ASBMB
JOURNAL OF LIPID RESEARCH

Chylomicron catabolism differs between Hooded and albino laboratory rats

Faye Jeffery and Trevor G. Redgrave[1]

Department of Physiology, University of Melbourne, Parkville, Victoria 3052, Australia

Abstract To extend previous reports that some aspects of lipid metabolism are different between Hooded and albino strains of laboratory rats, thoracic duct lymph chylomicrons were collected and their composition and metabolism were compared in this study. Chylomicrons from Hooded rats had more core components and fewer surface components than albino rats. After adding 1% cholesterol to the diet the ratio of cholesterol:phospholipid was higher in Hooded rat chylomicrons. Nascent lymph chylomicrons from Hooded rats contained less apolipoprotein A-IV, but after incubation with serum there was a gain of apolipoprotein A-IV and a loss of A-I from both types of chylomicron. The ratio of apoC to apoE was higher in Hooded than in albino rats. The metabolism of injected chylomicrons was slower in Hooded rats. Twenty minutes after a single injection, only 1–3% of chylomicron triacylglycerol and 8–13% of chylomicron cholesteryl ester remained in the plasma of albino rats, compared with 6–12% of triacylglycerol and 30–33% of cholesteryl ester in Hooded rats. At 30 min in the Hooded rats, the uptake of injected chylomicron cholesterol into the liver was decreased whereas uptake into other tissues, notably adipose tissue and muscle, was increased. During the steady intravenous injection of chylomicrons from the same strain, the fractional clearance rate of chylomicron triaclyglycerol was about three-fold faster in albino rats than in Hooded rats, and the fractional clearance rate of chylomicron cholesteryl ester was 70% faster in albino rats. When chylomicrons were allowed to circulate for 30 min after injection into functionally hepatectomized rats, with or without heparin, the remnant formation and chemical composition were similar in Hooded and albino rats. Hence the deficiency in chylomicron metabolism in Hooded rats was not due to an absolute impairment in the formation of remnants by the action of lipoprotein lipase in peripheral tissues, although our findings were most likely accounted for by some relative impairment of remnant formation in the Hooded strain. The deficiency in chylomicron metabolism correlated with an increased cholesterolemic response in Hooded rats compared with albino rats when 1% cholesterol was added to the diet.—**Jeffery, F., and T. G. Redgrave.** Chylomicron catabolism differs between Hooded and albino laboratory rats. *J. Lipid Res.* 1982. **23:** 154–160.

Supplementary key words chylomicron remnants • apolipoproteins • lymph • hypercholesterolemia • dietary cholesterol

In 1964 Dawson et al. (1) reported that in Hooded rats oleic acid was less well absorbed from the duodenum into thoracic-duct lymph than in Sprague-Dawley rats. The difference was abolished if the test meal was either placed in the stomach, given with taurocholate, or given as glyceryl trioleate. Possible consequences of the uniqueness in lipid metabolism of the Hooded strains of rat have been obscure, until 1977 when Hulan, Cramer, and Corner (2) observed that Hooded rats were resistant to the myocardial lesions produced in other rats by diets with a high content of erucic acid.

In the experiments reported here, the composition and metabolism of chylomicrons (CM) is compared between Hooded and albino strains of rats to test the hypothesis that differences in CM formation will result in differences in CM metabolism. The formation and composition of remnants have also been studied. Differences found between rat strains are due to defective CM metabolism in Hooded rats, and this defect correlates with an increased cholesterolemic response to added cholesterol in the diet.

METHODS

Animals

Male rats used throughout these studies were obtained from a number of sources. Albino Wistar rats were bred in a colony maintained in this department, or else purchased from Monash University, Clayton, Victoria. Albino Sprague-Dawley rats, and hooded Long-Evans rats were also purchased from Monash University. Other hooded rats were obtained from the Commonwealth Serum Laboratories, Parkville, Victoria.

Preparation of CM

The thoracic duct of 250-g albino or Hooded rats was cannulated (3) and at the same time a cannula was secured in the stomach via a gastrostomy. Post-operative management and collection of lymph was previously described (4). Animals were fed their usual pelleted chow and, when the lymph was milky on the first post-oper-

Abbreviations: CM, chylomicrons; FCR, fractional catabolic rate.

[1] Present address: Biophysics Institute, Boston University Medical Center, 80 East Concord Street, Boston, MA 02118.

Downloaded from www.jlr.org by guest, on June 19, 2012

ative day, 20 μCi of [1-^{14}C]palmitic acid, and 60 μCi of [1,2-^{3}H(n)]cholesterol (New England Nuclear, Boston, MA) were dissolved in 0.1 ml of 0.1 M KOH in 50% ethanol, added to 1 ml of cow's milk, and then injected over 1 hr via the gastrostomy. The CM present in the lymph was isolated and purified by centrifugation in a discontinuous density gradient (5) to float all particles >75 nm in diameter to the top of the centrifuge tube. By polyacrylamide gel electrophoresis there was no albumin contamination of CM prepared in this way.

Studies of CM clearance

For single injection studies, recipient rats without anesthesia were injected via a tail vein with 0.5 ml of CM suspension containing about 100 μg of protein. Recipient rats were not starved and experiments were commenced 3–5 hr from the onset of the dark phase in 12 hr light:12 hr dark light-cycled rats adapted for a minimum of 2 weeks. This protocol ensured that all rats had full stomachs and milky intestinal lymphatics when injected. Exactly 20 min later, under ether anesthesia, a blood sample was taken by cardiac puncture and the liver was removed.

For steady infusion studies the procedure was exactly as previously described (4, 6) except that femoral artery and vein were cannulated. Briefly, a CM suspension was injected steadily for 30 min into a vein to establish a steady state, then blood samples were taken from the arterial cannula for measurement of plasma triacylglycerol and cholesteryl ester radioactivity and hence for calculation of fractional catabolic rates.

Formation of remnant particles

Functionally hepatectomized rats were prepared under light ether anesthesia as previously described (5, 7) to compare remnant formation in Hooded and albino rats. CM was injected into a tail vein at a dose of about 1 mg of CM protein per kg body weight and allowed to circulate in the functionally hepatectomized rat for 30 min before blood was taken by cardiac puncture. Control experiments showed that, within the range 0.36 to 1.49 mg protein per kg body weight, the disappearance of CM triacylglycerol radioactivity from the plasma was not related to the dose of injected CM ($r = 0.293$, $P > 0.5$ for 12 pairs of observations). Remnants were isolated from EDTA plasma by density gradient centrifugation (5). The presence of 5,5-dithionitrobenzoic acid was shown not to affect remnant lipid or apoprotein composition, so it was not routinely added to plasma. Remnant formation was studied with or without the simultaneous injection of 10 U of heparin.

Chemical analysis

Protein determinations were according to Lowry et al. (8). Turbidity due to lipids was extracted with chloroform after color development. Lipids were extracted in chloroform–methanol 2:1(by volume) according to Folch, Lees, and Sloane Stanley (9). Phospholipids, cholesterol, fatty acids, triacylglycerol, and cholesteryl ester were separated by chromatography of the lipid extracts on sintered glass rods (10) in the solvent system light petroleum (bp 40–60°C)–diethyl ether–acetic acid 90:10:0.6 (by volume). Quantitation was by flame ionization detection in the Iatroscan TH-10 (Iatron Laboratories, Tokyo, Japan) using calibration factors calculated from standard mixtures 18.5A, B and C obtained from Nu-Chek-Prep, Inc., Elysian, MN. Similarly, phospholipid fractions were separated into lysolecithin, sphingomyelin, phosphatidylcholine and phosphatidylethanolamine in the solvent system chloroform–methanol–water 80:35:3.5 (by volume) and assuming constant detector responses for the different phospholipids. Polyacrylamide gel electrophoresis was on 5 or 10% gels containing 0.1% sodium dodecyl sulfate, lipoproteins were delipidated with ethanol–diethyl ether 3:2 (v/v). Quantitation was by densitometry on bands stained with Coomassie Blue.

RESULTS

Metabolism of CM triacylglycerol and cholesteryl ester

After the injection of a single dose of radioactive CM in four strains of albino rats, there was less than 4% of triacylglycerol and less than 14% of cholesteryl ester label remaining in the plasma after 20 min (**Table 1**), but for two strains of Hooded rats 6% or more triacylglycerol and about 30% of cholesteryl ester remained in the plasma. Consistent with the increased retention of injected CM radioactivity in the plasma, a lesser amount of CM cholesteryl ester radioactivity was recovered in the liver of Hooded rats, where it was taken up after catabolism to the CM remnant. Because most CM triacylglycerol was removed during catabolism to the remnant, no difference was seen in the recovery of triacylglycerol radioactivity in the liver between albino and Hooded strains (Table 1).

In another experiment, the recovery of injected CM cholesterol radioactivity in other tissues was measured. Results in **Table 2** again showed the retention of label in the blood plasma of Hooded rats and the reduced recovery in the liver. In kidneys, lungs, adrenals, adipose tissue, and muscle, the Hooded strain exhibited greater uptake than the albino strain. Some apparent uptake was probably accounted for by radioactivity in blood within the tissue, but similar uptakes in the spleen show that such a possibility could not account for the observed differences. Rats were exsanguinated as completely as pos-

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 1. Removal from plasma of a single injection of chylomicrons

	Recovery of Injected CM Radioactivity[a]			
	[^{14}C]Triacylglycerol		[^{3}H]Cholesteryl Ester	
	Plasma	Liver	Plasma	Liver
Albino rats				
Wistar strain A	1.2 ± 0.28	14.1 ± 1.28	7.7 ± 2.24	63.6 ± 6.49
Wistar strain B	2.4 ± 0.36	15.3 ± 0.86	13.4 ± 2.55	61.5 ± 3.80
Wistar strain C	1.5 ± 0.20	14.4 ± 0.88	10.9 ± 1.66	68.5 ± 2.81
Sprague-Dawley	3.2 ± 0.20	14.0 ± 0.91	13.4 ± 1.77	62.8 ± 4.05
Hooded rats				
Long-Evans strain D	6.0 ± 0.56[b]	11.3 ± 0.76	33.0 ± 4.76[b]	40.2 ± 1.76[b]
Long-Evans strain E	12.3 ± 2.74[b]	14.6 ± 0.72	29.7 ± 3.35[b]	56.9 ± 3.46[b]

[a] Results are given as the mean ± SEM of the proportion of the radioactivity recovered in plasma or liver 20 min after injection, expressed as a percentage of the injected CM radioactivity.
[b] $P < 0.001$ by analysis of variance.

sible but tissues were not free of blood. Although hepatic uptake was less in Hooded rats than in albino rats, the liver still accounted for 70% of the injected CM radioactivity that had disappeared from the plasma compartment. Failure to recover 100% of injected radioactivity was presumably due to uptake in tissues not sampled, or to errors in assumed muscle or adipose tissue mass.

The difference in CM clearance between albino and Hooded rats shown in the above experiments was confirmed when CM was steadily infused into a vein for 30 min. **Table 3** gives the fractional clearance rates (FCR) for the catabolism of CM triacylglycerol and cholesteryl ester. When given CM obtained from their own strain, albino rats cleared CM triacylglycerol faster by three-fold than Hooded rats. For both strains, clearance of CM cholesteryl ester was slower than triacylglycerol, but again albino rats showed a significantly faster clearance rate than Hooded rats. In an attempt to establish whether the difference in clearance was due to the infused CM or to the recipient rats, CM from albino rats was infused steadily into Hooded rats and vice versa. As shown for triacylglycerol in Table 3, Hooded rats cleared albino CM better than their own CM and albino rats also cleared albino CM better than CM from Hooded rats. The data therefore suggested that defective clearance in Hooded rats was accounted for by a CM factor. Nevertheless Hooded rats cleared albino CM less well than did albino rats, which showed that the recipient rat contributed to the observed effect. Data for clearance of CM cholesteryl ester paralleled CM triacylglycerol, but differences were not statistically significant.

Chemical composition of CM

To account for the differences in CM metabolism the compositions of albino and Hooded rat CM were compared. **Table 4** shows that differences in CM composition were small, with somewhat less core triacylglycerol and somewhat more surface phospholipids and protein in CM from albino rats. When cholesterol was present in the diet, both strains showed an increased content of core cholesteryl ester but also a significant increase in unes-

TABLE 2. Distribution of tissue radioactivity after injection of [^{3}H]cholesterol-labeled CM[a]

	Tissue Recovery of Injected Radioactivity (% of dose)		
	Albino Rats	Hooded Rats	
Plasma	6.1 ± 0.88	28.0 ± 3.45	$P < 0.001$
Liver	62.8 ± 2.55	50.6 ± 5.17	$P < 0.05$
Spleen	0.9 ± 0.05	1.0 ± 0.11	NS
Kidneys	0.2 ± 0.02	0.5 ± 0.09	$P < 0.01$
Lungs	0.5 ± 0.05	1.5 ± 0.34	$P < 0.01$
Adrenals	0.06 ± 0.01	0.09 ± 0.01	$P < 0.05$
Adipose	1.1 ± 0.21	1.8 ± 0.02	$P < 0.02$
Muscle	1.6 ± 0.22	4.8 ± 0.67	$P < 0.01$

[a] There were six rats in each group. After injection into a tail vein, radioactive CM labeled with [^{3}H]cholesterol was allowed to circulate for 15 min before the rat was killed and tissue lipids were extracted for counting of radioactivity. Adipose tissue was taken to be 0.078 × body weight (11), and muscle 0.42 × body weight (12).

TABLE 3. Steady state clearance of CM from plasma

	Fractional Clearance Rate (min^{-1})	
	[^{14}C]Triacylglycerol	[^{3}H]Cholesteryl Ester
A. Infused CM of same strain		
Albino rats	0.76 ± 0.040	0.17 ± 0.020
Hooded rats	0.26 ± 0.025	0.10 ± 0.009
	($P < 0.001$)	($P < 0.01$)
B. Infused CM of other strain		
Albino rats	[a]0.46 ± 0.075	0.16 ± 0.029
Hooded rats	[ab]0.52 ± 0.043	0.12 ± 0.014

[a] Significantly different from FCR of rats infused with CM of same strain, $P < 0.01$.
[b] Significantly different from FCR of albino rats infused with CM from albino rats, $P < 0.01$. Comparisons by Student's t test. There were six rats in each group.

Downloaded from www.jlr.org by guest, on June 19, 2012

TABLE 4. Chemical composition of rat CM, with and without 1% cholesterol in the diet[a]

Rat Strain	Normal Fed		Fed 1% Cholesterol	
	Albino (n = 23)	Hooded (n = 4)	Albino (n = 23)	Hooded (n = 4)
Triacylglycerol	84.8 ± 0.56	88.0 ± 0.89[b]	80.1 ± 0.90	83.0 ± 1.82
Cholesteryl Ester	1.4 ± 0.09	1.4 ± 0.13	5.5 ± 0.38[b]	3.7 ± 0.87[b]
Cholesterol	1.0 ± 0.07	1.1 ± 0.16	1.3 ± 0.08[b]	2.1 ± 0.24[b]
Phospholipids	11.3 ± 0.53	8.7 ± 0.67[b]	11.6 ± 0.72	9.1 ± 1.45
Protein	1.5 ± 0.11	0.9 ± 0.18	1.4 ± 0.09	2.1 ± 0.62
Chol/PL (molar)	0.18 ± 0.02	0.24 ± 0.03	0.22 ± 0.02	0.51 ± 0.11[c]

[a] Results are mean ± SEM of the percentage distribution of the components of thoracic duct lymph CM of diameter > 75 nm.
[b] $P < 0.01$ compared with normal-fed and albino rats by Student's t test.
[c] $P < 0.02$ compared with albino cholesterol-fed rats.

terified cholesterol content. This increase was significantly greater in CM of Hooded rats, producing a cholesterol:phospholipid molar ratio of 0.51, compared with 0.22 in albino cholesterol-fed rats. The distributions of apoproteins in the lymph CM of the two strains are given in **Table 5.** The distributions were similar except for less apoA-IV in Hooded rats. Because of the substantial exchanges that occur when lymph CM enters the plasma, CM apoproteins were also studied after incubation with serum from the same rat strain. As seen in Table 5B, the substantial loss of apoA-I, and the gain of apoA-IV were similar in the two strains, and resulted in the restoration of apoA-IV to CM from Hooded rats. Although other differences were small, it was noteworthy that the ratio of apoC: apoE after incubation with serum averaged 2.71 in the Hooded rats but only 1.42 in albino rats.

Formation of remnants in functionally hepatectomized rats

The differences in CM clearance between albino and Hooded rats might have been attributable to differences either in hydrolysis of CM triacylglycerol by lipoprotein lipase, or in subsequent uptake of the CM remnant by the liver. To distinguish between these alternatives, CM was injected into rats from which the entire splanchnic circulation, including the portal and arterial hepatic blood flow, had been occluded (7). After 30 min, the

TABLE 5. A. Distribution of lymph CM apoproteins with or without 1% cholesterol in the diet

Rat strain	Normal Fed		Fed 1% Cholesterol	
	Albino (n = 9)	Hooded (n = 9)	Albino (n = 5)	Hooded (n = 4)
Apoprotein C	33.8 ± 2.7	39.6 ± 3.3	33.4 ± 4.3	36.0 ± 1.7
Apoprotein A-I	32.1 ± 2.9	26.2 ± 2.5	32.4 ± 2.8	34.6 ± 3.3
Apoprotein E	18.6 ± 1.5	25.9 ± 2.4	19.1 ± 2.8	19.2 ± 4.0
Apoprotein A-IV	12.1 ± 2.0	4.5 ± 1.4[a]	11.1 ± 2.5	6.5 ± 1.1
Apoprotein B	3.4 ± 0.8	3.9 ± 0.7	3.8 ± 1.4	3.7 ± 1.3

B. Effect of incubation with serum on soluble CM apoproteins[b]

	Without Added Serum		With Added Serum	
	Albino (n = 4)	Hooded (n = 5)	Albino (n = 4)	Hooded (n = 5)
Apoprotein C	39.6 ± 3.6	42.8 ± 5.4	43.1 ± 4.8	56.1 ± 4.7
Apoprotein A-I	28.6 ± 3.7	28.0 ± 4.2	7.5 ± 1.1[a]	7.1 ± 1.8[a]
Apoprotein E	22.8 ± 1.9	25.3 ± 3.6	30.4 ± 6.4	20.7 ± 2.0
Apoprotein A-IV	9.2 ± 2.7	4.0 ± 1.8	19.1 ± 2.6	16.3 ± 2.2[a]

[a] $P < 0.01$ compared with albino rats, or before incubation by Student's t test.
[b] CM containing 300 μg of total protein was incubated for 15 min at 37°C with 3 ml of rat serum from the same strain. Controls contained 3 ml of 0.15 M NaCl. CM were then re-isolated by density gradient centrifugation as described in Methods after adding KBr to the incubation mixture to increase its density to 1.10 g/ml.
Results are given as g/100 g CM total protein in A, or g/100 g CM non-B protein in B.

ASBMB JOURNAL OF LIPID RESEARCH

Downloaded from www.jlr.org by guest, on June 19, 2012

turbidity of the injected CM had disappeared because of the reduction in size of the triacylglycerol-rich lipoprotein particles (5). The extent of the depletion of injected triacylglycerol as assessed by removal of [^{14}C]palmitate label was 71–73% without heparin, and was increased to 84–89% if heparin was injected simultaneously (**Table 6**). In the injected CM, 90% of the radioactivity was in triacylglycerols, 4% in cholesteryl ester, 3% in diacylglycerol, and 2% in phospholipids. In control experiments under identical conditions, 98.0 ± 2.8% of cholesterol label was recovered from the plasma when CM was injected.

The composition of the remnants is given in Table 6. Compared with the precursor CM (Table 4) remnants were depleted in triacylglycerol, but cholesteryl ester, cholesterol, and protein were proportionately increased. Heparin accentuated the depletion of triacylglycerols, and small amounts of free fatty acid now were found in the remnants. Probably because considerable phospholipid was lost during the conversion of CM to remnants (5) there was a marked increase in the molar ratio of cholesterol: phospholipid from about 0.2 in the precursor CM (Table 4) to about 1.0 in remnants, and even greater if heparin was present (Table 6). The phospholipids of remnants were predominantly phosphatidylcholine (70 ± 2.73%), with about 10% each of lysolecithin, sphingomyelin, and phosphatidylethanolamine. The polar lipids of the remnants included trace amounts of monoacylglycerol and of ceramide which were identified by two-dimensional thin-layer chromatography, but they did not account for more than 1% of total lipids. The apoproteins of remnants were predominantly apoproteins C and E, with apoB accounting for only 5–10% of remnant protein. Other apoproteins present on CM were absent from the remnants (**Fig. 1**). Antibody was prepared in rabbits against rat LDL of 1.040–1.050 g/ml (13), which showed only apoB on polyacrylamide gel electrophoresis. This antibody reacted with the remnants of albino and Hooded rats similarly.

The effect of cholesterol feeding on plasma lipids

Because previous observations have indicated a correlation between hyperlipidemia and defective CM metabolism (4, 6, 14–16), our observation of a difference in CM metabolism between albino and Hooded rats led us to predict that Hooded rats would show a greater cholesterolemic response to added cholesterol in the diet. **Table 7** shows that this prediction was borne out. In two separate experiments conducted in Melbourne and in Boston when Hooded rats were fed cholesterol in the diet for 3 weeks, plasma cholesterol was increased by an average 153% or 101%, respectively, compared with corresponding increases of only 70% and 62% in albino rats. The final cholesterol concentrations were significantly higher in Hooded rats than in albino rats by an average 53% ($P < 0.001$).

DISCUSSION

Factors controlling plasma lipid concentrations are poorly understood. In species such as the rabbit which readily develops hypercholesterolemia, a grading of susceptibility is apparent and genetic factors contribute to

TABLE 6. Formations of CM remnants (RM) in functionally hepatectomized rats with or without heparin[a]

	Without Heparin		With Heparin	
	Albino (n = 27)	Hooded (n = 14)	Albino (n = 9)	Hooded (n = 11)
Clearance of CM triacylglycerol (% of injected radioactivity)	73.5 ± 2.75	70.6 ± 4.64	89.0 ± 0.97	83.8 ± 1.19
Composition of RM (%)				
Cholesteryl esters	6 ± 0.6	7 ± 1.7	18 ± 3.6	15 ± 0.8
Triacylglycerols	70 ± 3.1	71 ± 3.1	55 ± 3.5	48 ± 2.1
Free fatty acids	0	0	2 ± 2.0	2 ± 1.7
Cholesterol	6 ± 0.3	6 ± 0.8	9 ± 1.0	11 ± 0.9
Phospholipids	12 ± 2.1	11 ± 1.4	11 ± 0.7	17 ± 0.8
Protein	7 ± 1.0	5 ± 0.4	5 ± 2.1	7 ± 0.9
Molar ratio C/PL	1.00	1.09	1.64	1.29
Recovery of RM from density gradient (% of load)	70 ± 3.2	76 ± 2.7	58 ± 7.4	62 ± 6.4
Body weight of recipient rats (g)	313 ± 12.3	375 ± 12.0	257 ± 13.0	349 ± 17.5
CM injected dose μg protein/g body wt	0.93 ± 0.04	0.71 ± 0.04	1.06 ± 0.17	0.87 ± 0.01

[a] After injection into a tail vein, CM labeled with [^{14}C]palmitate in triacylglycerol was allowed to circulate for 30 min in functionally hepatectomized rats under light ether anaesthesia. CM was then recovered by density gradient centrifugation from the plasma. Rats given heparin received 10 units with the CM injection. Donor rats were consuming normal chow without added cholesterol.

Downloaded from www.jlr.org by guest, on June 19, 2012

the variability (17). Similar genetic variability has been described in several other species including primates (18, 19). In man there is evidence for genetic influences on cholesterol metabolism and plasma lipids, but how these are expressed in the general population is far from clear (20). The rat, like the primate, is omnivorous and is relatively resistant to the development of hypercholesterolemia when cholesterol is present in the diet. Genetic variation in the cholesterolemic response of rats has been reported by Kim et al (21) and by Imai et al (22) in highly inbred strains that were selected for their hypercholesterolemic responses. In the latter study (22) it was suggested that hypercholesterolemia was due to remnants. Our study now demonstrates that commonly available outbred laboratory rats differ in their cholesterolemic response, and the difference correlates with a difference in CM metabolism.

Albino rats were used in earlier studies which showed the formation and clearance of CM remnant particles (7). Curiously, in 1962, Lossow, Brot, and Chaikoff (23) showed uptake of CM cholesterol by adipose tissue and muscle was 6–18% of the injected dose in Long-Evans (Hooded) rats, whereas, in similar experiments, Goodman (24) found only 1–3% in adipose tissue and muscle of Sprague-Dawley (albino) rats. Our data (Table 2) confirm these long-standing observations and suggest that these differences are probably explained by an intrinsic characteristic of the strain of rat.

Because our metabolic data in intact rats show a clear difference in the clearance of CM triacylglycerol, it is likely that Hooded rats have a relative defect in the first stage of CM clearance, which is hydrolysis by lipoprotein lipase in peripheral tissues such as muscle and adipose

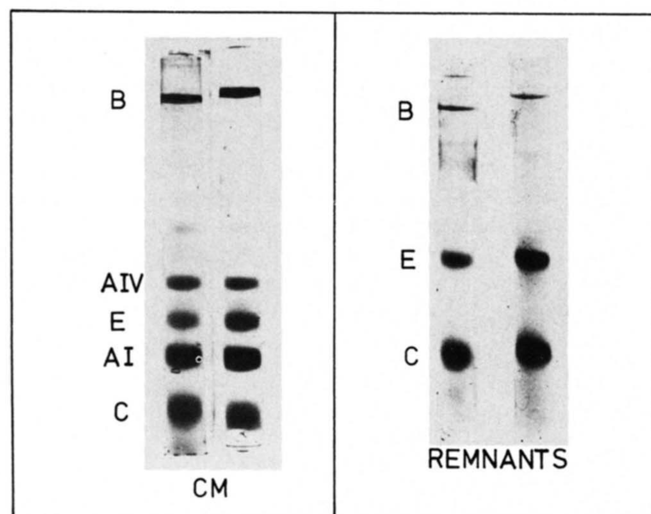

Fig. 1. The apolipoproteins of lymph CM and remnants on 5% polyacrylamide gels. In each case the proteins are from an albino rat on the left and a Hooded rat on the right and 20 μg of protein was applied. The remnants contain only apolipoproteins B, E, and C.

TABLE 7. Effect of dietary cholesterol on plasma lipids of albino and hooded rats[a]

Rat Strain	Plasma Cholesterol (mM/L) Normal Diet	Diet with Added Cholesterol
Albino Wistar		
Experiment 1 (n = 12)	1.23 ± 0.06	2.09 ± 0.05
Experiment 2 (n = 6)	1.49 ± 0.09	2.42 ± 0.09
Hooded Long-Evans		
Experiment 1 (n = 12)	1.41 ± 0.10	3.57 ± 0.05[b]
Experiment 2 (n = 6)	1.63 ± 0.10	3.27 ± 0.14[b]

[a] In each of two separate experiments, male rats of initial body weight 190–220 g were fed for 3 weeks the usual pelleted chow, or the same chow containing 1% w/w cholesterol. Experiment 1 was conducted in Melbourne, and experiment 2 was conducted by Dr. Susanne Bennett Clark at the Biophysics Section, Boston University Medical Center, Boston, MA.
[b] $P < 0.001$, compared with albino rats in the same experiment. Results are mean ± SEM.

tissue. Our studies in functionally hepatectomized rats failed to reveal any absolute difference between the two strains with regard to remnant formation. Hence the defect appears to be marginal, yet sufficient to hinder remnant formation and possibly remnant uptake by the liver. Defective remnant uptake by the liver would contribute to less efficient triacylglycerol clearance by virtue of the residual triacylglycerol content of CM remnants. Definition of the mechanism that underlies these differences in CM metabolism between rat strains awaits further study.

Further experiments are also necessary to compare remnant uptake by the livers of albino and Hooded rats. Windler, Chao, and Havel (25) have reported opposing regulatory roles for apoproteins C and E in hepatic uptake of lipoproteins, and the data of Table 5 are consistent with such an underlying mechanism, with a higher ratio of apoC to apoE in Hooded rats. Other metabolic differences have been documented in Hooded rats, for example an increased plasma glucose (26), so the differences in CM clearance could be part of a wide spectrum of metabolic diversity. Our data are consistent with but do not prove that CM catabolic products contribute to the development of hyperlipidemia in cholesterol-fed Hooded rats. Other lipoproteins such as hepatic VLDL might show similar differences between the strains and thus contribute to the cholesterolemic response. Our data demonstrate that the genetic factors regulating lipoprotein metabolism in the rat may be linked to the hh genotype responsible for Hooded pigmentation (27).

This work was assisted by grants from the National Health and Medical Research Council and the National Heart Foundation of Australia.

Manuscript received 21 January 1981, in revised form 29 April 1981, and in re-revised form 3 September 1981.

ASBMB JOURNAL OF LIPID RESEARCH
Downloaded from www.jlr.org by guest, on June 19, 2012

REFERENCES

1. Dawson, A. M., N. Gallagher, D. R. Saunders, and J. Webb. 1964. Resynthesis and transport of long chain fatty acids by the small gut. *In* Metabolism and Physiological Significance of Lipids. R. M. C. Dawson and D. N. Rhodes, editors, John Wiley and Sons Ltd., New York. 243–255.
2. Hulan, H. W., J. K. G. Cramer, and A. H. Corner. 1977. Myocardial lesions in rats fed rapeseed oil. I. Influence of strain of rats. *Can. J. Physiol. Pharmacol.* **55:** 258–264.
3. Bollman, J. L., J. C. Cain, and J. H. Grindlay. 1948. Techniques for the collection of lymph from the liver, small intestine, or thoracic duct of the rat. *J. Lab. Clin. Med.* **33:** 1348–1352.
4. Redgrave, T. G., and G. Martin. 1977. Effects of chronic ethanol consumption on the catabolism of chylomicron triacylglycerol and cholesteryl ester in the rat. *Atherosclerosis.* **28:** 69–80.
5. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64:** 162–171.
6. Redgrave, T. G. 1977. Catabolism of chylomicron triacylglycerol and cholesteryl ester in genetically obese rats. *J. Lipid Res.* **18:** 604–612.
7. Redgrave, T. G. 1970. Formation of cholesteryl ester-rich particulate lipid during metabolism of chylomicrons. *J. Clin. Invest.* **49:** 465–471.
8. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
9. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* **226:** 497–509.
10. Vandamme, D., V. Blaton, and H. Peeters. 1978. Screening of plasma lipids by thin-layer chromatography with flame ionization detection on chromarods. *J. Chromatogr.* **145:** 151–154.
11. Caster, W. O., J. Poncelet, A. B. Simon, and W. D. Armstrong. 1956. Tissue weights of the rat. I. Normal values determined by dissection and chemical methods. *Proc. Soc. Exp. Biol. Med.* **91:** 122–126.
12. Jackson, C. M., and L. G. Lowrey. 1912. On the relative growth of the component parts (head, trunk, and extremities) and systems (skin, skeleton, musculature, and viscera) of the albino rat. *Anat. Rec.* **6:** 449–474.
13. Lasser, N. L., P. S. Roheim, D. Edelstein, and H. A. Eder. 1973. Serum lipoproteins of normal and cholesterol-fed rats. *J. Lipid Res.* **14:** 1–8.
14. Redgrave, T. G., and D. A. Snibson. 1977. Clearance of chylomicron triacylglycerol and cholesteryl ester from the plasma of streptozotocin-induced diabetic and hypercholesterolemic hypothyroid rats. *Metabolism.* **26:** 493–503.
15. Redgrave, T. G., K. B. Dunne, D. C. K. Roberts, and C. E. West. 1976. Chylomicron metabolism in rabbits fed diets with or without added cholesterol. *Atherosclerosis.* **24:** 501–508.
16. Ross, A. C., and D. B. Zilversmit. 1977. Chylomicron remnant cholesteryl esters as the major constituents of very low density lipoproteins in plasma of cholesterol-fed rabbits. *J. Lipid Res.* **18:** 169–181.
17. Roberts, D. C. K., C. E. West, T. G. Redgrave, and J. B. Smith. 1974. Plasma cholesterol concentration in normal and cholesterol-fed rabbits: its variation and heritability. *Atherosclerosis.* **19:** 369–380.
18. Lofland, H. B., T. B. Clarkson, R. W. St. Clair, and N. D. M. Lehner. 1972. Studies on the regulation of plasma cholesterol levels in squirrel monkeys of two genotypes. *J. Lipid Res.* **13:** 39–47.
19. Parks, J. S., N. D. M. Lehner, R. W. St. Clair, and H. B. Lofland. 1977. Whole body cholesterol metabolism in cholesterol-fed African green monkeys with a variable hypercholesterolemic response. *J. Lab. Clin. Med.* **90:** 1021–1034.
20. Murphy, E. A., and P. O. Kwiterovich. 1977. Genetics of the hyperlipoproteinemias. *In* Hyperlipidemia. B. M. Rifkind and R. I. Levy, editors. Grune and Stratton, Inc. New York. 217–247.
21. Kim, W. M., C. Merskey, Q. B. Denning, H. N. Adel, H. Wolinsky, T. B. Clarkson, and H. B. Lofland. 1976. Hyperlipidemia, hypercoagulability, and accelerated thrombosis. Studies in congenitally hyperlipidemic rats and in rats and monkeys with induced hyperlipidemia. *Blood.* **47:** 275–286.
22. Imai, Y., H. Matsumura, A. Shino, K. Oka, and Z. Sukuoki. 1977. Induction of aortic lipid deposition in a high response (ExHC) rat fed a diet containing cholesterol and cholic acid. *Atherosclerosis.* **28:** 453–461.
23. Lossow, W. J., N. Brot, and I. L. Chaikoff. 1962. Disposition of the cholesterol moiety of a chylomicron-containing lipoprotein fraction of chyle in rat. *J. Lipid Res.* **3:** 207–215.
24. Goodman, D. S. 1962. The metabolism of chylomicron cholesterol ester in the rat. *J. Clin. Invest.* **41:** 1886–1896.
25. Windler, E., Y. Chao, and R. J. Havel. 1980. Regulation of the hepatic uptake of triglyceride-rich lipoproteins in the rat. *J. Biol. Chem.* **255:** 8303–8307.
26. Kozma, C. K., S. H. Weisbroth, S. L. Stratman, and M. Conejeros. 1969. Normal biological values for Long-Evans rats. *Lab. Animal Care.* **19:** 746–755.
27. Weisbroth, S. H. 1969. The origin of the Long-Evans rats and a review of the inheritance of coat colors in rats *(Rattus norvegicus)*. *Lab. Animal Care.* **19:** 733–737.

Downloaded from www.jlr.org by guest, on June 19, 2012