

# Plasma lipoprotein changes resulting from immunologically blocked lipolysis

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**Abstract** The role of lipoprotein lipase (LPL) in the generation of low density lipoprotein (LDL) and high density lipoprotein (HDL) was investigated. Intravenous injections of high titer goat antiserum against highly purified chicken LPL into fasted roosters quantitatively blocks the removal of plasma VLDL triglyceride (1976. *J. Lipid Res.* 17: 498–505). Analyses of the chemical components of lipoproteins after 8 hr of LPL inhibition showed that the very low density lipoprotein (VLDL) concentration increased over 10-fold, while LDL and HDL concentrations decreased by 5-fold and 48%, respectively. LDL and HDL cholesterol levels decreased logarithmically over the 8-hr period, with half-lives of 2.4 and 6 hr, respectively. The composition of these lipoprotein fractions on a percent weight basis changed significantly. Experimental LDL contained 37% less phospholipid, 64% less cholesterol, and 2.3-fold more triglyceride than control LDL. Experimental HDL contained 3.1-fold more triglyceride and 50% less unesterified cholesterol than control HDL. The Stokes' radii of HDL were determined by gel filtration on Biogel A5M and Ultragel AcA 22: the radius of experimental HDL (44.9 Å) was smaller than that of control HDL (55.4 Å). These measurements were confirmed by electron microscopy (43 and 54 Å, respectively). After rate zonal ultracentrifugations of plasma samples, control LDL was clearly resolved, while no LDL could be detected in the experimental samples. Rate zonal ultracentrifugations of plasma samples also indicated that control HDL had a higher flotation rate than experimental HDL. Equilibrium zonal ultracentrifugation showed experimental HDL to be more dense than control HDL with hydrated densities of 1.118 and 1.113 g/ml, respectively. These experiments provide in vivo evidence that LDL is a direct metabolic product of VLDL and that LPL plays a role in the transfer of surface constituents from VLDL to HDL. — Behr, S. R., J. R. Patsch, T. Forte, and A. Bensadoun. Plasma lipoprotein changes resulting from immunologically blocked lipolysis. *J. Lipid Res.* 1981. 22: 443–451.

**Supplementary key words** very low density lipoprotein · low density lipoprotein · high density lipoprotein · lipoprotein lipase

The hydrolysis of plasma VLDL and chylomicron triglycerides leads to the generation of new lipoproteins and to changes in the chemical composition and

physical properties of pre-existing circulating lipoproteins. Although VLDL degradation has not been directly linked to LDL production in vivo, it has been shown in vitro that “LDL-like particles” are formed from VLDL in the presence of milk lipoprotein lipase (1). Furthermore, it has been inferred that all or most LDL apoprotein B is derived from VLDL apoprotein B in normal humans (2). Direct synthesis of LDL by the liver has been demonstrated in hypercholesterolemic rats (3) and in the isolated perfused pig liver (4). Recently, it has been demonstrated that HDL<sub>2</sub>-like particles are formed in vitro during the lipolysis of human VLDL by bovine milk lipoprotein lipase in the presence of human HDL<sub>3</sub> (5). It has also been shown that when chylomicrons, labeled in the phospholipid and apoprotein moieties, are injected intravenously into normal or hepatectomized rats, these components are recovered in the HDL fraction (6, 7).

It has been previously demonstrated (8) that anti-lipoprotein lipase serum injected intravenously in roosters quantitatively blocked the catabolism of VLDL triglyceride. In this report we show that, as VLDL accumulates in roosters as a result of anti-LPL treatment, plasma LDL levels decrease and the chemical composition of LDL changes. Simultaneously, the HDL concentration decreases and the HDL particles become smaller and more dense and accumulate some triglyceride. These results provide in vivo evidence that VLDL is a direct precursor of LDL and that VLDL lipolysis provides components to the HDL density class.

Abbreviations: LPL, lipoprotein lipase; lipoprotein classes and respective densities employed for isolation: VLDL, very low density lipoproteins ( $d < 1.006$  g/ml); LDL, low density lipoproteins (1.006–1.063 g/ml); HDL, high density lipoproteins (1.063–1.21 g/ml).

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### Preparation of anti-lipoprotein lipase serum

Rooster adipose tissue lipoprotein lipase was purified by a method described elsewhere (9). Antisera against homogenous preparations were raised in goats and antibody titers were determined as previously described (8). Immunoglobulin fractions prepared from these antisera quantitatively inhibit partially purified LPL from adipose, heart, and liver tissues (9), but do not inhibit the NaCl-resistant liver lipase (10). Immunodiffusion experiments with purified anti-LPL immunoglobulins showed a single precipitation line against a crude extract of chicken adipose tissue concentrated by ammonium sulfate precipitation. A line of identity was seen between this crude preparation and highly purified LPL (11).

The antisera used in this study contained from 7,000 to 12,000 units/ml of antibody (one unit inhibits the hydrolysis of 1  $\mu$ eq fatty acid/hour). In most experiments, the antiserum and control serum were treated with 2% (W/V) Cab-o-sil for 4 hr at 4°C with occasional gentle shaking. The sera were then centrifuged at 20,000 rpm for 30 min in a Sorvall SS34 rotor. This method removes all lipoproteins from the serum (12). The recovery of anti-LPL titer in the supernatant was over 100%, while only 5% of the cholesterol was recovered in the supernatant. The removal of goat lipoproteins from the antisera eliminated the complicating effect of introducing foreign lipoproteins into the circulation of the rooster.

### Animal experimental procedures

White leghorn roosters weighing between 1.5 and 2.5 kg were fasted overnight. To inhibit LPL activity, 4,000 units of anti-LPL serum per kg of body weight were injected intravenously into the experimental bird. This single dose was followed by hourly injections of 2000 units per kg of body weight for the remainder of the treatment period (usually 8 hr). Previous results have demonstrated that this injection protocol blocks the removal of biologically labeled VLDL (8). Control birds received the same volumes of non-immune goat serum. For time course experiments, approximately 4 ml of blood was obtained from the wing vein at 0, 2, 4 and 6 hr, and by heart puncture at 8 hr. Clotting was prevented by mixing 1 ml of 0.15 M NaCl, 1% EDTA, pH 7.4, with 10 ml of whole blood. Plasma was separated immediately by low speed centrifugation at 4°C. Final concentrations of 0.05% sodium azide and 2 mM *p*-chloromercuriphenylsulfonic acid, an inhibitor of lecithin: cholesterol acyltransferase activity (13), were added to the plasma.

### Ultracentrifugation

For the time course experiments, all centrifugations were done at 4°C in a Beckman 40.3 rotor in 0.5 in.  $\times$  2.5 in. thick cellulose nitrate tubes. VLDL (density < 1.006 g/ml), LDL (density 1.006 to 1.063 g/ml) and HDL (density 1.063 to 1.21 g/ml) were isolated by preparative ultracentrifugation of 2 ml of plasma (14). Two-ml fractions were obtained from the supernatant by aspiration after centrifugation at each successive density. In order to minimize contamination of the LDL fraction with VLDL, the infranatant from the initial ultracentrifugation at density 1.006 g/ml was subjected to a second centrifugation at the same density. Lipoprotein preparations from larger volumes of plasma were spun at 4°C in 1.0 in.  $\times$  3.5 in. polyallomer tubes in a Beckman 60 Ti rotor at the same densities as above. Fractions were separated by slicing the tubes.

### Chemical analyses of lipoprotein constituents

Protein content was determined by the method of Lowry et al. (15). In the case of VLDL, the lipid was extracted with chloroform after the last step of the assay. For LDL and HDL, a variation of the assay of Lowry et al. was employed (16). In later analyses, HDL protein was measured by the Biorad method (17). This assay gave the same results as the modified Lowry assay when bovine serum albumin was used as a standard in the 10–50  $\mu$ g range. Total cholesterol was measured by gas-liquid chromatography. A known amount of beta-sitosterol was added to the sample as an internal standard in an approximate weight ratio with total cholesterol of 1. The sample was then saponified in 5 ml of 2% KOH in 95% ethanol for 1 hr at 60°C. The lipid was extracted in 5 ml of hexane, followed by 5 ml of distilled water (18). The hexane phase was collected, dried under nitrogen, redissolved in a small volume of hexane, and approximately 1  $\mu$ g of cholesterol was injected into a Hewlett Packard 5830 A Gas Chromatograph equipped with a flame ionization detector set at 300°C and dual 6 ft, 2 mm ID glass columns packed with 3% OV17 on Gas Chrom Q. The oven temperature was 260°C, the injection port temperature 300°C, and the flow of carrier nitrogen was 30 ml/min.

Unesterified cholesterol was measured as described except that the saponification step was omitted. This simple method was found to give results identical to a determination of free cholesterol by gas-liquid chromatography after the separation of unesterified cholesterol and cholesteryl esters by thin-layer chromatography. The coefficient of variation for ten determinations of 1  $\mu$ g of cholesterol was 1.2%.

Phospholipid was extracted using chloroform-methanol 2:1 (19) and was measured as phosphorus according to Bartlett (20). Triglyceride was determined by the colorimetric method of Sardesai and Manning (21). The results of the above chemical assays were added for estimating total lipoprotein mass in each density fraction.

### Gel filtration

Equal volumes of plasma from a rooster before antibody injection and from the same rooster after 8 hr of LPL inhibition were adjusted to a density of 1.21 g/ml with solid KBr and were centrifuged at 4°C and 60,000 rpm for 26 hr using a Beckman 60 Ti rotor. The supernatants containing VLDL, LDL, and HDL were collected and concentrated with a YM10 Amicon Membrane. The concentrate was applied to a 1.6 × 88 cm column containing Biogel A5M (BioRad Laboratories, Richmond, CA) equilibrated with 0.15 M NaCl, 1% EDTA, 0.02% NaN<sub>3</sub>, pH 7.4. The void volume of the column was determined with Dextran Blue 2000, and calibration was performed using bovine serum albumin, aldolase, catalase, and ferritin (Pharmacia Fine Chemicals, Uppsala, Sweden). All the gel filtration experiments were repeated using Ultrogel AcA 22 (1.6 × 95 cm) under identical conditions. Individual column eluent fractions were weighed and analyzed for their absorbance at 280 nm.

### Zonal ultracentrifugation

Analyses for VLDL and LDL were performed under rate flotation conditions and, for HDL, under both rate flotation and equilibrium conditions. Sample volumes from experimental and control roosters were kept equal to facilitate comparison. After filling Beckman Ti-14 zonal rotors with appropriate NaBr density gradients using a Beckman 141 gradient maker, ultracentrifugations were performed in Beckman L2-65B ultracentrifuges. The procedural details for loading and unloading the rotors as well as for monitoring the rotor contents by continuously reading the absorbance at 280 nm have been described previously (22).

Analyses for VLDL subfractions were performed employing a linear gradient in the density range 1.00–1.15 g/ml, at a rotor speed of 42,000 rpm at 15°C for 45 min (23). Analyses for LDL were obtained using a linear gradient (1.00–1.30 g/ml) at 42,000 rpm at 15°C for 140 min (22). HDL flotation characteristics were determined by rate zonal ultracentrifugation at 41,000 rpm at 15°C for 22 hr in a non-linear gradient in the density range of 1.00–1.40 g/ml (22). The hydrated densities of HDL fractions were further evaluated by density gradient ultra-

centrifugation under equilibrium conditions (24). At initiation of ultracentrifugation, the gradient was linear with respect to the rotor volume spanning the density range of 1.00–1.20 g/ml. Centrifugations were performed at 35,000 rpm at 20°C for 65 hr. The rotor effluent was monitored continuously at 280 nm and superimposed on a pre-run density gradient pattern determined by refractometry.

### Electron microscopy

Aliquots of control and experimental rooster HDL were dialyzed at 4°C against 0.13 M ammonium acetate buffer containing 345 μM Merthiolate. Lipoproteins were then negatively stained with sodium phosphotungstate as previously described (25) and examined in a JEM 100C (JEOL, Inc., Tokyo, Japan) electron microscope; micrographs were obtained at instrumental magnifications of 60,000 to 80,000×. Lipoprotein sizes were obtained on 150 to 300 free-standing particles by means of a computer program using a sonic digitizer (Science Accessories Corporation Graf-pen, Southport, CT) which records x-y coordinates and translates them into particle diameters.

## RESULTS

Three separate 8-hr time course experiments were performed, each with one experimental and one control rooster. The concentration of the chemical components of each lipoprotein fraction at each time point is expressed as the mean of the three experiments (Figs. 1, 3, and 5). In addition, nine pairs of animals were used to evaluate the compositional changes of each lipoprotein fraction after an 8-hr inhibition of LPL. The chemical compositions of the 8-hr fractions are expressed as the means of several determinations (Table 1).

### VLDL concentration during LPL inhibition

Administration of anti-LPL serum brought about a dramatic increase in plasma VLDL concentration. Fig. 1 illustrates this increase in terms of total cholesterol and triglyceride over an antiserum injection period of 8 hr. As observed previously (6), there is a linear increase of VLDL cholesterol and triglyceride for at least 2.5 hr. This is followed by a lower rate of increase of VLDL cholesterol and triglyceride in the last 4 hr. The rate zonal ultracentrifugal profile of VLDL is shown in Fig. 2. Whereas in the control rooster there is a continuum of particles from S<sub>r</sub> 20 to S<sub>r</sub> 400, the experimental VLDL particles have S<sub>r</sub> values above 120. The chemical composition of VLDL after 8 hr of LPL inhibition is shown in Table 1.



TABLE 1. Concentrations and chemical compositions of rooster plasma VLDL and LDL after 8 hours of inhibition of lipoprotein lipase

	Concentration <i>mg/100 ml</i>	Lipoprotein Composition <sup>a</sup>				n
		Protein	C	TG	PL	
		%	%	%	%	
Exp. VLDL	514	9.4	5.8	79.8	5.3	6
Cont. VLDL <sup>b</sup>	37.5	12.9	13.6	46.1	27.3	2
Exp. LDL	10.5	14.0	13.6	58.8	13.6	1
Cont. LDL	55.3	14.5	38.2	25.6	21.6	1

<sup>a</sup> Figures represent means of n observations. Exp. LDL analyses were conducted on pooled plasma samples from two animals. Lipoprotein concentration was calculated by adding constituents: protein, total cholesterol (C), triglyceride (TG), and phospholipid (PL).

<sup>b</sup> Composition determined previously (8).

The composition at 8 hr was not different from the VLDL composition after 2, 4, and 6 hr.

### LDL changes during LPL inhibition

With respect to LDL, the most striking effect of anti-LPL injections is the five-fold decrease in plasma LDL concentration observed at 8 hr after LPL inhibition. LDL cholesterol declined logarithmically (Fig. 3).

The composition of LDL after 8 hr of LPL inhibition was further investigated as follows. The plasma of two experimental roosters was pooled 8 hr after antiserum injection; the LDL density fraction was obtained by sequential ultracentrifugation and compared to that of a normal rooster. Chemical analyses of this material are reported in Table 1. The total LDL levels decreased from 55.3 mg/100 ml plasma in the normal rooster to 10.5 mg/100 ml plasma in the experimental rooster. On a weight percent basis,

the experimental LDL contained the same amount of protein, while the proportion of cholesterol and phospholipid decreased by 64% and 37%, respectively, and the proportion of triglyceride increased 2.3-fold.

The effect of LPL inhibition was confirmed by rate zonal ultracentrifugation of the  $d < 1.063$  g/ml fractions (Fig. 2) derived from the same volumes of control and experimental plasma and by rate zonal ultracentrifugation of whole plasma (Fig. 4). In these studies (four control and experimental samples analyzed), there was no significant absorbance in the density region where control LDL typically banded.

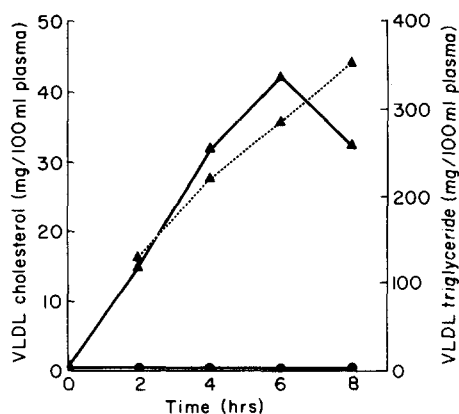


Fig. 1. Effect of anti-LPL serum on plasma VLDL concentration. VLDL cholesterol concentration was determined in plasma from fasted roosters injected with goat anti-LPL serum for 8 hr ( $\blacktriangle$ — $\blacktriangle$ ) or with control goat serum ( $\bullet$ — $\bullet$ ). Plasma triglyceride concentration ( $\blacktriangle$ — $\blacktriangle$ ) was determined only in animals receiving goat anti-LPL serum. Values are means of three separate time course experiments.

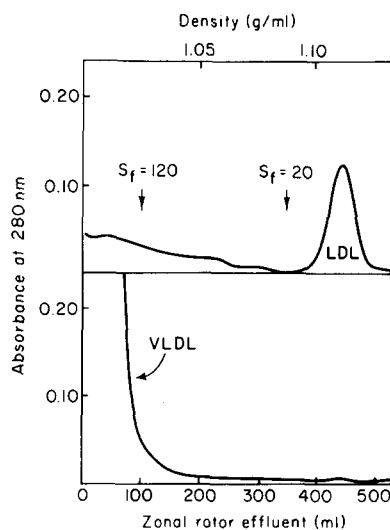
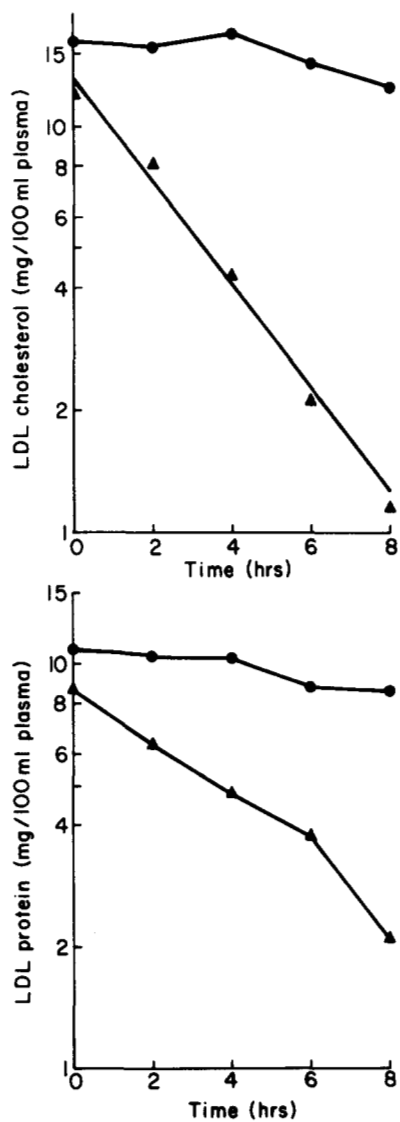


Fig. 2. Rate zonal ultracentrifugal analysis of control plasma (upper panel) and of experimental plasma (lower panel) for VLDL subclasses (17). Fifty ml of plasma from each control and experimental animal were subjected to ultracentrifugation in the Beckman 60 Ti rotor at solvent density of 1.063 g/ml and the top fractions were separated by slicing the polyallomer tubes. These  $d < 1.063$  g/ml lipoproteins were subjected to rate zonal ultracentrifugation at 42,000 rpm for 45 min using a linear gradient in the density range 1.00–1.15 g/ml. Arrows indicate effluent positions for VLDL with  $S_f = 120$  and  $S_f = 20$ , respectively, as obtained from a calibration curve (17).

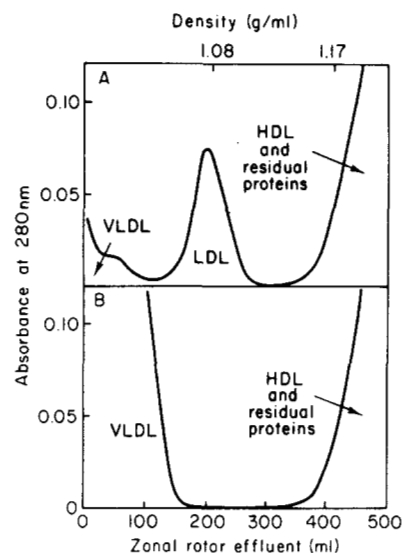


**Fig. 3.** Effect of anti-LPL serum on plasma LDL concentration. LDL cholesterol (top) and protein (bottom) concentrations were determined in fasted roosters injected with goat anti-LPL hourly for 8 hr (▲—▲) or with control goat serum (●—●). Values are means of three separate time course experiments.

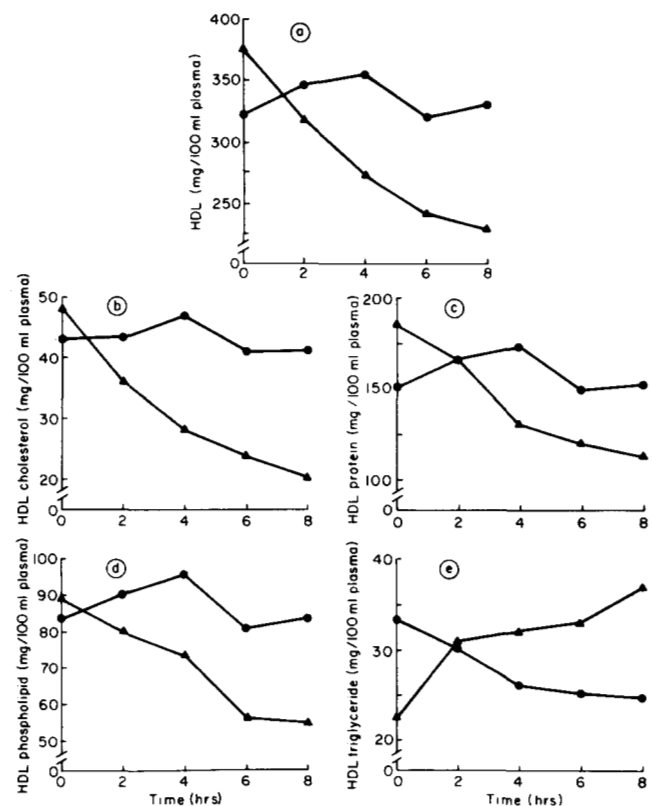
### HDL changes during LPL inhibition

The concentration of HDL in the plasma after 8 hr of LPL inhibition decreased an average of 48% from  $352.9 \pm 73.9$  mg/100 ml plasma (five observations) to  $182.9 \pm 67.6$  mg/100 ml plasma (five observations) (Table 1). The changes in total HDL concentration and in HDL constituent concentrations over time are illustrated in **Fig. 5**. The chemical composition changes in HDL are shown in **Table 2**. Control HDL clearly had more cholesterol and less triglyceride than experimental HDL.

For the analysis of HDL flotation characteristics, equal volumes of experimental and control plasma



**Fig. 4.** Rate zonal ultracentrifugal analysis of control (upper panel) and experimental plasma (lower panel) for LDL. Thirty-six ml of each plasma were directly (without prior ultracentrifugation in the angle head rotor) applied to the Ti 14 zonal rotor and spun in a linear gradient (d 1.00–1.30 g/ml) at 42,000 rpm and 15°C for 140 min (16).



**Fig. 5.** Effect of anti-LPL serum on the plasma HDL concentration. Total HDL (a), HDL cholesterol (b), HDL protein (c), HDL phospholipid (d), and HDL triglyceride (e) concentrations were determined in fasted roosters injected with goat anti-LPL hourly for 8 hr (▲—▲) or with control goat serum (●—●). Values are means of three separate time course experiments.

TABLE 2. Concentrations and chemical compositions of rooster plasma HDL after 8 hours of inhibition of lipoprotein lipase

	HDL Concentration <i>mg/100 ml</i>	HDL Composition <sup>a</sup>				
		Protein	C	TG	PL	C/CE <sup>b</sup>
		%	%	%	%	
Experimental HDL	182.9 ± 67.6	51.3 ± 4.0	10.2 ± 1.1	9.5 ± 1.2	29.1 ± 5.1	0.22 ± 0.03
Control HDL	352.9 ± 73.9	48.7 ± 2.6	13.7 ± 2.4	3.1 ± 0.8	34.5 ± 2.7	0.36 ± 0.06

<sup>a</sup> Figures represent means ± standard deviations of five observations; except for C/CE for which there were four observations.

<sup>b</sup> CE, cholesteryl ester.

were subjected to rate zonal ultracentrifugation (Fig. 6). The control HDL appeared as a single peak, larger than the experimental HDL peak, indicating that the control rooster had a higher concentration of HDL in the plasma. Also the control peak preceded the experimental peak in the rotor effluent profile indicating a higher flotation rate of control HDL. The equilibrium zonal ultracentrifugation of equal starting volumes of plasma clearly resolved symmetrical HDL fractions (Fig. 7). The experimental HDL was present as a smaller broader peak with a mean density of 1.118 g/ml. Control HDL was significantly less dense, at 1.113 g/ml.

### Gel filtration

Concentrated samples of lipoproteins obtained by ultracentrifugation from control and experimental roosters were applied to Biogel A5M and Ultrogel AcA 22 columns. The elution profiles of lipoproteins from a Biogel A5M column are presented in Fig. 8.

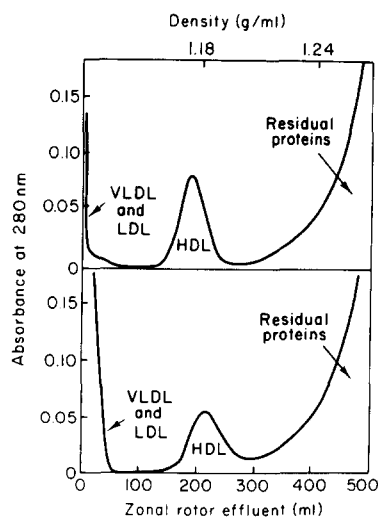


Fig. 6. Rate zonal ultracentrifugal analysis of control (upper panel) and experimental plasma (lower panel) for HDL. Five ml of each plasma were loaded in the Ti 14 zonal rotor and spun in a non-linear gradient in the density range of 1.00–1.40 g/ml (16) and 41,000 rpm and 10°C for 22 hr.

The lipoprotein profile from the control rooster showed a small peak eluting at 56.9 ml and a large HDL peak eluting at 124.8 ml. The experimental lipoprotein profile had a very large VLDL peak superimposing the first control peak and an HDL peak that was somewhat smaller than the control HDL peak and which eluted at 129.9 ml, 5 ml later than the control HDL. These elution volumes correspond to Stokes' radii of 57.3 Å for the control HDL and 43.0 Å for the experimental HDL. The Stokes' radii determined by Ultrogel AcA 22 chromatography were 53.5 Å and 46.7 Å, respectively.

### Electron microscopy

High density lipoproteins from control roosters consisted of round particles with a mean particle radius of 54 Å ± 16 Å S.D. Anti-LPL treated roosters possessed HDL which were similar in morphology to their untreated counterparts, but were 20% smaller in mean particle radius (43 Å ± 10 Å S.D.). The observed electron microscopic size of the HDL particles agrees well with those determined by gel filtration.

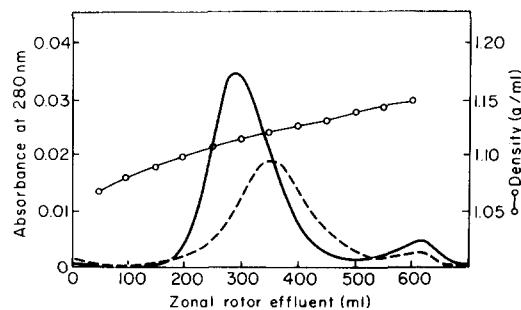


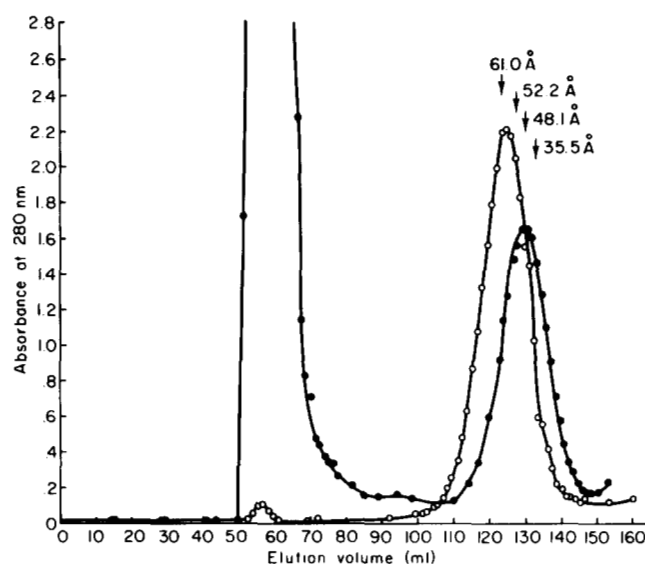
Fig. 7. Ultracentrifugal behavior of control HDL (—) and experimental HDL (---) under equilibrium conditions. Equal amounts of plasma from both control and experimental animals were subjected to ultracentrifugation in an angle head rotor and solvent densities of 1.063 and 1.210 g/ml, respectively, to isolated HDL. Control and experimental HDL were quantitatively applied to a Ti 14 zonal rotor and centrifuged separately using a linear gradient of NaBr in the density range of 1.00–1.20 g/ml under identical conditions: 35,000 rpm, 20°C, 65 hr.

## DISCUSSION

The increased VLDL concentration occurring during blocked lipolysis confirms and extends a previous observation (8) that VLDL triglyceride hydrolysis can be inhibited by anti-LPL serum *in vivo*. The intravenous injection of goat anti-LPL serum caused a linear increase in the concentration of VLDL triglyceride and cholesterol for several hours. The VLDL triglyceride and cholesterol concentrations between 4 and 8 hr did not increase as much as between 0 and 4 hr. Three possible mechanisms may explain this lack of linearity. First, the liver requires a large substrate pool in the synthesis of VLDL. Some of the fatty acids from VLDL triglyceride hydrolysis are returned to the liver; this fatty acid flux is negligible in the absence of LPL activity and therefore the liver fatty acid pool which serves as a precursor to VLDL triglyceride might become depleted and not be sufficient to maintain a constant VLDL triglyceride synthetic rate. Secondly, there may be a negative feedback of VLDL on its own synthesis and secretion in the liver. A recent report (26) demonstrated that proteins such as fatty acid-free bovine serum albumin and ovalbumin, as well as dextrans of different molecular weights, inhibit the synthesis of apoprotein B and the secretion of VLDL from cultured rat hepatocytes. At high concentrations, VLDL might have a potent effect on its own synthesis and secretion. Finally, at high VLDL levels another VLDL clearance pathway may become quantitatively significant. The existence of such a compensatory mechanism for VLDL catabolism has not been previously documented.

It has been shown that LDL-like particles are formed *in vitro* by incubating human VLDL with bovine milk LPL (1). Conversely, the present experiment demonstrated that, in the absence of LPL activity *in vivo*, LDL levels decreased rapidly while VLDL accumulated. Plasma LDL cholesterol decreased by a first order process with a half-life of 2.4 hr. Analyses of several plasma samples, obtained 8 hr after LPL inhibition was initiated, demonstrated that only  $6.9 \pm 0.7\%$  of the control LDL cholesterol remained in the plasma. Rate zonal ultracentrifugation in two different density gradients confirmed the clearance of LDL during LPL inhibition: LDL was clearly resolved in the plasma of control roosters, but could not be detected in plasma from experimental roosters (Figs. 2 and 4).

The inhibition of LPL activity caused a logarithmic decrease in HDL cholesterol with a half-life of 6 hr. Calculations based on the mean values of these separate time course experiments gave the following results: the total cholesterol increase in VLDL was



**Fig. 8.** Gel filtration of lipoproteins from control and anti-LPL treated roosters. Lipoproteins obtained by ultracentrifugation of equal volumes of control (○—○) and anti-LPL (●—●) treated rooster serum adjusted to density 1.21 g/ml were concentrated by ultrafiltration and applied to a  $1.6 \times 88$  cm Biogel A5M column. Column eluates were monitored by absorbance at 280 nm. Standard proteins, bovine serum albumin, aldolase, catalase, and ferritin with Stokes' radii of 61.0, 52.2, 48.1, and 35.5 Å, respectively, had elution volumes as indicated by arrows.

7.9 mg/100 ml plasma during the first hour of inhibition, while the decreases in LDL cholesterol and HDL cholesterol were 3.3 and 4.7 mg/100 ml plasma, respectively, or a total of 8.0 mg/100 ml of cholesterol removed from the plasma. The close correspondence of cholesterol secretion and removal during the first hour of LPL inhibition suggests that in the normal bird both the LDL and the HDL cholesterol originated as VLDL cholesterol which is transferred to the higher density fractions during the hydrolysis of VLDL triglyceride. These calculations are somewhat simplified since they do not consider the transfer of free cholesterol from HDL to nascent VLDL or the free cholesterol secreted in association with nascent HDL.

Both the LDL and the HDL fractions isolated after 8 hr of LPL inhibition had chemical compositions different from that of the control fractions. When the 1.006 to 1.063 g/ml density fraction from a large volume of plasma pooled from two LPL inhibited roosters was isolated by sequential ultracentrifugation and subjected to chemical analyses, the composition resembled that of VLDL (8): the experimental LDL contained much less cholesterol and more triglyceride per mg of LDL protein than the control LDL. These findings are compatible with the results of Sniderman, et al (27) which demonstrate that LDL is modified during its passage through the splanchnic bed in humans: the LDL from blood collected in the hepatic



vein contained 8 mg/100 ml less cholesterol and 7.8 mg/100 ml more triglyceride for the same amount of B protein as aortic LDL. In our experiments, the 8-hr experimental LDL fraction contained 24.6% less cholesterol and 33.2% more triglyceride on a weight percent basis. The large differences between our two LDL fractions may result from multiple passages of LDL particles through the splanchnic bed over the 8-hr experimental period in the absence of LDL production. Alternatively, the direct exchange of LDL cholesteryl ester and VLDL triglyceride by exchange proteins in the plasma compartment might also contribute to the observed compositional modifications of LDL (28).

Similarly to experimental LDL, the experimental HDL contained proportionately less cholesterol and more triglyceride than control HDL. The ratio of free cholesterol to cholesteryl ester decreased from 0.36 to 0.22 over the 8-hr experimental period. The decrease in the percent of total cholesterol of the HDL fraction is mostly due to a decrease in free cholesterol. The higher concentration of lipid surface components in the HDL of control birds might be due to a transfer of these components from the VLDL fraction to the HDL fraction during the lipolysis of VLDL triglyceride. Such a transfer of surface components from VLDL to HDL during lipolysis in vitro has been reported by Patsch et al. (5). When human VLDL and HDL<sub>3</sub> were incubated together in the presence of bovine milk LPL, a new particle similar to native human HDL<sub>2</sub> was formed. This "HDL<sub>2</sub>" fraction contained proportionately more free cholesterol and more phospholipid than HDL<sub>3</sub>. Equilibrium ultracentrifugation showed that the "HDL<sub>2</sub>" particle was less dense than HDL<sub>3</sub>. In the present in vivo studies, the control particles were clearly less dense and larger than the experimental HDL particles (Figs. 7 and 8).

The present studies delineate, in vivo, the effects on all lipoprotein fractions of a highly specific and quantitative blockage of lipolysis. The observed changes in concentration and composition of various lipoprotein fractions are consistent with the hypothesis that lipid and apolipoprotein constituents leaving the surface of triglyceride-rich lipoproteins during their catabolic degradation contribute to the mass of HDL subfractions. In addition, the results support the view that in normolipemic roosters most of the LDL fraction is a catabolic product of VLDL. ■

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## REFERENCES

1. Deckelbaum, R. J., S. Eisenberg, M. Fainaru, Y. Barenholz, and T. Olivecrona. 1979. In vitro production of human plasma low density lipoprotein-like particles. *J. Biol. Chem.* **254**: 6079-6097.
2. Sigurdsson, G., A. Nicoll, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. *J. Clin. Invest.* **56**: 1481-1490.
3. Noel, S-P., L. Nong, P. J. Dolphin, L. Dory, and D. Rubinstein. 1979. Secretion of cholesterol-rich lipoproteins by perfused livers of hypercholesterolemic rats. *J. Clin. Invest.* **64**: 674-683.
4. Nakaya, N., B. H. Chung, J. R. Patsch, and O. D. Taunton. 1977. Synthesis and release of low density lipoproteins by the isolated perfused pig liver. *J. Biol. Chem.* **252**: 7530-7533.
5. Patsch, J. R., A. M. Gotto, Jr., T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein<sub>2</sub>-like particles during lipolysis of very low density lipoprotein in vitro. *Proc. Natl. Acad. Sci. USA.* **75**: 4519-4523.
6. Tall, A. R., P. M. R. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apoproteins in the rat. *J. Clin. Invest.* **64**: 977-989.
7. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in the rat. *J. Clin. Invest.* **64**: 162-171.
8. Kompang, I. P., A. Bensadoun, and M. W. W. Yang. 1976. Effect of an anti-lipoprotein lipase serum on plasma triglyceride removal. *J. Lipid Res.* **17**: 498-505.
9. Bensadoun, A., and I. P. Kompang. 1979. Role of lipoprotein lipase in plasma triglyceride removal. *Federation Proc.* **38**: 2622-2626.
10. Bensadoun, A., and T. L. Koh. 1977. Identification of an adipose tissue-like lipoprotein lipase in perfusates of chicken liver. *J. Lipid Res.* **18**: 768-773.
11. Cheung, A. H., A. Bensadoun, and C. F. Cheng. 1978. Direct solid phase radioimmunoassay for chicken lipoprotein lipase. *Anal. Biochem.* **94**: 346-357.
12. Weinstein, D. B. 1979. A single-step adsorption method for removal of lipoproteins and preparation of cholesterol-free serum. *Circulation.* **59**, **60**: Suppl. II, Abstract 204.
13. Barter, P. J., and J. I. Lally. 1978. The activity of an esterified cholesterol transferring factor in human and rat serum. *Biochim. Biophys. Acta.* **531**: 233-236.
14. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. In *Advances in Lipid Research*. R. Paoletti and D. Kritchevsky, editors. Academic Press, New York. 1-68.
15. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
16. Bensadoun, A., and D. Weinstein. 1976. Assay of proteins in the presence of interfering materials. *Anal. Biochem.* **70**: 241-250.
17. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein



- utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**: 248–254.
18. Thompson, J. N., P. Erdody, R. Brien, and T. K. Murray. Fluorometric determination of vitamin A in human blood and liver. *Biochem. Med.* **5**: 67–89.
  19. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226**: 497–509.
  20. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234**: 466–468.
  21. Sardesai, V., and J. A. Manning. 1968. The determination of triglyceride in plasma and tissues. *Clin. Chem.* **14**: 156–161.
  22. Patsch, J. R., S. Sailer, G. Kostner, F. Sandhofer, A. Holasek, and H. Braunsteiner. 1974. Separation of the main lipoprotein density classes from human plasma by rate-zonal ultracentrifugation. *J. Lipid Res.* **15**: 356–366.
  23. Patsch, W., J. R. Patsch, G. M. Kostner, S. Sailer, and H. Braunsteiner. 1978. Isolation of subfractions of human very low density lipoproteins by zonal ultracentrifugation. *J. Biol. Chem.* **253**: 4911–4915.
  24. Patsch, J. R., K. C. Aune, A. M. Gotto, Jr., and J. D. Morrisett. 1977. Isolation, chemical characterization, and biophysical properties of three different abnormal lipoproteins: LP-X, LP-X<sub>2</sub>, and LP-X<sub>3</sub>. *J. Biol. Chem.* **252**: 2113–2120.
  25. Forte, T., K. R. Norum, J. A. Glomset, and A. V. Nichols. 1971. Plasma lipoproteins in familial lecithin: cholesterol acyltransferase deficiency: structure of low and high density lipoproteins revealed by electron microscopy. *J. Clin. Invest.* **50**: 1141–1148.
  26. Davis, R. A., S. C. Engelhorn, D. B. Weinstein, and D. Steinberg. 1980. Very low density lipoprotein secretion by cultured rat hepatocytes: inhibition by albumin and other macromolecules. *J. Biol. Chem.* **255**: 2039–2045.
  27. Sniderman, A., D. Thomas, D. Marpole, and B. Teng. 1978. Low density lipoprotein: a metabolic pathway for return of cholesterol to the splanchnic bed. *J. Clin. Invest.* **61**: 867–873.
  28. Barter, P. J., J. I. Lally, and D. Wattchow. 1979. Metabolism of triglyceride in rabbit plasma low and high density lipoproteins: studies in vivo and in vitro. *Metabolism.* **28**: 614–618.