.

2). By contrast, post-heparin HL activity was decreased by 15 and 16 % with the 0.5 % cholesterol diet and by 23 and 19 % with the 1 % cholesterol diet for SW and RICO rats respectively, thus showing responsiveness of the enzyme to dietary cholesterol.

DISCUSSION

It has been established that the RICO rat has a high cholesterol-synthesizing capacity [1,2]. Whether a decrease in clearance processes also contributes to RICO hypercholesterolaemia had not been investigated. It is shown here that with the control diet, a deficiency in post-heparin LPL (35 %) occurs in RICO rats. In contrast, HL activity was comparable in RICO and SW rats, regardless of whether the enzyme was measured in post-heparin plasma, in isolated hepatocytes or in liver homogenates. With the control diet, hypercholesterolaemia in the RICO rats results from the increase in $d > 1.006$ lipoprotein fractions. Thus the contribution of decreased post-heparin LPL activity to RICO hypercholesterolaemia is not clear, since this enzyme acts mainly on triacylglycerol-rich lipoproteins ($d < 1.006$). It was confirmed that the heparin doses used (20 units/100 g body wt.) and the experimental timing (2 min) were sufficient to liberate all of the endothelium-bound enzyme, thus ruling out any artefact. An abnormality in the composition of RICO chylomicrons was suggested by recent experiments showing (1) that [^3H]cholesterol-labelled homologous chylomicrons were cleared at a significantly lower fractional catabolic rate in RICO rats compared with SW rats [$7.7 \pm 0.6 \text{ h}^{-1}$ ($n = 5$) versus $10.2 \pm 0.7 \text{ h}^{-1}$ ($n = 5$)] and (2) that the fractional catabolic rate of heterologous chylomicrons was unaffected in RICO rats [$9.5 \pm 0.7 \text{ h}^{-1}$ ($n = 5$)], but was decreased when chylomicrons of RICO rats were injected into SW rats [$7.8 \pm 0.6 \text{ h}^{-1}$ ($n = 5$)] (K. Ouguerran, T. Magot & C. Lutton, unpublished work). Our present results show that RICO hepatocytes normally catabolize heterologous chylomicron remnants, thus displaying no defect at the receptor level (apolipoprotein E and/or apolipoprotein B/E receptors). Whether an abnormality in the chylomicron composition in RICO rats may account for their lower LPL activity is unknown. In any case, this provides no explanation for their decreased LPL activity under standard dietary conditions, since chylomicron and VLDL levels were similar to those of SW rats [3]. Interestingly, inhibition of rat LPL by HDL has been reported [19], and RICO rats do have high HDL levels when fed on the control diet [3]. These points deserve further investigation.

When cholesterol-rich diets were fed to the rats, post-heparin LPL activity was not modified and remained lower in the RICO rats. At the same time, post-heparin HL activity was significantly decreased in both groups of rats. The dramatic accumulation of chylomicrons observed, particularly in RICO rats fed on the cholesterol diet [3], clearly suggests, under these conditions, a defect in the clearance of these particles. It has been shown that cholesterol feeding down-regulates apolipoprotein B/E receptors [20–22], which, in turn, accounts for the chylomicron (and VLDL) accumulation in the plasma. In addition, lower plasma lipolytic activities may also be involved. It is now accepted that degradation of chylomicrons is a two-step mechanism, in which extensive hydrolysis of triacylglycerols is first achieved by LPL.

Subsequently, after the chylomicrons have lost some of their lipids and apolipoproteins, they become suitable substrates for HL. With the high-cholesterol diet, which induces intestinal chylomicron synthesis, a deficient LPL activity may then become rate-limiting in the clearance process in the RICO rat. Such a limiting role of LPL is suggested in high- and low-responding rhesus monkeys [23], in which a decrease in post-heparin LPL activity was measured when plasma cholesterol and triacylglycerols began to rise (high-responders). A depressed LPL activity was also measured in guinea pigs [24] rendered hypercholesterolaemic with a 1 % cholesterol diet. In the RICO and SW rats, LPL was not modified by either of the cholesterol-enriched diets but was apparently insufficient to prevent the hypertriacylglycerolaemia observed in both groups of rats and particularly in the RICO strain [3]. Under these conditions, the decreased HL activity may be an additional factor in the diet-induced hypertriacylglycerolaemia and hypercholesterolaemia in that strain of rats.

An interesting observation in the present study is the decrease in post-heparin HL activity caused by cholesterol-enriched diets. A previous paper [25] reported a 24 % decrease in HL activity in liver homogenates from rats fed on a 2 % cholesterol diet. In contrast, a 2-fold increase in HL activity in liver homogenates has been observed in guinea pigs fed on a 1 % cholesterol diet [26]. In rhesus monkeys [23], cholesterol-enriched diets led to an even more complex adaptation; post-heparin HL activity increased during the first 21 days of cholesterol feeding, and then decreased to basal values as plasma cholesterol accumulation began. Thus dietary adaptation of HL seems to differ between species but in all cases varies with the cholesterol content of the diets.

Thus, under standard nutritional conditions, the lower LPL activity is not rate-limiting, since no hypertriacylglycerolaemia was observed in RICO rats [1–3]. Feeding on a high-cholesterol diet, by contrast, leads both to a decrease in HL activity and to an important increase in concentrations of chylomicron and VLDL fractions [3]. With high dietary cholesterol intake, the conjunction of persisting lower LPL and decreased HL activities may become rate-limiting for the clearance of triacylglycerol-rich lipoproteins in the RICO rats.

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