Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein

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Abstract Hepatic lipase deficiency produces significant distortion in the plasma lipoprotein profile. Particles with reduced electrophoretic mobility appear in very low density lipoprotein (VLDL). Intermediate density lipoprotein (IDL) increases markedly in the circulation and plasma low density lipoprotein (LDL) levels fall. At the same time there is a mass redistribution within the high density lipoprotein (HDL) spectrum leading to dominance in the less dense HDL_2 subfraction. The present study examines apolipoprotein B turnover in a patient with hepatic lipase deficiency. The metabolism of large and small very low density lipoproteins was determined in four control subjects and compared to the pattern seen in the patient. Absence of the enzyme did not affect the rate at which large very low density lipoproteins were converted to smaller particles within this density interval (i.e., of VLDL). However, subsequent transfer of small very low density lipoproteins to intermediate density particles was retarded by 50%, explaining the abnormal accumulation of VLDL in the patient's plasma. Despite this, intermediate density particles accumulated to a level 2.4-times normal because their subsequent conversion to low density lipoprotein has been almost totally inhibited. Consequently, the plasma concentration of low density lipoprotein was only 10% of normal. On the basis of these observations, hepatic lipase appears to be essential for the conversion of small very low density and intermediate density particles to low density lipoproteins. The pathways of direct plasma catabolism of these species were not affected by the enzyme defect. In vitro studies were performed by adding purified hepatic lipase to the patient's plasma. This did not modify the size of HDL_2 significantly, but resulted primarily in triglyceride hydrolysis in the less dense apolipoprotein B-containing particles.—**Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd.** Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* 1988. **29:** 1603-1611.

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The lipolytic degradation of circulating triglyceride-rich lipoproteins involves simultaneous loss of core triglycerides and coat phospholipid from the particles. Two key enzymes, located on the endothelial surfaces of capillary beds, are believed to participate in this process (1). Lipoprotein lipase, the better characterized of the two, is associated primarily with adipose tissue and skeletal muscle. It shows particular affinity for larger lipoprotein particles (2) like chylomicrons and very low density lipoprotein (VLDL) with Svedberg flotation rates (S_f) greater than 100. Hereditary absence of the enzyme therefore results in accumulation of such particles in the circulation, leading to the phenotypic lipoprotein disorder called Type I hyperlipoproteinemia (3).

The function of the other enzyme, hepatic lipase, synthesized and secreted by hepatocytes, is less well documented (1). It possesses both triglyceride hydrolase and phospholipase activities in vitro (4) and has a higher affinity for smaller, denser lipoprotein particles (2). Animal studies (5, 6) have suggested that its actions may be directed primarily at the metabolism of small VLDL, intermediate density lipoprotein (IDL), and high density lipoprotein (HDL). Antibody infusions (5), designed to inhibit the enzyme, lead, within a few hours to the accumulation of S_f 20-100 VLDL and S_f 12-20 IDL in plasma, with a concomitant reduction in circulating low density lipoprotein (LDL). At the same time, the mass of phospholipid within the HDL_2 density interval (1.063-1.125 kg/l) increases (5, 6). However, it has also been proposed that hepatic lipase

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Abbreviations: S_f, negative sedimentation coefficient at d 1.063 kg · l^{-1} and 26°C; VLDL, very low density lipoproteins, d < 1.006 kg · l^{-1}; IDL, intermediate density lipoproteins, d 1.006-1.019 kg · l^{-1}; LDL, low density lipoproteins, d 1.019-1.063 kg · l^{-1}; TMU, 1,1,3,3-tetramethylurea; HDL, high density lipoproteins, d 1.063-1.210 kg · l^{-1}; HDL_2, high density lipoprotein subfraction 2, d 1.063-1.125 kg · l^{-1}; HDL_3, high density lipoprotein subfraction 3, d 1.125-1.210 kg · l^{-1}; LCAT, lecithin:cholesterol acyltransferase; FFA, free fatty acids.

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is involved in the elimination of HDL_2 constituents by the liver (7-10). Recently two pairs of brothers, one Canadian (11) and one Swedish (12), were reported to have a deficiency of postheparin hepatic lipase activity. The lipoprotein profile in all four patients was distorted, with the accumulation of small VLDL (β-VLDL) and HDL_2, but it was not clear from the observations whether the enzyme acted primarily on VLDL or HDL. Since both of these particles are linked metabolically, disturbances in the metabolism of one would have an impact on the other, possibly via the agency of plasma lipid transfer activities. The present report describes in vivo and in vitro studies on the lipoproteins of one of the Swedish patients.

MATERIALS AND METHODS

Subjects

The four subjects who formed the control group in this study were healthy males aged 32-65 years. Routine clinical and laboratory screening revealed no evidence of cardiologic, renal, hepatic, endocrine, or metabolic disease. Plasma lipid and lipoprotein levels were determined according to the Lipid Research Clinics protocol (13).

The hepatic lipase-deficient patient (GP, age 63 years), one of two affected Swedish brothers, has been described in detail elsewhere (12). He is an $apoE_3/E_4$ heterozygote. At the time of this study he had a moderate hypertriglyceridemia without any increase in VLDL cholesterol (**Table 1**) and with virtually immeasurable postheparin hepatic lipase but normal lipoprotein lipase activity. His general health remains good. Specifically, there was no clinical evidence of central or peripheral vascular disease. All subjects gave their informed consent to the study which followed the guidelines of the Ethical Committees of Glasgow Royal Infirmary and the Karolinska Hospital, Stockholm.

In vivo studies

Isolation and characterization of plasma lipoproteins was performed using two ultracentrifugation techniques. The cumulative flotation ultracentrifugation procedure, a modification (14) of the method of Lindgren, Jensen, and Hatch (15) was employed to isolate apolipoprotein B-containing particles in four subfractions: S_f 60-400 (large VLDL), 20-60 (small VLDL), 12-20 (IDL), and 0-12 (LDL). The detailed methodology is described in earlier publications (14,16). Since the majority of the apolipoprotein B-containing lipoproteins lie within the density interval 1.006-1.063 $kg \cdot l^{-1}$, this material was also subjected to continuous gradient analysis by the rate zonal ultracentrifugation method of Patsch et al. (17). To facilitate characterization of GP's profile, a marker of radioiodinated normal LDL (18) was added to his plasma specimen prior to analysis. The HDL profile in this patient has already been shown to be abnormal by ultracentrifugation (19) as well as polyacrylamide gel electrophoresis (12). It was re-examined by the rate zonal separation procedure (17).

Fractions isolated by the cumulative or rate zonal ultracentrifugation procedure were analyzed to determine their contents of free and esterified cholesterol, triglyceride, phospholipid, and protein as outlined elsewhere (20).

Kinetic studies

Total VLDL of density less than 1.006 $kg \cdot l^{-1}$ was prepared in a Beckman Ti 60 rotor (Beckman Instruments, Palo Alto, CA) by ultracentrifugation of 250 ml of fasting plasma for 18 hr at 10°C and 40,000 rpm. The lipoprotein was aspirated, pooled, and its density was increased to 1.182 $kg \cdot l^{-1}$ by addition of solid NaBr (0.384 $g \cdot ml^{-1}$). A discontinuous salt gradient from density 1.0988 $kg \cdot l^{-1}$ to 1.0588 $kg \cdot l^{-1}$ was constructed over 2.0-ml aliquots of the VLDL in an SW40 rotor, and the preparation was centrifuged according to a modification (14) of the procedure of Lindgren et al. (15) to isolate large and small VLDL frac-

TABLE 1. Plasma lipids and lipoproteins in control subject and a hepatic lipase-deficient subject

Subjects	Total Triglyceride	Total Cholesterol	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL
			mmol/l		
N1[a]	1.71 ± 0.38[b]	4.96 ± 0.54	0.79 ± 0.21	3.14 ± 0.35	1.01 ± 0.13
N2	2.55 ± 0.71	6.23 ± 0.79	1.02 ± 0.25	4.09 ± 0.50	1.36 ± 0.19
N3	0.94 ± 0.25	3.76 ± 0.40	0.33 ± 0.17	1.66 ± 0.32	1.78 ± 0.24
N4	2.00 ± 0.75	6.55 ± 1.00	1.08 ± 0.54	4.37 ± 0.83	1.10 ± 0.22
Mean	1.80 ± 0.58	5.38 ± 1.10	0.81 ± 0.29	3.32 ± 1.06	1.31 ± 0.30
HL(-)[c]	2.82 ± 0.49	5.10 ± 1.05	0.75 ± 0.41	2.18 ± 0.29	1.96 ± 0.25

[a]Normal subjects 1 to 4.
[b]Mean ± SD, n = 5.
[c]Hepatic lipase-deficient patient GP.

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tions of S_f 60–400 and 20–60, respectively. These fractions were than labeled with ^{131}I and ^{125}I by a modification (21) of the Macfarlane ICl procedure (22). Labeling efficiency varied between 5 and 15% and the conditions used gave less than 1 mol of iodine per 300,000 daltons of B protein as described previously (16). The labeled tracers were sterilized by membrane filtration (0.45 μm Amicon filters (Amicon Corp., Bedford, MA)) prior to reinjection into the donor. Preparation time overall was less than 48 hr. The tracers were administered at 8:00 AM after an overnight fast and, in order to minimize chylomicron production, the subjects received a hypocaloric fat-restricted (less than 5 g) diet during the first day of the study. Plasma samples were collected at frequent intervals over the first 72 hr and then daily in the fasting state for 14 days. Plasma from each time point was used to isolate large and small VLDL, IDL, and LDL by the method outlined above (14, 16). These lipoproteins were treated with 1,1,3,3-tetramethylurea (TMU) as described elsewhere (16) to prepare apolipoprotein B, whose specific activity was calculated following direct measurement of protein (16) and radioactivity content. The apolipoprotein B pool circulating with each of these lipoprotein fractions was determined by replicate analyses of plasma samples collected intermittently throughout the turnover study. Losses during centrifugation were corrected by comparing the total cholesterol mass recovered in all four fractions with the d < 1.063 kg $\cdot$ l^{-1} cholesterol content of the subject's plasma determined by the standard Lipid Research Clinics protocol (13). Further correction was made for possible B protein loss during selective TMU precipitation by comparing the apolipoprotein B recovered at the end of the procedure with the values calculated as the difference between total and TMU-soluble (apolipoproteins E and C) protein contents (23).

Kinetic analysis

The radioactivity associated with the B protein present in each fraction was calculated from the apolipoprotein B specific activities and the individual pool sizes. These were expressed as a percentage of the total B protein radioactivity present in the plasma 10 min after injection and the resulting values were used to construct decay curves which were analyzed by the SAAM 29 multicompartmental modeling program (24). The model that was employed is described elsewhere (16). Its main features allow for: *a*) apolipoprotein B input at the level of both large and small VLDL and LDL; *b*) stepwise delipidation of VLDL following the concept of Berman et al. (25); and *c*) parallel pathways of a B protein processing from small VLDL through IDL to LDL.

Rate constants were determined and, in combination with B protein pool sizes, were used to calculate flux ratios and steady-state synthetic input.

In vitro studies

These studies were performed in Sweden. The methods for lipid analysis, lipoprotein separations and isolations, and polyacrylamide gradient gel electrophoresis have all been described (12). Blood taken from the fasting subject was put into chilled EDTA-tubes kept on ice and plasma was recovered within 30 min by low speed centrifugation at 2°C. Hepatic lipase was isolated in Lund from postheparin plasma of healthy volunteers by repeated heparin-Sepharose chromatography (26) and shipped on dry ice to Stockholm. The enzyme preparation had a hepatic lipase activity of 9.2 U/mg protein (1 unit representing the release of 1 μmol fatty acid per min at 37°C) and was devoid of lipoprotein lipase activity, as determined by specific assays using radiolabeled trioleoylglycerol emulsions as substrate (27). Incubations were performed in triplicate in the dark at 37°C (to prevent auto-oxidation) for the indicated periods of time and were terminated by putting the incubation vessels on ice. Two series of incubations were performed. In one series whole plasma was used while in the other we used plasma from which VLDL and LDL had been removed by tube slicing after preparative ultracentrifugation at d 1.063 kg $\cdot$ l^{-1}. Before use, the preparation was dialyzed against 0.15 M NaCl and its original volume was restored with the same solution (28). To the incubation mixtures were added either 0.15 ml of enzyme solution or 0.15 ml of 0.15 M NaCl per ml of plasma.

Two identical studies performed 1 year apart gave the same results. In the first, the enzyme activity was 135 mU/ml (27); in the second, not reported here, the activity was 225 mU/ml. Enzyme activity was assayed the day before the experiment.

RESULTS

The lipid and lipoprotein profiles of the control subjects and of the hepatic lipase-deficient patient (GP) are shown in Table 1. The hypertriglyceridemia in GP, whose plasma VLDL cholesterol concentration was normal, is explained by the finding noted earlier (11, 19) that the majority of the triglyceride was present in LDL and HDL (1.42 and 0.64 mmol $\cdot$ l^{-1}, respectively). These lipoproteins were enriched in triglyceride (cholesterol/triglyceride ratio in LDL was 1.5, versus 6.0–16.0 in normals; in HDL the ratio was 3.0, versus 5.0–16.0 in normals). On the other hand, the cholesterol/triglyceride ratio in GP's VLDL was high (1.1 versus 0.4–0.8 in normals).

Compositional analysis of the four apolipoprotein B-containing lipoprotein subfractions is presented in **Table 2**. The large and small VLDL in GP had normal triglyceride contents but were reduced in cholesteryl esters and enriched

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TABLE 2. Compositions of apoB-containing lipoproteins in hepatic lipase deficiency

	Triglyceride	Cholesteryl Ester	Free Cholesterol	Phospholipid	Protein
			g/100 g (n = 3)		
$VLDL_1$[a]	62.2 ± 3.1[b,c]	9.3 ± 0.5	3.9 ± 1.8	14.6 ± 3.4	10.0 ± 0.2
	(56.2 ± 4.8)	(16.0 ± 4.3)	(1.7 ± 2.3)	(17.0 ± 1.4)	(9.1 ± 2.4)
$VLDL_2$	34.6 ± 0.9	12.7 ± 2.2	13.3 ± 2.6	23.4 ± 1.2	16.0 ± 0.6
	(35.1 ± 4.0)	(21.1 ± 5.9)	(8.1 ± 1.4)	(21.4 ± 2.4)	(14.4 ± 1.6)
IDL	27.9 ± 1.7	13.5 ± 3.7	12.7 ± 2.0	24.7 ± 1.2	21.0 ± 0.8
	(12.4 ± 2.0)	(33.4 ± 4.8)	(11.2 ± 2.3)	(23.9 ± 1.3)	(19.1 ± 2.3)
LDL	23.6 ± 1.4	24.5 ± 4.9	7.1 ± 0.2	26.4 ± 1.2	18.6 ± 4.9
	(5.1 ± 0.2)	(34.8 ± 2.2)	(13.5 ± 1.5)	(23.0 ± 1.6)	(23.6 ± 1.6)

Values in parentheses are from normolipidemic control subjects.
[a] $VLDL_1$, S_f 60-400 lipoproteins; $VLDL_2$, S_f 20-60 lipoproteins; IDL, S_f 12-20 lipoproteins; and LDL, S_f 0-12 lipoproteins.
[b] Mean ± SD.
[c] Each fraction was isolated from GP on three occasions and assayed as described in Methods.

in free cholesterol. However, the IDL and LDL fractions were quite abnormal in composition. Both fractions were enriched in triglyceride at the expense of cholesteryl ester. The phospholipid, protein and free cholesterol contents were unchanged. In the lipase-deficient subject, most VLDL apoB (**Table 3**) resided at the denser end of the spectrum (i.e., S_f 20-60); and the level of IDL apoB was raised severalfold.

Tracers of large and small VLDL were used to investigate the origins of the above abnormal distributions. The results are presented in **Fig. 1** and Table 3. The distribution of apolipoprotein B in the normal subjects reflected their lipoprotein pattern (Table 1) in that most of its mass was found in the LDL density interval. In contrast, most B protein in GP was associated with IDL. The normal subjects synthesized about 1000 mg of total VLDL apolipoprotein B each day, 70% of which appeared first in the large S_f 60-400 component. Approximately half of this protein was metabolized through IDL to LDL, the remainder leaking out of the delipidation cascade at the levels of large and small VLDL and IDL. GP produced 570 mg of total VLDL apolipoprotein B, most of which was secreted with small VLDL particles. That portion of protein that appeared first in large VLDL was transferred to the smaller VLDL range (S_f 20-60) at a normal rate. The mass of B protein in GP's small VLDL pool was normal. However its fractional clearance rate (Table 3; Fig 1) was retarded and its rate of transfer to the IDL fraction was delayed. On the other hand, its direct catabolism from the circulation was at least as high as normal. Despite the reduced input of protein from VLDL, the plasma pool of IDL was elevated 2.4-times, principally because the plasma clearance of this fraction was so slow (Table 3, Fig. 1). The transfer of B protein from IDL to LDL occurred only at about 5% of normal, although it should be noted that, as in the case of VLDL, the rate of direct removal of IDL apolipoprotein B from the circulation (0.25 pools · d^{-1}) was not reduced. A small amount of IDL apolipoprotein B did transfer into

TABLE 3. Apolipoprotein B metabolism in normal subjects

Subject	Large VLDL ApoB				Small VLDL ApoB				
			Fractional Rate					Fractional Rate	
	Synthesis	Plasma Pool[a]	Direct Catabolism	Transfer to $VLDL_2$	Synthesis Direct	Synthesis from $VLDL_1$	Plasma Pool[a]	Direct Catabolism	Transfer to IDL
	mg/day	*mg*	*pools/day*		*mg/day*	*mg/day*	*mg*	*pools/day*	
N1NC	802	79	3.7	6.5	393	512	246	0.36	3.3
N2CD	710	80	2.9	6.0	467	478	226	1.32	2.0
N3MQ	290	18	0.0	16.2	137	290	100	0.70	2.8
N4TS	954	115	5.0	3.2	238	372	258	0.35	1.9
Mean ± SD	689 ± 246	73 ± 35	2.9 ± 1.8	8.0 ± 4.9	259 ± 91	413 ± 88	208 ± 63	0.68 ± 0.39	2.5 ± 0.6
HL(-)	91	9	1.8	8.3	480	74	265	1.08	0.97

[a] Plasma pool derived from steady-state analysis using SAAM 29 program. This agrees with the observed apolipoprotein B pool to within ± 15%.

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the LDL fraction (Fig. 1, Table 3) which was degraded at a low normal fractional clearance rate. Table 2 indicates that this LDL was not normal in composition, and indeed from rate zonal ultracentrifugation of the IDL/LDL lipoproteins in GP's plasma (**Fig. 2**) it is clear that there was no discrete LDL peak. The small amount of material isolated as "LDL" by cumulative flotation ultracentrifugation probably represents the denser component of the IDL spectrum.

In vitro studies

Incubation of whole plasma from the hepatic lipase-deficient patient for 24 hr with or without added purified hepatic lipase resulted in the formation of about 1 mmol of cholesteryl ester per liter of plasma in both cases (**Table 4**), very close to earlier reported values (12). A corresponding decrease of phospholipids, the donor of the fatty acid in the esterification reaction, occurred in the two incubations. The percentage of plasma cholesterol that was esterified increased from 51% — a lower than normal value as pointed out before (12) — to 67%. These incubation-induced changes are due to lecithin:cholesterol acyltransferase (LCAT) activity as both α- and β-LCAT activity are normal in hepatic lipase deficiency (12).

The plasma triglyceride concentration remained unchanged during the control incubation, while the addition of hepatic lipase resulted in a decrease of the triglyceride content of about 0.5 mmol · 1^{-1}.

The incubations of the VLDL- and LDL-depleted plasmas, which contain HDL as the major lipoprotein class, resulted in changes similar to those observed for whole plasma (Table 4). The cholesteryl ester concentration rose by 0.7 mmol · 1^{-1} whether or not hepatic lipase was present. The decrease in phospholipids was of the same order.

The hepatic lipase caused a slight decrease in the triglyceride content of HDL (0.1 mmol · 1^{-1}). At the same time the concentration of free fatty acids (FFA) increased by 0.14 mmol · 1^{-1} as compared to 0.08 mmol · 1^{-1} without added lipase. This latter increase is due to release of FFA from lecithin during the LCAT reaction (29).

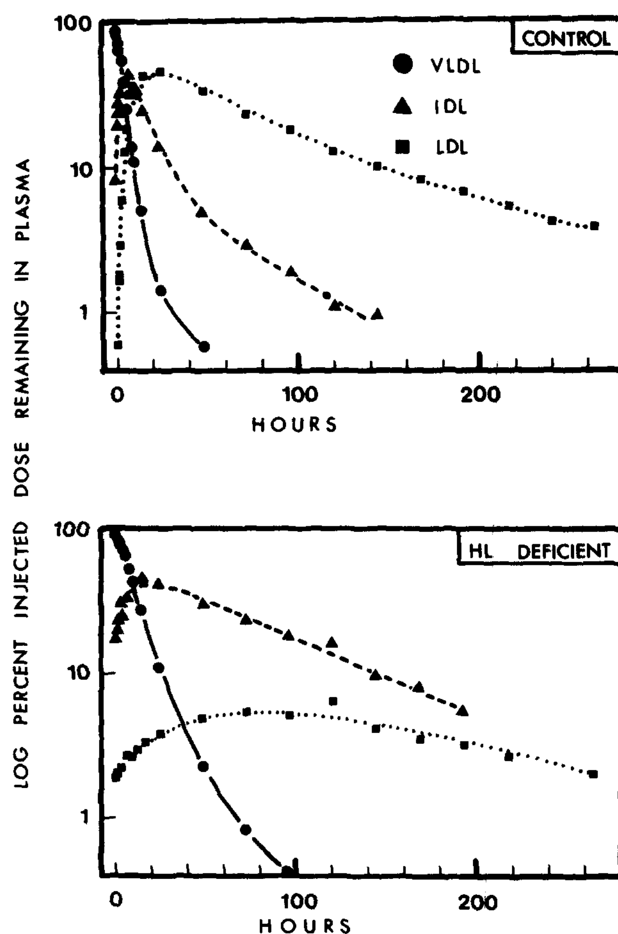

Fig. 1. Plasma decay curves of apolipoprotein B in small VLDL, IDL, and LDL from control subject (N1) and patient GP following injection of ^{125}I-labeled small VLDL. Symbols represent observed data and lines represent the computer-derived fit.

The HDL particles of GP's plasma migrate exclusively as large HDL_2 particles on gradient gel electrophoresis (12). In conformity, when GP's HDL was examined using the standard rate zonal method (**Fig. 3**), the majority of particles were associated with the HDL_2 density interval. HDL_3 was virtually absent. The size of the HDL particles of this

and in a hepatic lipase-deficient subject (HL(−))

IDL ApoB				LDL ApoB			
		Fractional Rate					
Synthesis from $VLDL_2$	Plasma Pool[a]	Direct Catabolism	Transfer to LDL	Synthesis Direct	Synthesis from IDL + $VLDL_2$	Plasma Pool[a]	Fractional Catabolic Rate
mg/day	*mg*	*pools/day*		*mg/day*	*mg/day*	*mg*	*pools/day*
817	337	0.77	1.65	0.0	556	1750	0.32
445	420	0.09	0.90	279	379	2630	0.25
275	210	0.32	0.98	0.0	290	635	0.46
499	496	0.15	0.85	90	423	2650	0.20
509 ± 196	366 ± 106	0.33 ± 0.27	1.10 ± 0.33	92 ± 114	412 ± 96	1916 ± 824	0.31 ± 0.10
262	886	0.25	0.05	0.0	43	215	0.21

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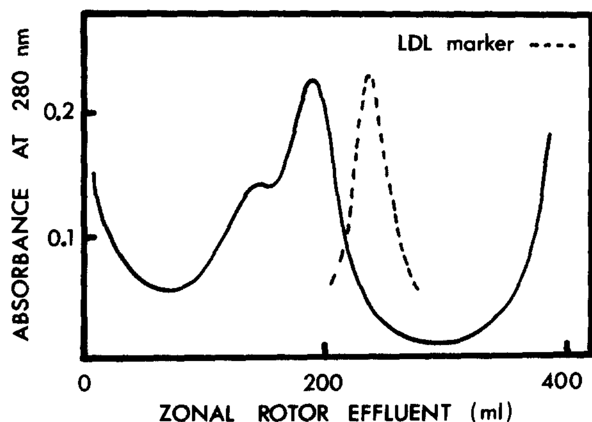

Fig. 2. Rate zonal ultracentrifugation profile of d 1.006–1.063 kg · l^{-1} lipoproteins in patient GP. The radioactive marker indicates the flotation characteristics of LDL prepared from a control subject.

hepatic lipase-deficient patient was not modified by the incubation with hepatic lipase (**Fig. 4**).

DISCUSSION

Intravenous heparin administration triggers the release into the plasma of two lipases which are distinct in their structure and function (1, 30). The better characterized lipoprotein lipase plays a primary role in the metabolism of large triglyceride-rich lipoproteins. Its deletion, therefore, results in the accumulation in the circulation of gut-derived chylomicrons containing apolipoprotein B-48- and B-100-containing large VLDL of hepatic origin (31). Endogenously labeled VLDL triglyceride is cleared slowly from the plasma of such patients (32). However, Nicoll and Lewis (2) have shown that lipoprotein lipase cannot be solely responsible for the catabolism of these particles since, even in its absence, VLDL (d < 1.006 kg · l^{-1}) is converted to IDL, albeit at a reduced rate. Clearly the other lipase in plasma must contribute, and indeed these authors (2) and others (33) have demonstrated that it is able to use small VLDL and IDL as efficient substrates for triglyceride hydrolysis. Certainly its action is inversely correlated with the levels of IDL in plasma (34); and, as indicated from the antibody inhibition experiments of Goldberg et al. (5), abolition of its activity causes an acute increase in the circulating mass of small VLDL and IDL with a reduction in LDL in cynomolgus monkeys. Such studies would implicate hepatic lipase in the VLDL delipidation cascade although the literature is not altogether consistent in this regard since Reardon, Sakai, and Steiner (35), in a study of the metabolism of large and small VLDL, were led to the conclusion that hepatic lipase has no role to play here. Certainly, hepatic lipase seems to have little activity against VLDL in vitro (Nilsson-Ehle, P., unpublished data). Most of the available evidence (7–10) suggests that the high density fraction, particularly HDL_2, is the preferred substrate for the enzyme. The recent discovery of hepatic lipase-deficient patients allows us to make a direct assessment of the mode of action of the enzyme. Our earlier studies (12) suggested that the disease was associated with delayed clearance of both VLDL and HDL with the resultant accumulation of β-VLDL and HDL_2.

The patient GP synthesized VLDL apolipoprotein B at about half of the rate seen in controls. Most (80%) of the material which he produced comprised smaller particles of S_f 20–60. This abnormal pattern of secretion, favor-

TABLE 4. Effects of incubation at 37°C for various times on lipid concentrations in whole plasma and VLDL- and LDL-depleted plasma from case GP, with and without added hepatic lipase (HL)

		Concentration of[a]							
		Phospholipids		Cholesteryl Ester		Triglycerides		Free Fatty Acids	
	Incubation Time	Without HL	With HL	Without HL	With HL	Without HL	With HL	Without HL	With HL
	hr	*mmol/l plasma*							
Whole plasma	0		4.86	2.85	2.79	4.22	3.96		
Whole plasma	0.5	4.51	4.92	2.87	2.83	4.16	4.28		
Whole plasma	1.5	4.53	4.73	2.93	2.92	3.98	4.10		
Whole plasma	6	4.55	4.16	3.13	3.18	4.13	3.46		
Whole plasma	24	4.12	3.90	3.81	3.68	4.24	3.62		
Depleted plasma	0	1.77	1.83	0.83	0.81	0.82	0.82	0.10	0.12
Depleted plasma	0.5	1.97	1.85	0.86	0.82	0.90	0.86	0.15	0.15
Depleted plasma	1.5	1.85	1.81	0.91	0.88	0.85	0.82	0.14	0.19
Depleted plasma	6	1.75	1.63	1.03	1.03	0.87	0.71	0.15	0.20
Depleted plasma	24	1.44	1.40	1.47	1.47	0.82	0.71	0.18	0.26

[a]Mean values; triplicate incubations.

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ing smaller particles, is responsible for the apparent cholesterol enrichment which we noted in the total VLDL (Table 1, ref. 12). The rate at which the large VLDL in GP was converted to smaller remnants was normal, in accord with our previous suggestion that this process is entirely lipoprotein lipase-dependent. Subsequent processing of the smaller VLDL, however, occurred at about one-half of the normal rate. This seems, therefore, to be the first point at which hepatic lipase plays a significant part in the delipidation process. Certainly the decrease in the observed fractional rate of VLDL to IDL conversion cannot be attributed to an expansion of the VLDL pool since this was obviously not present in GP (Table 3). The most remarkable defect in GP's apolipoprotein B metabolism was the virtual complete block in particle transfer between IDL and LDL. This resulted in a major increase in the circulating mass of IDL and a 90% decrease in LDL. In fact, examination of GP's LDL zonal profile raises doubts about the existence of any true (i.e., "normal") LDL in this patient. What was identified by the cumulative flotation procedure may in fact have been contaminating IDL. Thus, we would conclude that GP largely or entirely lacks the ability to form normal LDL. The IDL that accumulated in his plasma is removed at a rate reminiscent of LDL, and earlier studies from this laboratory (36) suggest that a receptor pathway may be implicated in the process. Indeed, examination in Table 3 of the fractional rates of direct removal of any of the four B protein-containing particles indicates that there was no defect in these pathways. Thus, lipoprotein and hepatic lipases are discrete in their activities. Lipoprotein lipase focuses on large VLDL catabolism and also contributes approximately 50% to the conversion of smaller VLDL to IDL. Hepatic lipase, on the other hand, while sharing in the latter process, is almost entirely responsible for catalysis of the conversion of IDL to LDL. Extrapolation from this suggests that such a process must occur in the liver, in agreement with published transhepatic catheterization studies (37). The mechanism itself, though still speculative, may well also involve the agency of receptors since it is known that individuals with homozygous familial hypercholesterolemia also accumulate IDL in the plasma (38). A separate, though possibly linked, function for the enzyme relates to the interconversion of HDL subspecies. Hepatic lipase has been proposed to be the principal enzyme involved in removing phospholipid and triglyceride from HDL, and individuals with low hepatic lipase activities have been found to have higher HDL_2/HDL_3 ratios (7). Again, what is remarkable in this patient is the virtual absence of HDL_3, as recorded by rate zonal ultracentrifugation (Fig. 4) or nondenaturing gradient gel electrophoresis (12). The HDL that accumulates is triglyceride-rich. According to current concepts, this lipid comes from triglyceride-rich particles in the circulation and its transfer is mediated by cholesteryl ester transfer protein. It has been suggested that HDL_2 phospholipids would normally be hydrolyzed by hepatic lipase with simultaneous shrinkage of the particle to the size of HDL_3 (9). Indeed, Rao et al. (39) have observed that heparin infusions in lipoprotein lipase-deficient patients lead to an acute mass transfer from HDL_2 to HDL_3. However, our in vitro studies failed to confirm this suggestion. When plasma from GP was incubated with purified hepatic lipase, no decrease in HDL_2 particle size was observed and what triglyceride hydrolysis did take place

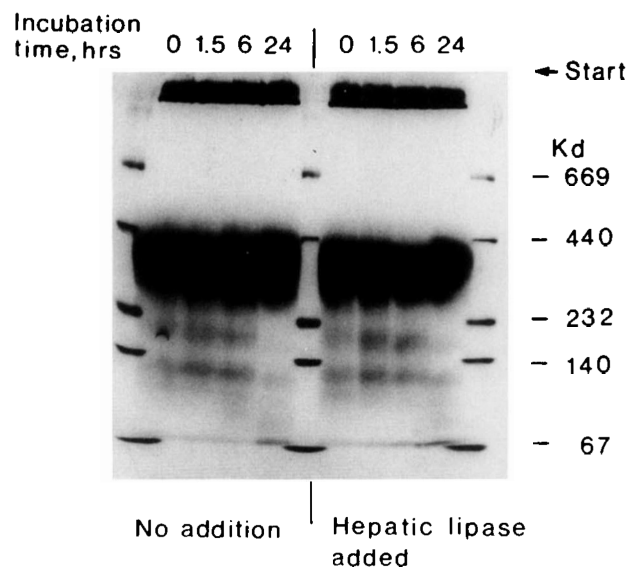

Fig. 4. Polyacrylamide gradient gel electrophoresis of total plasma lipoprotein isolated after incubation of plasma from patient GP at 37°C with or without added purified hepatic lipase. Marker proteins as in ref. 12. Note *1*) the presence of large HDL particles in the region corresponding to molecular masses of 300–400 kDa as the totally dominating HDL species, and *2*) the lack of effect of incubation with or without the lipase on HDL particle size.

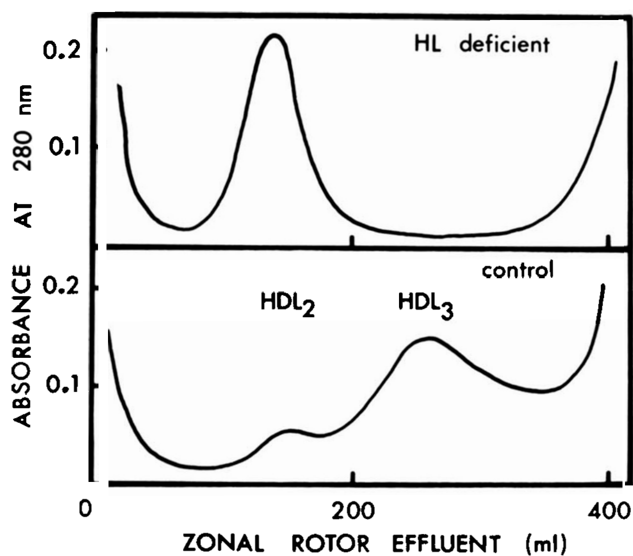

Fig. 3. Rate zonal ultracentrifugation profile of HDL from a control subject and patient GP.

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seemed to result from an action of the enzyme on VLDL and LDL. But, this must be viewed with caution since it is possible that the composition of GP's HDL_2 is abnormal and the particle may have decreased affinity for the enzyme.

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