Modifications of plasma lipoproteins after lipase activation in patients with chylomicronemia

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Abstract Lipoprotein lipase (LPL) and hepatic lipase (HL) are enzymatic activities involved in lipoprotein metabolism. The purpose of this study was to analyze the physicochemical modifications of plasma lipoproteins produced by LPL activation in two patients with apoC-II deficiency syndrome and by HL activation in two patients with LPL deficiency. LPL activation was achieved by the infusion of normal plasma containing apoC-II and HL was released by the injection of heparin. Lipoproteins were analyzed by ultracentrifugation in a zonal rotor under rate flotation conditions before and after lipase activation. The LPL activation resulted in: *1*) a reduction of plasma triglycerides; *2*) a reduction of fast-floating very low density lipoprotein (VLDL) concentration; *3*) an increase of intermediate density lipoprotein (IDL), which maintained unaltered flotation properties; *4*) an increase of low density lipoproteins (LDL) accompanied by modifications of their flotation rates and composition; *5*) no significant variations of high density lipoprotein (HDL) levels; and *6*) an increase of the HDL flotation rate. The HL activation resulted in: *1*) a slight reduction of plasma triglycerides; *2*) a reduction of the relative triglyceride content of slow-floating VLDL, IDL, LDL_2, and HDL_3 accompanied by an increase of phospholipid in VLDL and by an increase of cholesteryl ester in IDL; and *3*) a reduction of the HDL flotation rate. These experiments in chylomicronemic patients provide in vivo evidence that LPL and HL are responsible for plasma triglyceride hydrolysis of different lipoproteins, and that LPL is particularly involved in determining the levels and physicochemical properties of LDL. Moreover, in these patients, the LPL activation does not directly change the HDL levels, and LPL or HL does not produce a step-wise conversion of HDL_3 to HDL_2 (or vice versa) but rather modifies the flotation rates of all the HDL molecules present in plasma.—**Manzato, E., S. Zambon, R. Marin, G. Baggio, and G. Crepaldi.** Modifications of plasma lipoproteins after lipase activation in patients with chylomicronemia. *J. Lipid Res.* 1986. **27:** 1248–1258.

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The metabolism of plasma lipoproteins is regulated to a great extent by lipolytic activities located on the capillary endothelial wall. After an intravenous injection of heparin, these activities increase in plasma and at least two different lipases can be recognized, one of hepatic and one of extrahepatic origin (1–3). Plasma postheparin lipolytic activities of extrahepatic origin are collectively referred to as lipoprotein lipase (LPL) and require apoC-II as an activator, while the activities of hepatic origin (also called hepatic lipase, HL) do not require an activator.

The effects of LPL and HL on plasma lipoproteins have been studied in vitro (4–9) and in vivo (9–16). It has been demonstrated that LPL hydrolyzes chylomicron and VLDL triglycerides, and in this way it regulates LDL and HDL production. Less clear is the physiological role of HL which seems to be particularly involved in the metabolism of VLDL, IDL, and HDL. Three metabolic diseases in which the plasma lipoproteins are grossly altered due to a deficiency of LPL or of its activator (i.e., apoC-II) (17), or to a deficiency of HL (18) have been identified.

LPL in apoC-II-deficient patients can be activated by the infusion of normal plasma. Such an infusion has been used to study the effects of LPL activation on plasma lipoproteins in apoC-II-deficient patients (19–21). Miller et al. (20) used preparative and analytical ultracentrifugation and electroimmunoassay to study the effects on lipoproteins of an intravenous infusion of plasma in one subject with apoC-II deficiency. They found: *1*) a decrease in chylomicron mass and sequentially in the S_f 100–400 and S_f 20–100 VLDL subclasses; *2*) an increase in IDL and LDL_2; and *3*) a decrease in HDL_2 and HDL_3 during the first 24 hr followed by an increase in both fractions. Catapano et al. (21) using preparative ultracentrifugation observed a reduction in VLDL mass and an increase in LDL and HDL cholesterol up to 24 hr after infusion in an 8-year-old girl with apoC-II deficiency. LPL in apoC-II-deficient patients can also be activated by the infusion of a biologically active synthetic fragment of apoC-II (residues 44–79), establishing that apoC-II alone can correct the metabolic defect in apoC-II deficiency (22).

With heparin infusion it is possible to increase the HL activity in patients with LPL deficiency. Rao et al. (23) used this technique to study the effects of HL activity on

Abbreviations: LPL, lipoprotein lipase; HL, hepatic lipase; d, density; VLDL, very low density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; HDL, high density lipoproteins; apoC-II, apolipoprotein C-II; EDTA, disodium ethylenediaminetetraacetate; S_f, flotation rate expressed in Svedbergs (10^{-13} sec).

plasma lipoproteins in eight subjects with LPL deficiency. Using preparative ultracentrifugation, they observed a VLDL reduction, a reduction of HDL_2 phospholipid and protein concentration, and simultaneously an increase of HDL_3 phospholipid concentration.

None of these studies (19–23) presents a complete protein-lipid composition of plasma lipoproteins, and only in the study performed by Miller et al. (20) were the lipoprotein physical properties analyzed.

The purpose of our study was to analyze the changes in the plasma lipoproteins after LPL and HL activation in two patients with apoC-II deficiency and two patients with LPL deficiency, respectively. To this aim we used ultracentrifugation in a zonal rotor under rate flotation conditions. Our experiments indicate that, in these patients, the LPL activation results in the lipolysis of large VLDL, in the production and modification of LDL, and increases the HDL flotation rate. No HDL_2 production can be directly related to the LPL activation. HL activation produces a reduction of the relative triglyceride content of small VLDL, IDL, LDL_2, and HDL_3 accompanied by an increase of phospholipid in VLDL, and by an increase of cholesteryl ester in IDL, and reduces the HDL flotation rate.

METHODS

Subjects

The four subjects for this study were a brother and sister (Subjects 1 and 2), aged 43 and 41, respectively, who presented with apoC-II deficiency syndrome, and another brother and sister (Subjects 3 and 4), aged 40 and 53, respectively, who presented with LPL deficiency. The clinical aspects and lipoprotein characterization of these patients have been published previously (22, 24). The diagnosis of LPL deficiency was based on clinical findings, serum lipid levels, lipase activities in plasma after heparin, and serum lipid modifications after a fat-free diet.

This study was performed following a 2-week period of isocaloric, mildly fat-restricted diet (20% protein, 55% carbohydrates, 25% fat). The patients were in good health and were taking no medication.

Informed consent was obtained from each patient, and the study protocol was approved by our institutional authorities.

Lipase activation

LPL in apoC-II-deficient patients was activated by the infusion of 200 ml of cross-matched normal human plasma which was free of hepatitis B antigen. The cholesterol and triglyceride levels of the infused plasma were 192 and 104 mg/dl, respectively. The calculated amount of apoC-II infused with the normal plasma in each patient was approximately 10 mg (25). Plasma infusion started at 2 PM and lasted for 30 min.

A baseline blood sample was collected at 8 AM 6 hr prior to the infusion (time = −6 hr). Subsequent blood samples were collected at 2 PM (time = 0 hr), and 8 PM (time = +6 hr), during the second day at 2 PM (time = +24 hr), and during the third, fourth, and fifth day at 10 AM (time = +44, +68, and +92 hr, respectively).

During the experimental period the patients consumed the same diet as the 2 preceding weeks. They had their breakfast at 8:30 AM, lunch at 12:30 PM, and dinner at 7:30 PM.

HL activation in the LPL-deficient patients was obtained by the intravenous injection of 100 U/Kg body weight (7600 U in the male and 6200 U in the female) of sodium heparin (Liquemin, La Roche). The heparin injection was performed after a 14-hr overnight fast. Blood samples were collected immediately before and 20 min after the injection, when HL is reported to be maximally active (2, 3, 14).

Lipid and lipoprotein analysis

Blood samples were collected in 0.01% EDTA and immediately processed. The $d < 1.006$ g/ml lipoprotein fraction (containing chylomicrons and VLDL) was obtained by preparative ultracentrifugation at 40,000 rpm, at 8°C for 18 hr in a solvent density of 1.006 g/ml in a Beckman Ti 50 rotor using a Beckman L5-65 ultracentrifuge.

The subfractionation of lipoproteins of $d < 1.006$ g/ml on the basis of their differing flotation rates was performed in a Ti 14 Beckman zonal rotor as previously described (26). An aliquot (corresponding to 3 ml of plasma) of the $d < 1.006$ g/ml fraction was subjected to zonal ultracentrifugation at 42,000 rpm, 14°C for 45 min in a linear sodium bromide gradient in the density range 1.0–1.15 g/ml. Four VLDL subfractions with different flotation properties were collected according to the reported zonal rotor calibration (26). The $S_f > 200$ (containing chylomicrons and fastest floating VLDL), S_f 100–200, S_f 60–100, and S_f 20–60 fractions were obtained from 0–25, 25–125, 125–175, and 175–325 ml of rotor effluent volume, respectively.

Two aliquots (each corresponding to 9 ml of plasma) of the $d > 1.006$ g/ml fraction isolated by preparative ultracentrifugation were used, respectively, for LDL and HDL analysis in the Ti 14 Beckman zonal rotor as previously described (27). In one aliquot, LDL were subfractionated using a linear density gradient (d = 1.00–1.30 g/ml) formed with sodium bromide at 42,000 rpm, 15°C for 140 min. In the other aliquot, HDL were subfractioned in a sodium bromide nonlinear density gradient (d = 1.00–1.40 g/ml) at 41,000 rpm, 12°C for 24 hr.

The zonal rotor effluent was monitored continuously at 280 nm, and 25-ml fractions were collected. Fractions belonging to the same lipoprotein peak were pooled, and dialyzed exhaustively at 4°C against 100 mM NaCl, 1 mM NaN_3, and 1 mM EDTA, pH 7.6. Lipoprotein fractions were concentrated by ultrafiltration in Amicon cells.

Cholesterol (28), triglyceride (29), phospholipid (30), and protein (31) were measured by standard methods. The reported values are the mean of duplicate determinations. Cholesterol was measured directly in the effluent from the zonal rotor as previously reported (32).

In expressing the percent composition of lipoproteins, the constituents were calculated in weight percent of the total lipoprotein mass. Cholesteryl esters were calculated as the total cholesterol minus free cholesterol times 1.68.

The day preceding the experiment, LPL and HL were quantitated in each subject as described by Krauss, Levy, and Fredrickson (2) using protamine sulfate for LPL inhibition. The assay for LPL was performed with and without 100 μl of normal plasma (containing apoC-II). The LPL activity in the presence of normal plasma in apoC-II-deficient patients was 6.2 (Subject 1) and 5.1 (Subject 2) μmol/ml per hr, while in LPL-deficient patients no LPL activity was found. The HL activity in apoC-II-deficient patients was 21 (Subject 1) and 16 (Subject 2) μmol/ml per hr, and in LPL-deficient patients was 19 (Subject 3) and 12 (Subject 4) μmol/ml per hr. The normal range in our laboratory for LPL and HL is 4–8 and 10–25 μmol/ml per hr, respectively.

RESULTS

LPL activation in subjects with apoC-II deficiency

The infusion of normal human plasma in the two subjects with apoC-II deficiency syndrome produced a reduction of plasma triglycerides, which was evident 24 hr after the infusion. The reduction persisted in Subject 1 (male) throughout the study, while in Subject 2 (female) the concentration gradually rose to baseline values around 68 hr (**Table 1**). At the same time, the cholesterol levels were reduced in the d < 1.006 g/ml fraction in both subjects, while in the d > 1.006 g/ml fraction only Subject 1 presented an increase. The slight apparent cholesterol increase in the d > 1.006 g/ml fraction of Subject 2 could have been due to errors in the ultracentrifugal separation or in the cholesterol measurement.

The modifications of the absorbance profiles from LDL and HDL analysis in zonal rotor differed in Subject 1 in comparison to Subject 2. The LDL absorbance profile of Subject 1 before the infusion showed three different peaks, as already reported in these patients (33) (**Fig. 1**). The IDL peak was present at 135 ml, the LDL_2 peak at 205 ml, and LDL_3 peak at 255 ml effluent volume. Following the plasma infusion, the IDL peak maintained its effluent position at 135 ml indicating a substantial stability of the flotation properties of this class of lipoproteins. However, the LDL_2 and LDL_3 peaks underwent a progressive merging of each other resulting at the 44th hour in a unique LDL_2 peak at 230 ml of effluent volume. In Subject 2, no substantial modification of the LDL absorbance profile was observed during the study period (**Fig. 2**). Modifications of the relative height of lipoprotein absorbance peaks were also observed in both subjects.

Before the plasma infusion, the HDL absorbance profile in both subjects (**Fig. 3** and **Fig. 4**) showed only a broad HDL_3 fraction, which had a peak effluent volume at 295 ml (the entire HDL_3 fraction eluted from 100 to 345 ml) and 280 ml (from 100 to 330 ml) in Subjects 1 and 2, respectively. The peak effluent volume of these HDL_3 is greater than normal HDL_3 (which ranges from 220 to 250 ml) indicating a lower flotation rate of these particles.

Following the plasma infusion, the HDL_3 peak of Subject 1 moved to 235 ml of effluent volume by the 6th

TABLE 1. Lipid levels in plasma and in d < 1.006 g/ml and d > 1.006 g/ml lipoproteins in two patients with apoC-II deficiency syndrome before (time = −6 hr and 0) and after (time = from +6 to +92 hr) the activation of LPL by the infusion of 200 ml of normal human plasma

	Subject 1 (Male)						Subject 2 (Female)					
	Cholesterol			Triglycerides			Cholesterol			Triglycerides		
Time	Plasma	d < 1.006	d > 1.006	Plasma	d < 1.006	d > 1.006	Plasma	d < 1.006	d > 1.006	Plasma	d < 1.006	d > 1.006
hr		*mg/dl*			*mg/dl*			*mg/dl*			*mg/dl*	
−6	288	208	75	2062	1954	68	192	123	63	1392	1302	66
0	290			2186			192			1156		
+6	216	120	104	1278	1271	78	190	126	69	1550	1473	83
+24	214	95	121	590	508	78	136	72	68	765	672	76
+44	224	97	136	600	521	74	135	66	69	905	809	94
+68	208	70	144	427	370	50	182			1210		
+92	236			826								

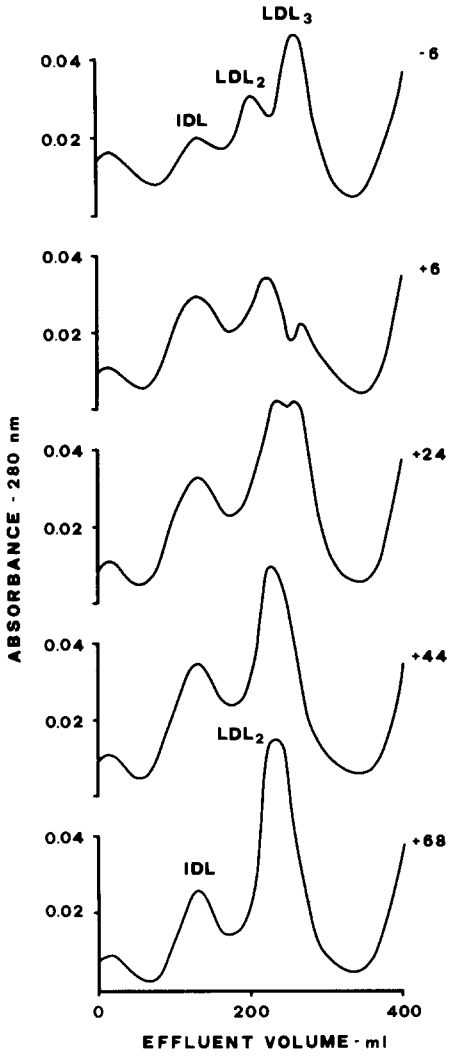

Fig. 1. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a linear density gradient (d = 1.00–1.30 g/ml) at 42,000 rpm, 15°C, for 140 min of d > 1.006 g/ml lipoprotein fraction from a patient with apoC-II deficiency syndrome (Subject 1) before (time = −6 hr) and after (time = from +6 to +68 hr) the activation of LPL by the infusion of 200 ml of normal human plasma.

hour and afterwards returned to its original position (Fig. 3). A similar behavior was observed in the HDL_3 peak of Subject 2. The HDL_3 peak moved to 200 ml of effluent volume by the 6th hour and a "shoulder" developed on the HDL_2 side of the HDL_3 peak. At the 24th hour, a discrete HDL_2 fraction was present (Fig. 4) in this subject as was the case in the preceding analysis (24, 33). This fraction had disappeared by the 44th hour when the HDL profile returned to baseline.

The lipoprotein cholesterol levels of the two subjects were measured in the effluent from the zonal rotor (**Table 2**). The plasma infusion resulted in a reduction of cholesterol of the $S_f > 100$ lipoproteins in both subjects with Subject 1 having a greater reduction. Subject 1 had an increase not seen in Subject 2 in S_f 20–60 lipoprotein cholesterol. The IDL cholesterol concentration increased in both subjects consistent with the increase in peak size (Figs. 1 and 2), while only in Subject 1 did the LDL_2 cholesterol undergo a significant increase. Only marginal variations were observed in HDL cholesterol levels in both subjects.

The protein-lipid composition of the lipoproteins following the infusion is given in **Table 3** and **Table 4.**

HL activation in patients with LPL deficiency

The heparin injection in the two subjects with LPL deficiency produced, after 20 min, a slight reduction of

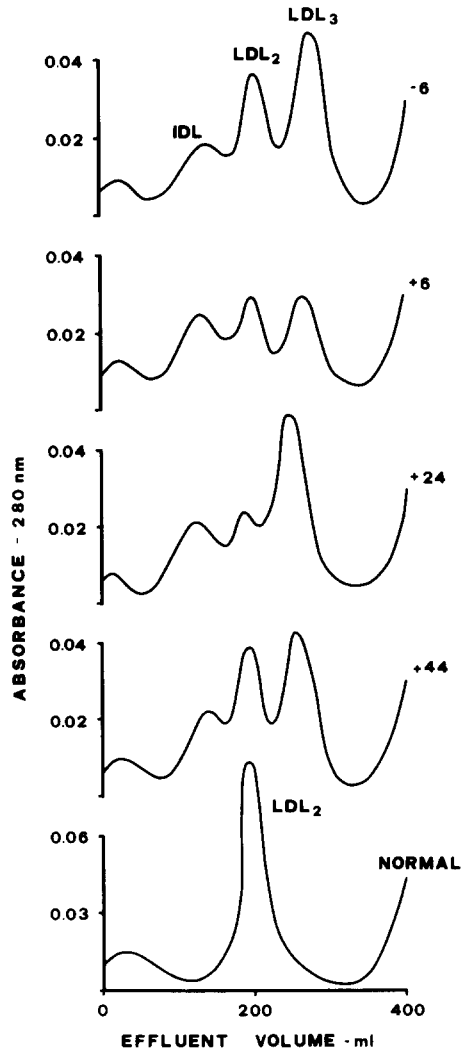

Fig. 2. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a linear density gradient (d = 1.00–1.30 g/ml) at 42,000 rpm, 15°C, for 140 min of d > 1.006 g/ml lipoprotein fraction from a patient with apoC-II deficiency syndrome (Subject 2) before (time = −6 hr) and after (time = from +6 to +44 hr) the activation of LPL by the infusion of 200 ml of normal human plasma. The absorbance profile of a normal subject is included for comparison.

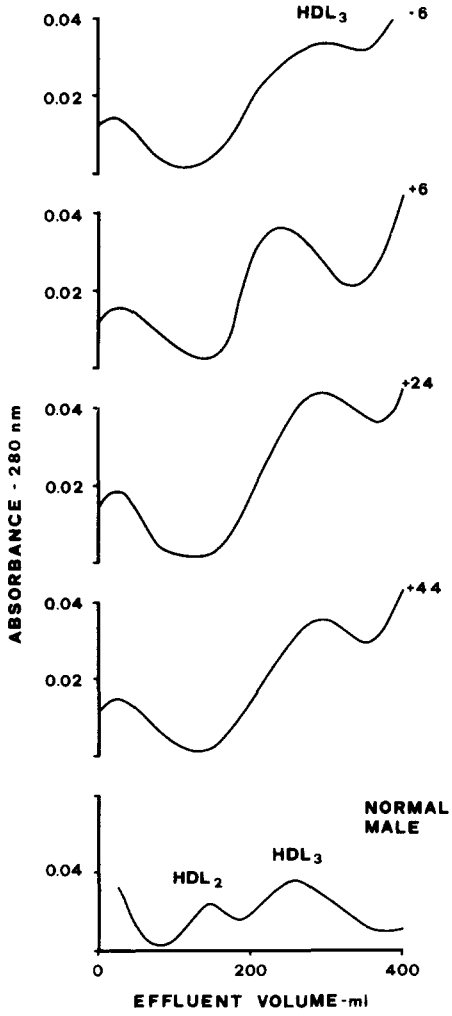

Fig. 3. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a nonlinear density gradient (d = 1.00–1.40 g/ml) at 41,000 rpm, 12°C, for 24 hr of d > 1.006 g/ml lipoprotein fraction from a patient with apoC-II deficiency syndrome (Subject 1) before (time = −6 hr) and after (time = from +6 to +44 hr) the activation of LPL by the infusion of 200 ml of normal human plasma. The absorbance profile of a normal male subject is included for comparison.

the cholesterol plasma levels of $S_f > 100$ particles (**Table 5**).

After heparin injection in both subjects, the LDL absorbance profile (**Fig. 5**) did not present significant variations. The HDL profile showed an increase from 100 to 150 ml in the peak effluent volume of HDL_2 in Subject 4 (female). The HDL_3 peak changed from 260 to 280 ml in Subject 3 (male) and from 235 to 280 ml in Subject 4 (**Fig. 6**) suggesting a reduction of the flotation rates of these lipoproteins. These HDL_3 peaks after heparin were also sharper than the baseline peaks.

The triglyceride content of slow floating VLDL, IDL, LDL_2, and HDL_3 was reduced and accompanied by an increase of phospholipid in VLDL and by an increase of cholesteryl ester in IDL after the heparin injection (**Table 6**).

DISCUSSION

The objectives of these studies were to characterize the changes in the plasma levels and physicochemical properties of the lipoprotein subfractions in two apoC-II-deficient patients following an infusion of normal human plasma and in two LPL-deficient patients following an injection of heparin. The analysis of plasma lipoproteins reported in this study was by ultracentrifugation in a zonal

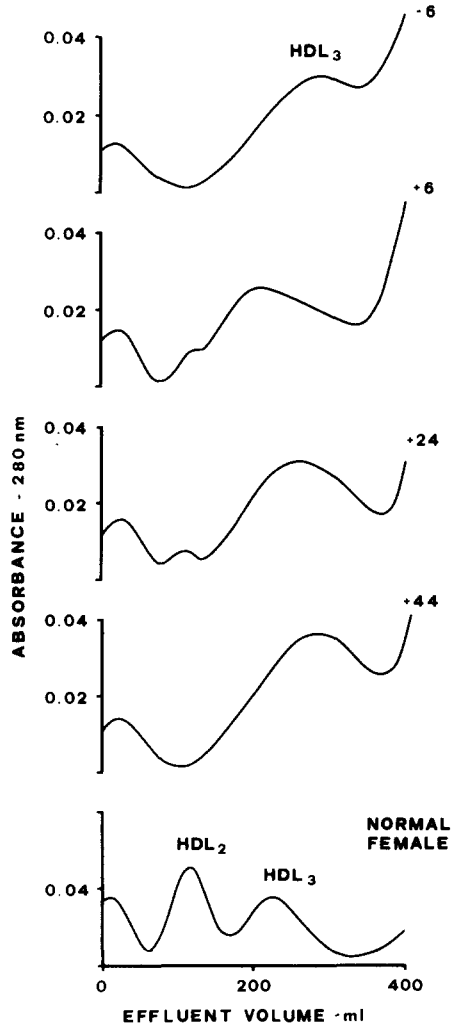

Fig. 4. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a nonlinear density gradient (d = 1.00–1.40 g/ml) at 41,000 rpm, 12°C, for 24 hr of d > 1.006 g/ml lipoprotein fraction from a patient with apoC-II deficiency syndrome (Subject 2) before (time = −6 hr) and after (time = from +6 to +44 hr) the activation of LPL by the infusion of 200 ml of normal human plasma. The absorbance profile of a normal female subject is included for comparison.

TABLE 2. Cholesterol concentrations in lipoprotein fractions isolated by ultracentrifugation in a zonal rotor in two patients with apoC-II deficiency syndrome before (time = −6 hr) and after (time = from +6 to +68 hr) the activation of LPL by the infusion of 200 ml of normal human plasma

	Subject 1 (Male)								Subject 2 (Female)								
	S_f								S_f								
	>200	100–200	60–100	20–60	IDL	LDL_2	LDL_3	HDL_3	>200	100–200	60–100	20–60	IDL	LDL_2	LDL_3	HDL_2	HDL_3
hr	*mg/dl*								*mg/dl*								
−6	84	70	33	21	10	24	27	17	52	39	14	18	7	13	22		19
+6	45	26	15	30	22	43	20	15	73	26	9	18	14	18	21	2	16
+24	8	24	22	41	28	48	25	17	19	21	14	18	13	12	28	1	19
+44	10	29	22	33	39	80		17	17	20	11	17	12	22	20		19
	d < 1.006 g/ml																
+68	70				23	99		14									

rotor under rate flotation conditions. With this technique it is possible, at the same time, to analyze the lipoprotein distribution, to evaluate the flotation properties of these molecules, and to obtain homogeneous fractions on the basis of the absorbance profile. The lipoprotein separation obtained with this method is primarily based on the particle flotation rate. Although this property does not necessarily reflect a physiological meaning, it may be particularly useful when the preparative ultracentrifugation at predetermined densities could result in inaccurate evaluation of lipoprotein distribution (e.g., in disease states or in particular metabolic situations).

From VLDL subclass modifications we observed after LPL activation, we have confirmed that large triglyceride-rich lipoproteins are the preferential substrate for this enzyme as already reported by others (2, 14, 34). When lipase was activated in our patients, there was a shift in distribution of the VLDL subclasses from more buoyant to less buoyant particles. LPL preferentially lipolyzed large particles resulting in the cholesterol lowering seen in $S_f > 100$ subclasses. At the same time the cholesterol concentration in $S_f < 100$ particles was only slightly reduced and newly synthesized particles in $S_f > 100$ did not accumulate in the plasma. However, the S_f 20–60 cholesterol increase observed in apoC-II-deficient Subject 1 is not indicative of an increase in the total lipoprotein mass (calculated as the sum of each lipoprotein constituent) since it is due to a cholesterol enrichment of these molecules after LPL activation. In fact, considering the total lipoprotein mass in the apoC-II-deficient patients,

TABLE 3. Protein-lipid composition (weight percent) of lipoprotein fractions isolated by ultracentrifugation in a zonal rotor in a patient with apoC-II deficiency (Subject 1) before (time = −6 hr) and after (time = from +6 to +68 hr) the activation of LPL by the infusion of 200 ml of normal human plasma

	S_f > 200					S_f 100–200					S_f 60–100					S_f 20–60				
Time	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
hr																				
−6	4	4	80	10	2	8	4	69	15	4	5	6	63	19	7	6	6	56	23	9
+6	4	4	80	10	2	5	5	69	14	7	5	6	61	19	9	6	6	59	21	8
+24	9	6	70	11	4	9	5	64	15	7	9	5	59	20	7	11	6	54	22	7
+44	10	4	70	14	2	8	5	65	16	6	10	5	58	20	7	11	5	57	19	8
	d < 1.006 g/ml																			
+68	8	5	70	13	4															

	IDL					LDL_2					LDL_3					HDL_3				
	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
−6	21	11	28	28	12	28	11	20	20	15	23	9	14	24	30	10	3	8	23	56
+6	23	15	21	27	14	30	15	12	25	18	27	9	13	19	27	14	1	8	22	55
+24	31	8	23	26	12	38	8	11	25	18	35	5	8	20	32	17	1	6	23	53
+44	37	9	20	23	11	43	7	11	21	18						15	1	6	20	58
+68	38	9	19	24	10	44	7	9	22	18										

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TG, triglyceride; PL, phospholipid; PR, protein.

TABLE 4. Protein-lipid composition (weight percent) of lipoprotein fractions isolated by ultracentrifugation in a zonal rotor in a patient with apoC-II deficiency syndrome (Subject 2) before (time = −6 hr) and after (time = from +6 to +44 hr) the activation of LPL by the infusion of 200 ml of normal human plasma

	$S_f > 200$					S_f 100–200					S_f 60–100					S_f 20–60				
Time	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
hr																				
−6	7	3	81	8	1	8	3	76	10	3	8	3	66	17	6	8	3	61	19	9
+6	3	2	87	7	1	2	1	86	8	3	4	3	71	15	7	8	3	62	18	9
+24	6	5	80	8	1	7	5	69	13	6	11	4	56	18	11	13	4	50	21	12
+44	9	4	77	9	1	7	4	74	11	4	9	6	59	17	9	9	5	60	17	9

	IDL					LDL_2					LDL_3					HDL_2					HDL_3				
	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
−6	28	7	26	23	16	29	5	28	23	15	26	3	19	16	36						15	2	14	28	41
+6	27	5	22	24	22	28	6	27	24	15	29	3	14	18	36	15	2	17	30	36	13	1	10	25	51
+24	27	7	28	27	11	29	6	27	22	16	29	4	16	17	34	19	1	7	33	40	14	1	9	27	49
+44	27	9	25	27	12	30	7	25	24	14	39	4	16	20	30						15	2	12	27	46

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TG, triglyceride; PL, phospholipid; PR, protein.

the total mass of $S_f > 100$ lipoproteins was reduced by LPL activation while the S_f 20–100 total mass remained almost unchanged.

These data suggest that, while LPL in chylomicronemic patients is a key enzyme in the regulation of large-sized VLDL plasma levels, the small VLDL are only partially affected by LPL activity. Since in normolipidemic subjects the VLDL fraction is represented mainly by S_f 20–100 particles (31), it could be possible that plasma triglyceride levels in normal subjects are regulated not only by LPL activity but also by other factors (e.g., HL, apoproteins, receptors).

The effects of HL activation on VLDL are in agreement with previously reported results (9, 12, 15, 23). Even though in LPL-deficient patients the heparin-release protocol results in a nonphysiological bulk phase lipolysis of particles that HL does not encounter in the normal situation, our data might suggest that in vivo HL has a triglyceride-hydrolyzing activity on small-sized VLDL. This activity could be partially responsible for the low VLDL levels observed in type I hyperlipoproteinemic patients (17). Moreover, the accumulation of S_f 20–100 lipoproteins in patients with HL deficiency (18) might be related to this HL activity on VLDL of low flotation rate.

The IDL and LDL_2 increase observed in both subjects with apoC-II deficiency after the LPL activation could be accounted for by changes in S_f 20–100 particles, when these particles are rapidly catabolized by LPL in a cascade fashion. However, this is only a possible explanation and we cannot exclude a direct synthesis of IDL and LDL.

It is clear from our results that the density distribution of IDL is very stable and homogeneous, regardless of the lipolytic activities. On the other hand, LDL_2 (and LDL_3) are greatly affected by the LPL activity and/or the plasma triglyceride levels, as already observed (35, 36). The changes in LDL composition after LPL activation could justify their density modification. This relation between lipase activities and LDL might also be important to understand the metabolic basis of LDL heterogeneity. In fact, multiple LDL subpopulations with differing structure

TABLE 5. Lipid levels in plasma, in d < 1.006 g/ml, and d > 1.006 g/ml lipoproteins isolated by preparative ultracentrifugation, and in lipoprotein fractions isolated by ultracentrifugation in a zonal rotor in two patients with LPL deficiency before and 20 min after the activation of HL by the intravenous injection of heparin (100 U/kg body weight)

	Cholesterol			Triglycerides			Cholesterol								
							S_f								
Subject	Plasma	d < 1.006	d > 1.006	Plasma	d < 1.006	d > 1.006	>200	100–200	60–100	20–60	IDL	LDL_2	LDL_3	HDL_2	HDL_3
	mg/dl			*mg/dl*			*mg/dl*								
#3 (Male)															
Before	328	296	35	3375	3180	56	145	102	25	23	3	11	12		9
After	318	285	34	3135	3078	89	138	90	25	29	3	9	13		8
#4 (Female)															
Before	163	112	46	1020	924	92	43	34	17	17	6	8	12	3	16
After	158	111	41	1008	874	92	40	31	16	20	8	8	14	2	10

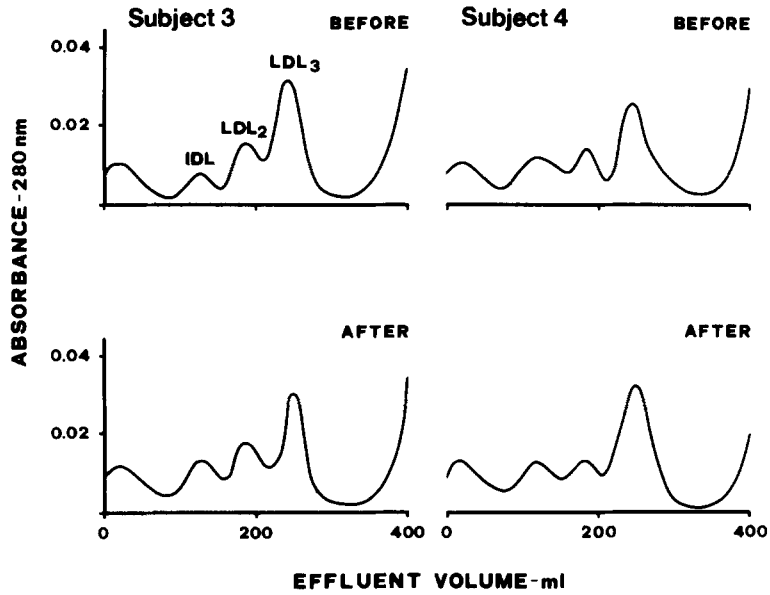

Fig. 5. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a linear density gradient (d = 1.00–1.30 g/ml) at 42,000 rpm, 15°C, for 140 min of d > 1.006 g/ml lipoprotein fraction from two patients with LPL deficiency before and 20 min after the activation of HL by the intravenous injection of heparin (100 U/Kg body weight). Subjects 3 and 4 on the left and right, respectively.

and composition have already been reported in normal (37, 38) and in hyperlipoproteinemic subjects (39–41).

The HL activation in LPL-deficient patients had no effect on LDL density distribution. However, the HL activation resulted in a cholesterol-enrichment and triglyceride-depletion of IDL, suggesting a role for HL in IDL metabolism (9, 12).

Several observations (for review see ref. 42) support the current hypothesis that HDL can be formed in part in plasma as a result of lipolytic activity (43). However, some studies in hypertriglyceridemic patients did not report an increase in HDL cholesterol following triglyceride reduction (44–47). When an increase in HDL was observed, it occurred later than the VLDL reduction and

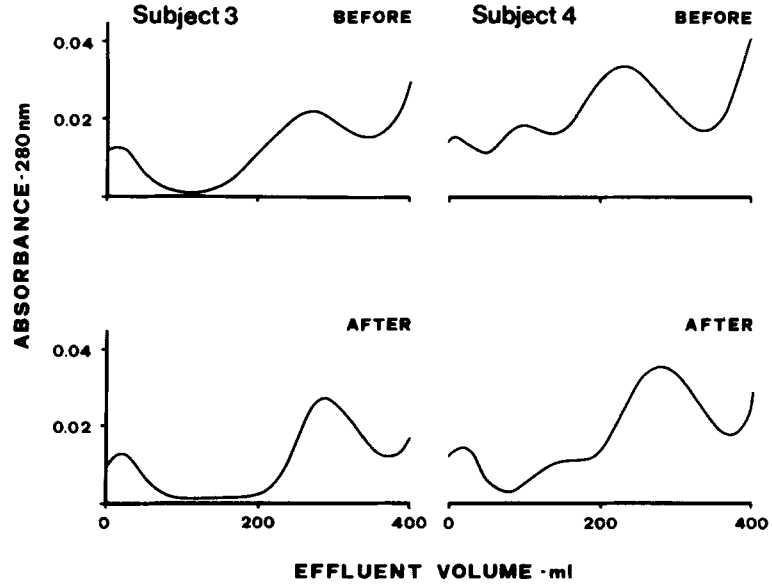

Fig. 6. Absorbance profiles (280 nm) obtained from ultracentrifugation in a zonal rotor using a nonlinear density gradient (d = 1.00–1.40 g/ml) at 41,000 rpm, 12°C, for 24 hr of d > 1.006 g/ml lipoprotein fraction from two patients with LPL deficiency before and 20 min after the activation of HL by the intravenous injection of heparin (100 U/Kg body weight). Subjects 3 and 4 on the left and right, respectively.

TABLE 6. Protein-lipid composition (weight percent) of lipoprotein fractions isolated by ultracentrifugation in a zonal rotor in two patients with LPL deficiency before and 20 min after the activation of HL by the intravenous injection of heparin (100 U/kg body weight)

	$S_f > 200$					$S_f 100–200$					$S_f 60–100$					$S_f 20–60$				
Subject	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
#3																				
Before	5	4	80	8	3	4	4	80	8	4	3	7	68	13	9	7	6	66	12	9
After	5	4	80	8	3	5	4	79	9	3	6	6	66	13	9	7	6	60	16	11
#4																				
Before	10	5	75	7	3	4	9	67	13	7	9	9	53	17	12	9	9	50	19	14
After	12	4	71	10	3	11	10	47	23	9	8	8	46	26	12	8	8	41	31	12

	IDL					LDL_2					LDL_3					HDL_3				
	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR	CE	FC	TG	PL	PR
#3																				
Before	16	11	21	26	26	25	6	19	22	28	21	4	25	19	31	10	2	10	23	56
After	23	9	18	27	23	23	9	14	27	27	21	4	27	19	29	11	2	6	24	57
#4																				
Before	15	9	28	23	25	12	8	28	21	31	14	4	27	18	37	11	2	11	22	54
After	20	8	13	28	21	21	8	17	21	33	19	5	20	19	37	10	3	8	24	55

Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TG, triglyceride; PL, phospholipid; PR, protein.

the LDL increase (48). No significant variations in HDL subclasses based on zonal rotor ultracentrifugation analysis were reported in hypertriglyceridemic patients after treatment (36, 47) even though the LPL activity significantly increased from below normal to the normal range (36).

The in vitro transformation of HDL_3 into HDL_2-like particles during VLDL lipolysis (4) was not observed in vivo. Tall et al. (49) and Groot and Scheek (50) demonstrated, in humans, an increase in mass in both HDL_2 and HDL_3 accompanied by a reduction in density of these particles after fat ingestion. Forte et al. (51), after heparin-induced lipolysis, observed an increment in total HDL mass in two type IV hyperlipoproteinemic patients and a decrease in total HDL mass in a type V patient. At the same time, the HDL flotation rate increased slightly in the type IV and remained almost unchanged in the type V patient.

Our data on HDL subclasses after LPL activation are in agreement with these results. The apoC-II-deficient patients in our study failed to show an increase in HDL during the first 48 hr following plasma infusion, as reported by Miller et al. (20) in similar conditions. Our present findings indicate that the LPL activation in chylomicronemic patients does not produce an immediate increase in HDL levels or an acute transformation of HDL_3 into HDL_2. We did observe significant variations in the flotation properties of HDL, concomitant with LPL activation, suggesting an increase of the flotation rate of HDL subclasses during LPL activity.

The trace amount of HDL_2 lipoproteins observed in Subject 2 after LPL activation can hardly be related to the LPL activity since the triglyceride reduction observed in this subject was far less than that observed in the other apoC-II-deficient subject, and since similar HDL_2 concentrations were sometime present in these type I hyperlipoproteinemic subjects (22, 33). However, we cannot exclude that the data might be consistent with low-level conversion of HDL_3 to HDL_2.

The results of HL activation observed in our study demonstrate that this lipase in LPL-deficient patients reduces the overall flotation rate of HDL. The HL activation produced variations on HDL flotation rates that are opposite to those produced by LPL activation. Thus we confirm that lipase activities modify HDL but this event, at least in our experimental conditions, was not due to immediate HDL production and the HDL flotation modifications were not due to a transformation of HDL_3 into HDL_2 (or vice versa).

With our data, the transient modifications in concentration and composition of individual density classes produced by lipase activation cannot be described in terms of metabolic interconversion of lipoproteins. It is also possible that the effects observed in apoC-II-deficient patients would have been different if normal levels of plasma apoC-II could have been achieved by the infusion of a larger amount of this protein. However, we produced evidence in vivo of some effects of LPL and HL on lipoprotein metabolism. Further studies are needed to definitely clarify the relationships between these two enzymatic activities and plasma lipoproteins.

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