Metabolism of lipoproteins containing apolipoprotein B in hepatic lipase-deficient mice

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Abstract **Mice lacking hepatic lipase have been reported to express mild hyperlipidemia characterized by increased concentrations of large high density lipoproteins, but normal concentrations of lipoproteins containing apolipoprotein B. Whereas hepatic lipase has been implicated in the clearance and processing of chylomicron remnants in rats, no such defect was found in these mice. We have further characterized the abnormal lipoprotein phenotype in young hepatic lipase-deficient mice and have found more pronounced elevations of high density lipoproteins associated in particular with a 5-fold increase in plasma concentrations of apolipoprotein E. In addition, there was a reduction in the concentration of low density lipoproteins containing apolipoprotein B-100 and B-48 relative to precursor lipoproteins of lower density and a pronounced deficiency of apolipoprotein B-containing low density lipoproteins with density exceeding 1.029 g/mL. Conversion of radiolabeled rabbit intermediate density lipoproteins to low density lipoproteins was reduced by 6-fold as compared with wild-type mice. Although clearance of cholesteryl ester-labeled chylomicrons from the blood was unimpaired in the deficient mice, that of chylomicron remnants was reduced. Furthermore, endocytosis of chylomicron cholesteryl esters into liver cells occurred more rapidly than in wild-type mice. The unimpaired hepatic clearance of injected chylomicron particles in hepatic lipase-deficient mice may be the result of greater acquisition of apoE from high density lipoproteins during remnant formation. These studies thus demonstrate a critical role for mouse hepatic lipase in the formation of small, dense low density lipoproteins, as well as participation in the normal clearance and processing of chylomicron remnants.**—Qiu, S., N. Bergeron, L. Kotite, R. M. Krauss, A. Bensadoun, and R. J. Havel. **Metabolism of lipoproteins containing apolipoprotein B in hepatic lipase-deficient mice.** ***J. Lipid. Res.*** **1998.** 39: **1661–1668.**

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Two lipases, lipoprotein lipase (LPL) and hepatic lipase (HL), are responsible for the extracellular processing of triglyceride-rich lipoproteins (1). Humans with LPL deficiency chiefly manifest dietary fat-dependent chylomicronemia (2) whereas human HL deficiency is accompanied by relatively small increases in the concentration of small very low density lipoproteins (VLDL) and intermediate density lipoproteins (IDL), and low concentrations of low density lipoproteins (LDL) (3). Clearance of triglycerides in chylomicrons containing apolipoprotein (apo)B-48 and large VLDL containing apoB-100 from plasma is defective in human LPL deficiency (4, 5), whereas abnormal metabolism of apoB-100 involving defective conversion of IDL to LDL is observed in human HL deficiency (3) and in cynomolgus monkeys after administration of hepatic lipase antiserum (6). In vitro studies indicate that LPL is active chiefly upon triglycerides in chylomicrons and large VLDL, whereas hepatic lipase is active upon triglycerides and phospholipids in small VLDL and IDL (7). HL is much more active upon lipoprotein-phospholipids than LPL (1) and human HL deficiency is also accompanied by increased size and lipid-enrichment of high density lipoproteins (HDL) (3).

Studies in rats have led to the proposal that HL may also participate in chylomicron metabolism after chylomicron remnants are produced through the action of LPL (8, 9). Thus, HL may serve as a ligand for the initial binding of chylomicron remnants to cell surfaces in the liver, followed by transfer to endocytic receptors, chiefly the LDL receptor (10). In mice (11) and rabbits (12), overexpression of HL causes striking reductions in the concentration and size of HDL.

Recently, mice lacking HL have been produced by homologous recombination (13). The principal phenotypic abnormality in these mice is increased concentration of large HDL. Lipoproteins containing apoB-100 and apoB-

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; HL, hepatic lipase; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; LPL, lipoprotein lipase; VLDL, very low density lipoproteins.

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48 do not appear to accumulate, and the initial removal of chylomicron remnant particles by the liver is apparently unimpaired (13). In rats, as in most other mammals, HL is almost entirely bound to cell surfaces, mainly in the liver, but in mice, most functional HL is found in blood plasma, owing to reduced affinity of the mouse enzyme for glycosaminoglycans on cell surfaces (14). The role of HL in the metabolism of chylomicron remnants and the processing of VLDL to LDL in mice (a species lacking cholesteryl ester transfer protein) is thus unclear. In this report, we describe the results of further analysis of the abnormal lipoprotein phenotype in homozygous (−/−) HL-deficient mice. We also show that HL has a critical role in the conversion of IDL to dense species of LDL in mice and that, as in rats, it participates in the initial hepatic binding of chylomicron remnants.

METHODS

Animals

Mice in which HL had been deleted by homologous recombination HL (−/−) were raised from breeding pairs kindly provided by Dr. Nobuyo Maeda. These mice were a cross of C57BL/6J with mice of the 129 strain, in which apolipoproteins apoC-II and A-IV are derived from the 129 strain. Control animals were C57BL/6 mice, whose lipoprotein concentrations and distributions are essentially the same as normal litter mates of the HL (−/−) animals (personal communication from Dr. Maeda). Unless otherwise noted, all animals were studied at 10–14 weeks of age and had been fed standard mouse chow. Male New Zealand white rabbits weighing 2.5–3.0 kg were obtained from commercial sources and fed standard rabbit chow for at least 1 week before blood was taken for lipoprotein isolation.

Materials

Goat antiserum against rat HL was purified as described (15). [1,2-^{3}H]cholesterol, 40 to 60 Ci/mmol, was from New England Nuclear. Bovine serum albumin and protease inhibitors were from Sigma. Tetrahydrolipostatin (Hoffman LaRoche, Basel, Switzerland) was a gift from John Brunzell.

Separation and characterization of mouse lipoproteins

Blood samples were obtained in the morning by exsanguination of anesthetized, non-fasted male or female wild-type or HL (−/−) mice, mixed with disodium EDTA (final concentration 0.02%), and immediately chilled on ice. Plasma from wild-type mice was also mixed with tetrahydrolipostatin to give a final concentration of 0.1 μg/mL. This amount is sufficient to inhibit HL activity for at least 24 h. Pooled plasma from 5–10 animals was subjected to sequential ultracentrifugation at ρ 1.006, 1.019, 1.055, 1.085, and 1.21 g/mL (16). Total and free cholesterol (17), triglycerides (18), phospholipids (19), and total protein (20) were estimated in plasma and lipoprotein fractions and their concentrations in original plasma were calculated as mg/dL. The concentrations of apoB-100, B-48, and E were estimated by a quantitative SDS gel electrophoretic procedure (21). In addition, the concentrations of apoA-I and total apoC were estimated based upon chromogenicity factors for human apoA-I and apoC-III (L. Kotite, R. Havel, unpublished data). The electrophoretic mobility of lipoprotein fractions was determined by staining with Sudan black B after separation by agarose gel electrophoresis (22). Non-denaturing 2–14% gradient gel electrophoresis of plasma, with lipid staining, and calculation of lipoprotein particle sizes from calibration curves was performed as described previously (23). Polyacrylamide gels were prepared by published procedures (24).

Preparation of lipoproteins for injection

IDL were prepared from femoral arterial blood of non-fasted rabbits tranquilized with xylazine and ketamine. The blood was mixed with disodium EDTA, 1 mg/mL, sodium azide, 0.5 mg/mL, and gentamycin, 0.1 mg/mL, and placed on ice. Plasma, separated by centrifugation (1000 *g* at 4°C for 20 min), was subjected to sequential ultracentrifugation (16) to separate IDL ($1.006 < \rho < 1.019$ g/mL), which were concentrated by a second ultracentrifugation to a protein concentration of about 0.4 mg/mL. IDL were radioiodinated with ^{125}I by the method of MacFarlane (25). ^{125}I-labeled IDL were separated from the reaction mixture on a column of Sephadex G50, which had been pretreated with 0.2 mL rabbit plasma and 0.2 mL 5% bovine serum albumin. After dialysis overnight against 0.15 M NaCl containing 0.01% disodium EDTA and 0.02% sodium azide in tubing pretreated with the plasma–albumin mixture, the preparation contained less than 5% of lipid-soluble ^{125}I. Greater than 80% of the ^{125}I was associated with apoB-100, as determined by SDS gel electrophoresis. For injection, the labeled IDL was diluted with 0.15 M NaCl to contain ~4,000,000 cpm/mL.

Chylomicrons were isolated from intestinal lymph of rats fitted with indwelling intestinal lymph and intraduodenal cannulae and infused through the latter with a lipid emulsion (Intralipid, Vitrum, Stockholm, Sweden) for large chylomicrons, or 10% glucose in 0.15 M NaCl for small chylomicrons respectively, as described (26). In each case, albumin-dispersed [^{3}H]cholesterol was added to the intraduodenal infusate to label the chylomicron cholesteryl esters and cholesterol (27). Small chylomicron remnants were prepared in functionally eviscerated rats, as described (27).

In vivo studies

In the morning lipoprotein preparations were injected into an exposed jugular vein of non-fasted mice anesthetized by intraperitoneal injection of sodium pentobarbital, as described (28). For experiments with labeled IDL, 0.3 mL samples of mouse plasma from inferior vena caval blood obtained by exsanguination at intervals up to 3 h after injection were adjusted to ρ 1.019 g/mL with D_20 and placed in 6.5 mL Beckman ultracentrifuge tubes. Then 2.5 mL of fresh rabbit plasma and 1.0 mL of the ρ 1.019 ultracentrifugal infranate of rabbit plasma were added as carrier, and the tubes were filled with a solution of D_20 in 0.15 M NaCl adjusted to the same density. After ultracentrifugation at 93,000 *g* at 12°C for 20 h (Beckman 40.3 rotor), 2.5 mL of the top portion of the tube was collected after tube-slicing. The middle 2.5 mL of the tube (intermediate fraction) was then carefully drawn off and saved. The remaining bottom fraction was adjusted with D_20 to a density of ρ 1.055 g/mL, made up to 6.5 mL with D_20/0.15 M NaCl of the same density and subjected to ultracentrifugation as above. In each experiment, as a control for ultracentrifugal redistribution of the ^{125}I-labeled IDL, 50 μL of the labeled IDL was mixed with 0.3 mL fresh plasma from wild-type or HL (−/−) mice and subjected to ultracentrifugation as described above.

After injection of labeled chylomicron preparations in a volume of 250 μL, blood samples of ~50 μL were obtained from the orbital plexus or, at the end of experiments, by exsanguination (~600 μL) from the exposed inferior vena cava (28). The liver was then flushed with cold 0.15 M NaCl, removed, and weighed (28). A partially purified endosome fraction was prepared in experiments with chylomicrons and chylomicron remnants (28, 29).

Radiochemical analysis

Lipids were extracted from samples of blood plasma, liver, and subcellular fractions into chloroform–methanol (30), and ^{3}H was estimated by liquid scintillation spectrometry in the extracts and in cholesterol and cholesteryl esters separated by thin-layer chromatography (31). ^{125}I was estimated in plasma and tissue samples by scintillation spectrometry with appropriate correction for radioactive decay. ApoB-100 and other proteins were separated by SDS electrophoresis in 3–10% gradient gels (21) at loads of 50 μg protein or greater. After staining with Coomassie brilliant blue (21), the gels were dried under a cellophane wrap. ^{125}I in excised segments containing apoB-100 and in other regions of the gel was estimated by scintillation spectrometry. Contents of radionuclides were calculated for total plasma, estimated as 4.5% of body weight, and for total liver and endosome fractions with appropriate corrections for samples removed during subcellular fractionation.

Calculations

Differences between experimental groups were evaluated by two-tailed Student's *t* test. Results are given as mean of n experiments ± SD.

RESULTS

Plasma lipoprotein-lipid and apolipoprotein concentrations

In males, the concentrations of total and free cholesterol and phospholipids were about 3-fold higher, and in females about 3- to 4-fold higher in animals lacking HL than in wild-type animals (**Table 1**). The increase in these lipids occurred predominantly in LDL and the light and heavy HDL fractions and was more pronounced in the fractions of lower density. Concentrations were thus increased 4- to 5-fold in LDL, 10- to 15-fold in light HDL and only 2- to 3-fold in heavy HDL in HL (−/−) males and females alike. By contrast, the concentrations of triglycerides in these lipoprotein fractions remained low and did not differ from those found in wild-type mice.

Homanics et al. (13) reported only mild elevations of HDL in HL (−/−) mice, chiefly involving large species with densities overlapping those of LDL. The more striking lipid and HDL elevations in our HL (−/−) mice may be attributable to nutritional state (non-fasted as compared with overnight-fasted animals in the study of Homanics et al. (13) as well as the older age (ca. 6 months or more) of their animals (H. de Silva, personal communication). More recently, high plasma cholesterol and phospholipid concentrations comparable to those reported here have been found in male HL (−/−) mice 2 to 3 months old and fasted for 4 h (32).

The accumulation of lipids in LDL and light HDL in our mutant mice was accompanied by many-fold elevations of apoE, apoA-I and C-apoproteins (**Table 2**), and agarose gel electrophoresis showed that α-migrating lipoproteins were greatly increased in these fractions (not shown). In heavy HDL, the concentration of apoE was increased about 4-fold, whereas concentrations of apoA-I and C apolipoproteins were increased only modestly, especially in females. The striking increase of apoE in α-migrating lipoproteins was manifested in 4- to 6-fold elevations of plasma apoE concentrations in the mutant animals.

VLDL-lipid concentrations were about 2-fold higher in HL (−/−) than in wild-type mice, particularly in females (Table 1). In IDL, lipid concentrations were similar in HL (−/−) and wild-type male mice, but were moderately increased in female HL (−/−) animals. The concentrations of apoB-100 and apoB-48 were similar in VLDL and IDL of the two groups of males, but were lower in LDL of HL (−/−) male mice (Table 2). Female HL (−/−) mice had higher concentrations of these apoproteins in VLDL than wild-type animals, but comparable concentrations in IDL and LDL. The concentrations of apoE, apoA-I, and C apo-

TABLE 1. Lipoprotein–lipid concentrations in wild-type and hepatic lipase (−/−) mice

	Wild-Type				Hepatic Lipase (−/−)			
	Total Cholesterol	Free Cholesterol	Phospholipids	Triglycerides	Total Cholesterol	Free Cholesterol	Phospholipids	Triglycerides
	mg/dL				*mg/dL*			
Males								
Plasma	77.3 (13.7)	13.7	95.6	94.0 (26.1)	180.6 (23.8)[a]	39.5	276.4	148.0 (25.1)[a]
VLDL	5.2	2.5	14.1	64.2	6.1	3.3	22.7	110.0
IDL	1.3	0.6	3.5	7.4	1.4	0.6	2.8	6.7
LDL	7.4	2.5	6.3	7.8	30.5	11.0	31.5	8.0
HDL_L	3.8	0.8	4.3	3.4	43.2	11.4	59.5	3.6
HDL_H	45.9	4.5	69.1	2.4	87.5	13.2	139.9	4.9
Females								
Plasma	63.3 (8.6)	8.3	65.7	65.3 (14.8)	181.3 (38.4)[a]	39.2	313.7	119.4 (14.7)[a]
VLDL	2.9	0.9	5.6	41.4	4.5	2.0	20.9	83.0
IDL	1.0	0.5	1.4	5.9	2.0	0.7	4.8	6.4
LDL	6.9	2.3	5.1	6.8	27.5	9.8	42.2	6.9
HDL_L	3.1	0.8	6.4	4.6	51.8	12.4	83.6	3.7
HDL_H	42.0	5.0	61.4	6.3	94.9	14.3	156.9	3.0

Data are means from two pools of plasma from 8–10 mice each. Standard deviations (in parentheses) for total cholesterol and triglycerides in plasma are from individual analysis from the male and female mice used to make these pools.

[a]Significantly higher than wild-type mice ($P < 0.01$).

TABLE 2. Apolipoprotein concentrations in wild-type and hepatic lipase (−/−) mice

	Wild-Type					Hepatic Lipase (−/−)				
	B-100	B-48	E	A-I	Cs	B-100	B-48	E	A-I	Cs
	mg/dL					*mg/dL*				
Males										
Total	6.87	3.17	3.49	57.6	48.1	4.32	2.29	16.2	86.2	117.1
VLDL	0.61	0.97	0.58	0.08	5.86	0.63	0.95	0.52	0.13	4.63
IDL	0.38	0.17	0.18	0.02	1.00	0.53	0.13	0.19	0.19	0.78
LDL	5.74	0.90	1.17	0.36	1.06	2.97	0.37	5.29	2.50	3.70
HDL_L	0.14	0.44	0.48	2.63	1.06	0.19	0.39	3.99	13.1	15.2
HDL_H	—	0.69	1.08	54.5	39.1	—	0.45	6.24	70.3	92.8
Females										
Total	3.97	1.11	2.53	58.4	61.5	3.47	1.88	14.6	73.1	86.5
VLDL	0.23	0.42	0.36	0.05	2.95	0.42	0.83	0.57	0.21	2.36
IDL	0.12	0.07	0.07	0.03	0.81	0.13	0.09	0.41	0.36	0.49
LDL	3.23	0.36	0.55	0.12	0.61	2.65	0.35	4.41	4.61	4.49
HDL_L	0.39	0.26	0.39	2.31	1.18	0.27	0.25	3.17	14.6	11.0
HDL_H	—	—	1.16	55.9	55.9	—	0.36	6.07	53.3	68.2

Data are means from two pools of plasma from 5–10 mice each. Values for total plasma are summed concentrations of those in lipoprotein fractions.

lipoproteins were not consistently altered in VLDL and IDL. As noted above, the increased lipid and apolipoprotein concentrations in the LDL density interval ($1.019 < \rho < 1.055$ g/mL) of HL (−/−) mice were related to profound increases in the concentrations of α-migrating lipoproteins; lipoproteins containing apoB, by contrast, did not apparently contribute to the increased concentration of LDL-lipids. Previously, Homanics et al. (13) have noted that apoE accumulates in α-migrating lipoproteins in the 1.02–1.04 density interval of HL (−/−) mice, accompanied by increased concentrations of total cholesterol and phospholipids.

Because HL has been implicated in the conversion of VLDL via IDL to LDL, we calculated the ratios of apoB-100 and B-48 in VLDL + IDL to LDL in wild-type and HL (−/−) mice. For both apoB-100 and apoB-48, these ratios were invariably about 2-fold higher in HL (−/−) than in wild-type mice of both sexes (**Table 3**), consistent with a relative reduction of apoB-containing particles in LDL of HL (−/−) mice. To explore this further, the size distribution of particles of LDL-size (and containing predominantly apoB-100) was determined by gradient gel electrophoresis (**Fig. 1**). In wild-type mice, LDL were found to be polydisperse, with three major subspecies accounting for an appreciable portion of the lipoprotein mass. By contrast, in HL (−/−) mice only the largest species (peak diameter 271 Å) could be discerned, with little lipoprotein mass in particles of smaller size. Preparative ultracentrifugation at ρ 1.029 g/mL showed that all the large LDL particles were present in the supernatant (data not shown). These results suggested that some IDL may be converted to large, buoyant LDL in HL (−/−) mice, but processing to smaller species was limited.

TABLE 3. Relative concentrations of B-apolipoproteins in wild-type and hepatic lipase (−/−) mice

	Ratio: VLDL + IDL/LDL		Hepatic Lipase (−/−) / Wild-Type
	Wild-Type	Hepatic Lipase (−/−)	
ApoB-100			
Males	0.172	0.391	2.27
Females	0.108	0.207	1.92
ApoB-48			
Males	1.18	2.92	2.47
Females	1.36	2.63	1.94

Ratios were calculated from data in Table 2.

VLDL metabolism

To assess the effect of HL deficiency on the formation of LDL, we injected ^{125}I-labeled-IDL from chow-fed rabbits into wild-type and HL (−/−) mice and measured the accumulation of ^{125}I in LDL at intervals up to 180 min after injection. Rabbit IDL was used in order to obtain sufficient lipoprotein to evaluate the metabolism of a single form of apoB (apoB-100), not to quantify apoB transport in mouse plasma. For this purpose, the mass of apoB-100 injected in this experiment was selected to yield an initial concentration about 3-fold that found in IDL of normal and HL (−/−) mice. As described in Methods, mice were bled at selected times after injection and data obtained from each animal at a given bleeding time were pooled for analysis. Clearance of labeled apoB-100 from IDL was somewhat more rapid in female than male mice, but the results did not differ significantly, and data from 5–6 animals at each time after injection were pooled for analysis. Of the injected ^{125}I, 85% was in apoB-100 and most of the remainder was in C apolipoproteins. When labeled IDL were mixed with mouse plasma to yield an initial concentration similar to that observed immediately after injection and subjected to ultracentrifugation at ρ 1.019 g/mL, 82% of the apoB-100 was recovered in the top 1.5 mL, 12% in the middle 2.5 mL, and 3% in LDL recovered from the bottom 2.5 mL of the 6.5-mL centrifuge tube. The same portions of the tube were harvested during the in vivo experiments and comparable distributions of ^{125}I-labeled apoB-100 were found in samples obtained 90 sec

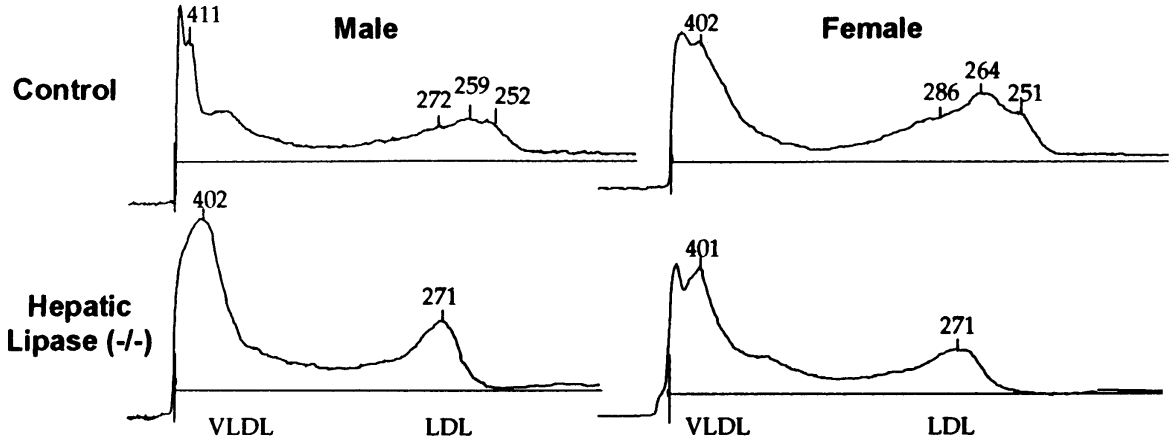

Fig. 1. Densitometric tracings of lipid-stained 2–14% polyacrylamide gradient gels after electrophoresis of plasma samples from representative HL (−/−) and wild-type mice. Shown are calculated diameters of the major VLDL and LDL species in Å.

after intravenous injection of labeled IDL. Results of two experiments involving 15 wild-type and 18 HL (−/−) mice are shown in **Fig. 2**. In wild-type mice 29% of the injected ^{125}I-labeled apoB-100 was recovered in IDL 90 min after injection and about 3% after 180 min, whereas in HL (−/−) mice, 60% and 40% remained in IDL at these respective time intervals. In the middle of the centrifuge tube containing lipoproteins close to a density of 1.019 g/mL, the amount of the ^{125}I-labeled apoB-100 increased to 12% after 90 min in wild-type mice and subsequently declined, whereas in HL (−/−) mice, the amounts recovered increased progressively to 16% after 180 min. In wild-type mice ^{125}I-labeled apoB-100 increased progressively in LDL to 30% of the injected ^{125}I-labeled apoB-100 after 180 min, whereas in HL (−/−) mice accumulation of the label in apoB-100 of LDL was about 6-fold lower, reaching 5% at this time. These data show that conversion of IDL is sluggish in HL (−/−) mice, with conversion appearing to be almost blocked at densities slightly exceeding 1.019 g/mL, the limiting upper density of the injected lipoprotein. Based upon an assumed plasma volume of 4.5% of body weight, the fraction of injected ^{125}I-labeled apoB-100 remaining in plasma after 180 min was 30.4 ± 10.0% in wild-type mice and 61.3 ± 15.7% in HL (−/−) mice, ($P <$

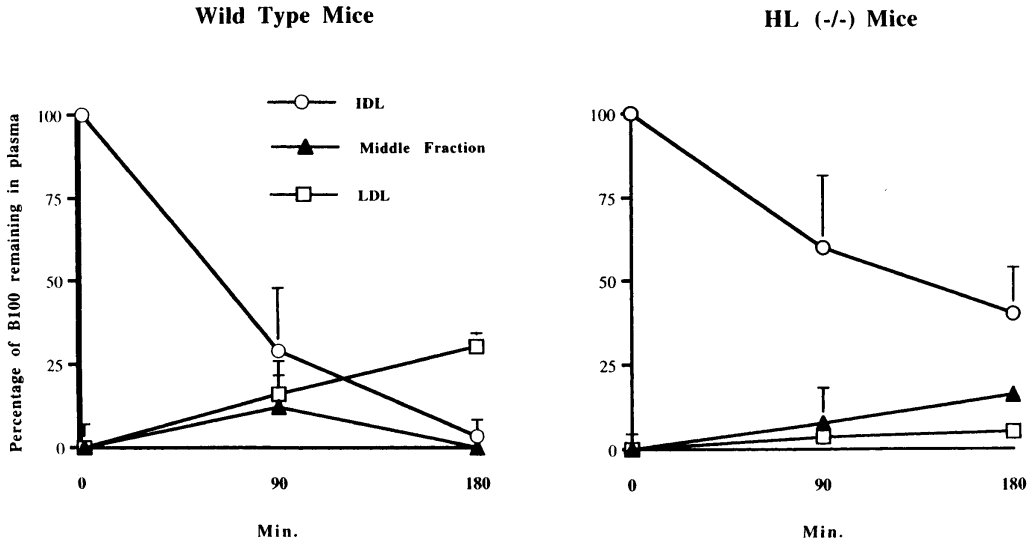

Fig. 2. Metabolism of ^{125}I-labeled apoB-100 of rabbit IDL in wild-type mice and HL (−/−) mice. In in vitro experiments in which the labeled IDL was mixed with mouse serum and subjected to ultracentrifugation, most of the ^{125}I-labeled apoB-100 was recovered in the top portion of the centrifuge tube and the remainder in the middle and bottom portions (see text). The bottom portion was adjusted to a density of 1.055 g/mL with D_2O and recentrifuged to obtain LDL. The observed percentages in the middle portion of the tube and in LDL were set to zero to correct for this redistribution of the injected ^{125}I-labeled apoB-100 and the values observed in the top portion 90 seconds after intravenous injection of the labeled IDL were set to 100%. Values shown are means from three male and two female wild-type mice and three male and three female HL (−/−) mice bled from the inferior vena cava 90 seconds, 90 min, and 180 min after injection. Bars indicate 1 SD (where not shown, values are contained within data point). Clearance of ^{125}I-labeled apoB-100 from plasma was slower in HL (−/−) mice and conversion to LDL was about 6-fold lower than in wild-type mice.

0.001), suggesting that HL promoted the hepatic uptake of IDL as such or after lipolysis. Even in the absence of HL, some conversion of IDL to products of slightly higher density occurred, which could reflect lipolysis by LPL.

Chylomicron metabolism

Recovery of [^{3}H]CE in the liver 20 min after injection of a physiological load of labeled small chylomicrons was similar in HL (−/−) and wild-type mice (**Table 4**). Recovery of hepatic [^{3}H]CE in endosomes, however, was almost 3-fold greater in HL (−/−) mice. This latter result resembles that found in rat experiments in which the rate of endocytosis of chylomicron remnants taken up by perfused livers was significantly greater when HL was inhibited by prior exposure of the livers to HL antiserum or in which HL was first washed out of the liver by perfusion with heparin (9). The magnitude of the effect observed here was considerably larger, however. In the rat experiments inhibition or removal of HL resulted in a significantly reduced rate of clearance of chylomicron remnants by the liver (9). Accordingly, we next injected [^{3}H]CE-labeled small chylomicron remnants into wild-type and HL (−/−) mice. In this case, virtually all of the [^{3}H]CE was recovered in the liver 20 min after injection into wild-type mice, but only a little more than one-half was recovered in the livers of HL (−/−) mice (Table 4). As with chylomicrons, recovery of hepatic [^{3}H]CE in endosomes was greater in the liver of HL (−/−) mice.

It has been proposed that HL is much more important for the initial removal of large chylomicron remnants than small chylomicron remnants by the perfused rat liver (33). However, in the mouse, recovery of injected [^{3}H]CE of large chylomicrons in the liver 20 min after intravenous injection was similar in wild-type and HL (−/−) mice (Table 4). This result is similar to that previously reported by Homanics et al. in HL (−/−) mice (13). Furthermore, the effect of HL deficiency on recovery of hepatic [^{3}H]CE in endosomes was comparable to that observed after injection of small chylomicrons. Endosomal recovery of [^{3}H]CE was, however, somewhat lower after injection of large chylomicrons, consistent with a reduced rate of endocytosis.

TABLE 4. Hepatic recovery of labeled cholesteryl esters 20 min after intravenous injection of chylomicron particles into wild-type (WT) and hepatic lipase (−/−) (HL(−/−)) male mice

Particles Injected, Mice	% of [^{3}H]CE Injected	
	Total Liver[a]	Hepatic Endosomes[b]
Small chylomicrons (n = 12)		
WT	41.0 ± 13.1	4.49 ± 2.36
HL (−/−)	34.8 ± 10.8	12.23 ± 4.16[c]
Small chylomicron remnants (n = 4)		
WT	101.3 ± 17.6	6.05
HL (−/−)	58.9 ± 3.9[c]	9.23
Large chylomicrons (n = 8)		
WT	39.2 ± 12.2	2.47 ± 0.84
HL (−/−)	46.0 ± 8.7	9.23 ± 0.99[c]

Values are means ± SD.

[a] For total liver, n indicates the number of WT and HL (−/−) mice in each group.

[b] For hepatic endosomes, livers from two mice were pooled for isolation of endosomes; therefore, the number of samples is n/2.

[c] Significantly different from corresponding WT value, $P < 0.01$.

DISCUSSION

The studies reported here are mainly concerned with alterations in the metabolism of lipoproteins containing apoB in mice lacking HL. Although Homanics et al. (13) found no appreciable accumulation of lipoproteins containing apoB-100 or apoB-48 in HL (−/−) mice 6 months old or more, in our younger animals, VLDL-lipid concentrations were increased by about 2-fold. We also found higher concentrations of apoB-100 and B-48 in VLDL and IDL and reduced concentrations in LDL of our HL (−/−) female mice, as well as increased concentrations of these proteins in VLDL and IDL relative to LDL in mutant animals of both sexes. These observations, which suggest a defect in conversion of IDL containing either apoB-48 or apoB-100 to LDL in the mutants, are strongly supported by the virtual absence of LDL species smaller than 271 Å in peak diameter in the mutants, whereas they comprised an appreciable fraction of wild-type LDL species. Based upon Western blots, Homanics et al. (l3) found reduced concentrations of apoB-containing particles with densities of 1.04–1.08 g/mL in their HL (−/−) mice, but we found no such difference in our mutant animals (Table 2).

Although the altered concentrations of apoB-containing lipoproteins species were subtle in HL (−/−) mice, the metabolic experiments demonstrated a striking defect in conversion of injected rabbit IDL to LDL. We selected IDL from rabbits, a species with naturally low levels of HL activity (34), for these studies because IDL concentrations are high and the particles are enriched in triglycerides (35). In chow-fed rabbits, about 30% of apoB-100 of IDL is converted to LDL (36). In the mutant mice, there was appreciable conversion of the IDL to species with density slightly greater than 1.019 g/mL (which could reflect further lipolysis by LPL) but very little conversion to species of higher density. This result is remarkably consistent with the comparably striking reduction in species of LDL with diameters below 271 Å, as observed in nondenaturing gradient gel electrophoretograms. Taken together, these results indicate an essential role for HL in the remodeling of large, buoyant LDL of $\rho < 1.03$ g/mL to small, dense LDL in the mouse. In species such as humans in which an appreciable fraction of VLDL is converted to LDL, the phenotypic abnormality associated with this function of HL may be much more pronounced. Indeed, large and triglyceride-enriched LDL are apparently characteristic of human HL deficiency (37). Triglyceride enrichment of LDL (as well as IDL and HDL species) was not observed in HL(−/−) mice, consistent with absence of cholesteryl ester transfer protein activity in rodents (38).

Our metabolic studies also indicate abnormal metabolism of chylomicron remnants in HL (−/−) mice, consistent with earlier observations in rats injected with homologous

HL antiserum (8, 9). Whereas the clearance of intravenously injected chylomicron remnants was delayed, that of large and small chylomicrons was not. Previously, Homanics et al. (13) also failed to observe a reduction in the hepatic uptake of intravenously injected chylomicrons in HL (−/−) mice, which they attributed to replacement of HL-binding sites in the liver by LPL. Two of our observations suggest a different interpretation. First, clearance of remnants derived from injected chylomicrons was normal, but that of preformed chylomicron remnants was substantially impaired; second, the concentration of apoE in plasma was about 5-fold higher in the mutant animals, predominantly involving large HDL species. During the formation of chylomicron remnants in rats (39) and humans (40, 41), apoE transfers from HDL to the chylomicron particles. Much more apoE was evidently available to transfer upon chylomicron injection in HL (−/−) than wild-type mice. This would likely increase the affinity of the remnant particles for endocytic receptors in the liver, compensating for loss of HL-binding of remnants, hydrolysis of their component lipids, or both. By contrast, the preformed remnant particles that we produced in rats, a species with much higher plasma apoE concentrations than mice (42), may have already been saturated with apoE with respect to receptor-affinity. With these particles, impaired clearance associated with deficiency of HL function was clearly manifested.

The increased rate of endocytosis of chylomicron-derived particles in HL (−/−) mice was more pronounced than that observed in rat livers pre-perfused with HL antiserum or heparin (9). The more rapid endocytosis in HL (−/−) mice is consistent with a role for this enzyme in binding and processing chylomicron remnants in the mouse. Recent studies have shown that, as proposed earlier (10, 43), HL is located primarily on the microvillous surface of hepatic parenchymal cells in rats (44) and HL-transgenic rabbits (45), consistent with a liganding function of HL for chylomicron remnants that are subsequently transferred to endocytic receptors (9, 46). Although mouse HL binds poorly to glycosaminoglycans and most of the functional enzyme is in the blood (14), its liganding function could be subserved by either circulating HL, which could bind chylomicron remnants in the blood, or by surface-bound HL in the liver.

Based upon observations of the effect of varying LDL receptor activity in rat liver on the rate of removal of large and small chylomicron remnants by perfused rat livers, a reduced rate of removal of large chylomicron remnants from heparin-preperfused livers, and a much slower rate of endocytosis of large remnants than small remnants, Windler et al. (33) have recently proposed that small chylomicron remnants bind initially to the LDL receptor and undergo rapid endocytosis, but large chylomicron remnants bind to other surface components such as HL. Whereas our data showing more rapid endocytosis of chylomicron remnants in HL (−/−) mice than in wild-type mice are consistent with initial binding of at least some chylomicron remnants to HL, our in vivo data provide little support for a specific role of HL in initial binding of large chylomicron remnants. The rate of endocytosis of remnants derived from small and large chylomicron alike was increased 3- to 4-fold in HL (−/−) mice. Furthermore, in another study we have found that small chylomicron remnants are removed by livers of LDLR (−/−) mice pretreated with excess HL antiserum at an appreciable rate (S-Q. Qui, A. Bensadoun, R. Havel, unpublished data). Two-thirds of injected [^{3}H]CE of small chylomicron remnants was recovered in the liver 20 min after injection, which can be compared with the value of 58.9% in HL (−/−) mice (Table 4). Endocytosis of the remnants was sluggish in the LDLR (−/−) mice, but was increased 2-fold after pretreatment with the antiserum. These data suggest that hepatic surface components other than the LDLR or HL can efficiently bind chylomicron remnants. An important role for the LDLR in endocytosis of chylomicron remnants is, however, supported by these data, which as reported previously (28), are endocytosed at a much slower rate when this receptor is absent. This slow rate evidently cannot be attributed to requirement for transfer of the remnant particle from HL to an endocytic receptor. Methodological rather than species differences are likely to explain the different results and conclusion of Windler et al. (33) and those reported here. As emphasized recently, rates of hepatic removal of chylomicron remnants may be influenced heavily by the number of particles injected (47). Indeed, Homanics et al. (13) found a prolonged increase in plasma triglycerides of HL (−/−) mice after gastric intubation of 1.0 mL, but not 0.4 mL olive oil. The extent to which the LDLR, HL, or other hepatic surface components bind chylomicron remnants is, therefore, likely to be a function of particle load during postprandial lipemia.

In summary, our data confirm an important role for HL in the metabolism of HDL and indicate that accumulation of apoE in α-migrating lipoproteins is the most prominent apolipoprotein abnormality in HL (−/−) mice. We propose that increased availability of apoE compensates for the effect of HL-deficiency on the metabolism of chylomicron remnants so that there is little disturbance in hepatic clearance. The actions of HL upon the clearance and endocytosis of chylomicron remnants by mouse liver are, however, essentially the same as those found earlier in rats, a species lacking circulating HL. Our data also show that, as in humans lacking HL, conversion of apoB-l00-containing IDL to LDL in HL-deficient mice is limited to large, bouyant species; despite the lack of cholesteryl ester transfer protein activity in mice, this defect is accompanied by a virtual absence of small, dense LDL.

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