A novel explanation for the reduced LDL cholesterol in severe hypertriglyceridemia

Anne Minnich, Børge G. Nordestgaard,[1] and Donald B. Zilversmit[2]

Division of Nutritional Sciences and Section of Biochemistry, Molecular and Cell Biology, Division of Biological Sciences, Cornell University, Ithaca, NY 14853

Abstract When [^{3}H]cholesteryl ester-labeled low density (LDL) and intermediate density lipoproteins (IDL) from a normotriglyceridemic, hypercholesterolemic rabbit were injected into severely hypertriglyceridemic, hypercholesterolemic rabbits, 60% of the label appeared in very low density lipoproteins (VLDL) at 3 hr. A similar experiment showed that 40% of injected ^{131}I-protein-labeled LDL appeared in the IDL fraction at 4 hr. Taken together, these data suggest that the exchange of LDL cholesteryl ester for VLDL triglyceride results in a density shift of injected LDL to the IDL density range. Furthermore, the percent of injected ^{131}I-labeled LDL from normotriglyceridemic rabbits that appeared in the IDL fraction increased in rabbits with increasing levels of plasma triglyceride. This LDL density shift was reproduced in vitro by incubating iodinated LDL from normotriglyceridemic, hypercholesterolemic rabbits with concentrations of VLDL from hypertriglyceridemic, hypercholesterolemic rabbits similar to those in plasma. With such a system, it was shown that the percentage of LDL that appeared in the IDL fraction increased with time, was enhanced fourfold by the addition of plasma lipid transfer protein, increased with increasing molar ratio of triglyceride to cholesteryl ester in VLDL, but apparently did not increase with increasing VLDL particle number. These studies suggest that a pronounced decrease in density of lipoproteins that would normally appear in the LDL density range, resulting from loss of cholesteryl ester in exchange for VLDL triglyceride, may explain, at least in part, the reduced LDL levels in severe hypertriglyceridemia. —**Minnich, A.; B. G. Nordestgaard and D. B. Zilversmit.** A novel explanation for the reduced LDL cholesterol in severe hypertriglyceridemia. *J. Lipid Res.* 1989. **30:** 347–355.

Supplementary key words alloxan-diabetic rabbit • hypercholesterolemia • hyperlipoproteinemia • LDL density shift

LDL cholesterol is markedly reduced in severe forms of human hypertriglyceridemia, such as lipoprotein lipase deficiency (1–3), apolipoprotein C-II deficiency (2–5), and type V hyperlipoproteinemia (3, 6, 7). Severe hypertriglyceridemia (5,500 mg/dl) in alloxan-diabetic rabbits fed a cholesterol- and triglyceride-enriched diet is also associated with low LDL as well as IDL cholesterol levels (8). Thus, the diabetic, hypertriglyceridemic, hypercholesterolemic rabbit may be an appropriate animal model in which to study a mechanism explaining the low LDL cholesterol concentration observed in severe hypertriglyceridemia.

The low LDL cholesterol levels in severe hypertriglyceridemia may be a result of the continual exchange of VLDL triglyceride for LDL cholesteryl ester (9, 10) in species with plasma lipid transfer activity (11, 12). However, the present study reports a new finding that suggests an additional means by which LDL may be diminished. Upon injection into hypertriglyceridemic, hypercholesterolemic rabbits, iodinated LDL from normotriglyceridemic, hypercholesterolemic rabbits appeared in the IDL density range. This shift in density of injected LDL is a likely consequence of the exchange of LDL cholesteryl ester for VLDL triglyceride (13, 14), as suggested by the extent and rate of cholesteryl ester transfer out of LDL and IDL observed in vivo in the hypertriglyceridemic, hypercholesterolemic rabbit. With an in vitro system that models these in vivo phenomena, we attempted to identify factors that are important in determining the extent of the appearance of labeled LDL in fractions of lower densities.

MATERIALS AND METHODS

Materials

Alloxan monohydrate, chloramphenicol, gentamicin sulfate, ϵ-amino-n-caproic acid, benzamidine, aprotinin, cholesteryl methyl ether, and triheptadecanoin were purchased from Sigma (St. Louis, MO). PPO and POPOP were from Eastman Kodak Company (Rochester, NY). Bovine albumin was from Miles Laboratories, Inc. (Pen-

Abbreviations: HDL, high density lipoproteins; LDL, low density lipoproteins; IDL, intermediate density lipoproteins; VLDL, very low density lipoproteins.

[1]Present address: Department of Chemical Pathology and Metabolic Disorders, St. Thomas' Hospital, London SE1 7EH, United Kingdom.

[2]To whom reprint requests should be addressed at: Division of Nutritional Sciences, Savage Hall, Cornell University, Ithaca, NY 14853.

tex Division, Naperville, IL). Cholesterol was from ICN Biochemicals, (Cleveland, OH). NPH insulin was from Lilly (Indianapolis, IN). Dextrostix were from Miles Laboratories, Inc. (Ames Division, Elkhart, IN). Glucose (Cat. No. 124001) and triglyceride (Cat. No. 701912) enzymatic assay reagents were from Boehringer Mannheim Diagnostics (Indianapolis, IN). [1,2(n)^{3}H]Cholesterol, ^{131}I, and ^{125}I were purchased from Amersham (Arlington Heights, IL), and [1,2,6,7-^{3}H(N)]cholesteryl oleate from DuPont NEN Research Products (Boston, MA). PD-10 disposable gel filtration columns (Sephadex G-25M) were obtained from Pharmacia (Piscataway, NJ). Centricon 30 microconcentrators were purchased from Amicon (Danvers, MA) and 30k CentriCell centrifugal ultrafilters were from Polysciences, Inc. (Warrington, PA). Filters (0.22 μm) were from Millipore Corporation (Bedford, MA). Pre-coated thin-layer chromatography plates (silica gel 60) were purchased from EM Science (Cherry Hill, NJ).

Animals

Female New Zealand White rabbits (Becken Research Animal Farm, Sanborn, NY) weighing 2–4 kg were housed separately and maintained on 100 g/day Purina Rabbit Laboratory Chow (Ralston Purina Co., St. Louis, MO). To induce hypertriglyceridemia secondary to diabetes, alloxan monohydrate (150–200 mg/kg body weight) as a 5% solution in 0.9% NaCl was infused intravenously at a rate of approximately 1 ml/min. Animals with resulting fasting plasma glucose $\geq$ 250 mg/dl, as measured enzymatically (glucose oxidase, horseradish peroxidase) or with Dextrostix, were considered diabetic. Some rabbits required repeated infusions with increasing doses of alloxan before they became diabetic. In order to achieve differences in plasma triglyceride in the hypertriglyceridemic rabbits, different daily doses of insulin (0–13 units) were given to the different diabetic rabbits prior to the experiments.

Both diabetic and nondiabetic animals were placed on diets (100 g/day) containing 0.5% cholesterol and either 2.5% Wesson oil or 7.5% Mazola corn oil 2–15 weeks before the experiments. Diabetic rabbits were fed an additional 50–100 g/day unaltered chow to prevent excessive weight loss. Unless otherwise noted, blood was collected into Na_2EDTA (1.5 mg/ml) and NaN_3 (4 mg/ml) and kept on ice until plasma was isolated at 4°C. All experimental protocols were in accordance with University guidelines.

Isolation and labeling of lipoproteins

LDL was isolated at 4°C from blood containing Na_2EDTA (2 mg/ml), chloramphenicol (40 μg/ml), gentamicin sulfate (0.1 mg/ml), ϵ-amino-n-caproic acid (2.6 mg/ml), benzamidine (10 μg/ml), and aprotinin (10 kallikrein units/ml) or Na_2EDTA (1.5 mg/ml) and NaN_3 (4 mg/ml) by sequential ultracentrifugation in solvent densities of 1.019 and 1.063 g/ml in a Beckman 65 rotor (Beckman Instruments, Inc., Palo Alto, CA) for 2.1 $\times$ 10^8 g_{av} · min, or in a Beckman 60Ti rotor for 2.8 $\times$ 10^8 g_{av} · min. Similarly, IDL was isolated between the densities 1.006 and 1.019 g/ml. Fractions were collected by tube slicing.

For in vivo experiments, LDL and IDL were labeled with 5 μCi [^{3}H]cholesteryl ester (>97% pure) by the liposome method of Hough and Zilversmit (15). For in vitro experiments, [^{3}H]cholesteryl ester-labeled LDL and IDL were obtained from plasma collected 18 hr following oral administration of 290 μCi [^{3}H]cholesterol (>95% pure) to a hypercholesterolemic donor animal (16). On the average, 80% of the label was in cholesteryl ester.

LDL and IDL were iodinated by the iodine monochloride method of McFarlane (17) at pH 10 in order to minimize lipid labeling (18). For in vivo experiments, 0.5–5.0 mCi ^{125}I or 1.0–1.6 mCi ^{131}I was used to label 2–13 mg LDL protein. For in vitro experiments, 20–100 μCi ^{131}I was used to label 15–58 mg LDL or IDL protein. Unreacted iodine was removed by PD-10 gel filtration and subsequent dialysis at 4°C against phosphate-buffered saline (1.14% $Na_2HPO_4 \cdot 7\text{-}H_2O$, 0.10% $NaH_2PO_4 \cdot H_2O$, 0.53% NaCl, 0.01% Na_2EDTA, 0.02% NaN_3, pH 7.4) if necessary. Labeling efficiency averaged 21% and 12% for ^{125}I and ^{131}I, respectively, which converts to 1.5 and 0.9 mole of I per mole of LDL, respectively. For labeled LDL, this procedure consistently resulted in >95% trichloroacetic acid precipitability and <7% lipid extractability of label with chloroform–methanol 2:1 (v/v). For labeled IDL, 90% of iodine precipitated with trichloroacetic acid and 73% of iodine precipitated with apolipoprotein B (19). For in vivo experiments, labeled lipoproteins were passed through 0.22-μm filters immediately before injection into recipient rabbits.

In vivo experiments

In eight pairs of rabbits, LDL from a diabetic, hypertriglyceridemic, cholesterol-fed and from a normal, cholesterol-fed rabbit were isolated simultaneously. LDL from the hypertriglyceridemic and from the normal rabbits were iodinated with ^{131}I and ^{125}I, respectively, or vice versa, and mixed. Aliquots of this dose were injected intravenously into both rabbits. Two additional hypertriglyceridemic cholesterol-fed rabbits were injected with iodinated LDL from a normal, cholesterol-fed rabbit. Blood samples were drawn at specified times for 3–6 hr. Lipoproteins from plasma and the injected dose with added carrier plasma were isolated at 10°C by sequential ultracentrifugation in a Beckman 50.3 Ti rotor for 1.4 $\times$ 10^8 g_{av} · min at solvent densities of 1.006, 1.019, and 1.063 g/ml. Lipoprotein fractions were dialyzed overnight at 4°C against 0.9% NaCl before counting in a Beckman Gamma 8000 counter. Ultracentrifugation recoveries were 84 $\pm$ 1% (n = 11). Plasma triglycerides were mea-

sured enzymatically (lipase, glycerol kinase, glycerol phosphate oxidase, and peroxidase). Most rabbits injected with iodinated LDL were also used to study arterial uptake of LDL, which will be reported in another paper.

Three additional hypertriglyceridemic, cholesterol-fed rabbits were injected intravenously with [^{3}H]cholesteryl ester-labeled LDL + IDL from a normal cholesterol-fed rabbit. VLDL, IDL, and LDL + HDL were isolated from blood samples drawn at specified times, and lipid extracts (20) of these fractions were dried under N_2 and assayed for ^{3}H in toluene-based scintillant (14.2 g PPO, 105 mg POPOP, 20 ml ethanol per liter) in a Beckman LS 8100 scintillation counter. Ultracentrifugation recoveries were 93 ± 3% (n = 6).

In vitro experiments

Experiments were performed in which labeled LDL or IDL from normal, cholesterol-fed rabbits was incubated with VLDL from hypertriglyceridemic, cholesterol-fed rabbits, in the presence or absence of plasma lipid transfer protein. The pooled d > 1.21 g/ml fractions from plasma from hypertriglyceridemic and normal cholesterol-fed rabbits, after dialysis against 0.9% NaCl, were used as a source of lipid transfer protein. To achieve a final composition in the incubation mixture similar to that in hypertriglyceridemic rabbit plasma, components were concentrated by ultracentrifugation and/or ultrafiltration. Fractions were kept on ice before being mixed and subsequently incubated at 37°C. For every set of incubations, an incubation containing an equal volume of phosphate-buffered saline in place of transfer protein was included. Aliquots were taken from the incubation at various time points from 0 to 6 hr, immediately placed on ice, and sequentially ultracentrifuged at 1.006 and 1.019 g/ml as described above. For density gradient ultracentrifugation, the solvent density of aliquots containing up to 1 mg lipid was raised to 1.054 g/ml. Density gradients were constructed by successive overlaying with 2.3 ml each of NaCl-solutions of densities 1.040, 1.030, 1.019, 1.010, 1.006, and 1.000 g/ml. Gradients were centrifuged for 5.5 × 10^8 g_{av} · min at 20°C in a Beckman SW 41 rotor. Fractions were collected by aspiration and their densities were determined gravimetrically.

Aliquots of the VLDL, IDL, and LDL fractions with added carrier plasma, after standing overnight in 15 volumes of chloroform–methanol 1:1 (v/v), were centrifuged to pellet the protein. The supernatant was removed, made to 2:1 (v/v) chloroform–methanol, and washed with 0.2 volumes water. Lipid extracts were dried under N_2 and assayed for iodine radioactivity simultaneously with the protein pellets and water washes. The distribution of iodine radioactivity between protein pellets, lipids, and water washes was 86 ± 0.3%, 3 ± 0.0%, and 11 ± 0.3% (n = 36), respectively. In cases where LDL was labeled in cholesterol as well as in protein, lipid extracts (20) of additional aliquots were applied to thin-layer chromatography plates to separate free and esterified cholesterol (see below) and radioactivity in esterified cholesterol was determined as described. For experiments with ^{131}I-labeled IDL, iodine radioactivity was determined on apolipoprotein B precipitates (19) of plasma fractions.

Chemical determinations

Except where otherwise noted, mass analyses of plasma and lipoprotein fractions were performed on lipid extracts (20) which were dried under N_2, applied to thin-layer chromatography plates, and developed in hexane–diethyl ether–acetic acid 80:20:1 (v/v/v). Free and esterified cholesterol were eluted from the silica gel with chloroform–methanol 9:1 (v/v). These eluates were dried under N_2 and saponified (21). Subsequently, cholesterol was determined by the method of Zak et al. (22) or by gas–liquid chromatography on 10-m fused silica capillary columns (Hewlett Packard HP-17), 0.53 mm internal diameter at 260°C with N_2 carrier flow rate of 10 ml/min and cholesteryl methyl ether as an internal standard. Triheptadecanoin was added as a triglyceride internal standard before thin-layer chromatography and the triglyceride-containing silica gel bands were transmethylated in 4 ml 2% H_2SO_4 in anhydrous methanol for 1 hr at 70°C. Fatty acid methyl esters were extracted into 4 ml of hexane after addition of 4 ml of water, and analyzed by gas–liquid chromatography at 170°C as described. Apolipoprotein B was precipitated by the method of Klein and Zilversmit (19) and protein was measured by the method of Lowry et al. (23) with dry bovine serum albumin as a standard.

RESULTS

Plasma triglyceride and lipoprotein cholesterol

Induction of diabetes in rabbits and subsequent addition of 0.5 g/day cholesterol and 2.5 or 7.5 g/day triglyceride to the diet resulted in plasma triglyceride concentrations from 100 to 16,000 mg/dl. Nondiabetic rabbits were fed a diet containing 0.5% cholesterol and, hence, were also hypercholesterolemic. In diabetic rabbits with plasma triglyceride averaging approximately 6,000 mg/dl, LDL + HDL and IDL cholesterol levels were 6% and 10%, respectively, of those in normal cholesterol-fed rabbits, all of which were normotriglyceridemic (**Table 1**). Since HDL cholesterol in hypercholesterolemic rabbits typically averages <5% of cholesterol in the HDL + LDL fraction (24), the relative decrease of LDL + HDL cholesterol represents mainly a decrease in LDL cholesterol. Also apparent from Table 1 is that LDL + HDL and IDL cholesterol levels are similar in diabetic, mildly hypertriglyceridemic, and normal, cholesterol-fed rabbits. This observation diminishes the possibility that some factor of diabetes other than hypertriglyceridemia is responsible

TABLE 1. Lipoprotein lipids in diabetic and normal rabbits after 2 weeks of cholesterol and triglyceride feeding

	Plasma Triglyceride	Total Cholesterol		
		VLDL	IDL	LDL + HDL
	mg/dl	*mg/dl (% esterified cholesterol)*		
Diabetics (n = 6)	6254 ± 1021	1193 ± 253 (59 ± 2)	23 ± 3 (58 ± 4)	21 ± 6 (68 ± 3)
Diabetics (n = 4)	146 ± 37	385 ± 108 (82 ± 3)	271 ± 115 (74 ± 1)	293 ± 90 (70 ± 2)
Normals (n = 5)	22 ± 3	247 ± 71 (80 ± 1)	223 ± 37 (69 ± 3)	346 ± 19 (73 ± 3)

Values are expressed as means ± standard error.

for the low IDL and LDL + HDL cholesterol levels in the hypertriglyceridemic rabbits. In another group of six diabetic rabbits, inverse relationships between plasma triglyceride and cholesterol content of IDL and LDL were demonstrated (25).

In vivo experiments

The low LDL and IDL cholesterol levels in hypertriglyceridemic rabbits may be due to exchange of IDL and LDL cholesteryl ester for VLDL triglyceride. When IDL + LDL from a normal cholesterol-fed rabbit, labeled in the cholesteryl ester moiety, were injected intravenously into three hypertriglyceridemic rabbits, labeled cholesteryl ester moved from IDL and LDL into VLDL (**Fig. 1**). At 3 hr, 64% of the injected label appeared in VLDL, and only 11% and 5% remained in IDL and LDL, respectively. Thus, rapid transfer of lipoprotein core lipids occurs in plasma of diabetic, hypertriglyceridemic, hypercholesterolemic rabbits.

When iodinated LDL from a normal, cholesterol-fed rabbit was injected intravenously into a hypertriglyceridemic, cholesterol-fed rabbit, label disappeared from the LDL fraction and appeared in the IDL fraction (**Fig. 2**, left panel). After 4 hr, 40% of the label had appeared in the IDL fraction. Since approximately 90% of the label in LDL is in apolipoprotein B (data not shown), which is nonexchangeable, movement of iodine label represents a density shift of the whole LDL particle to the IDL density range. It is likely that the shift in density of LDL from normal, cholesterol-fed rabbits to the IDL fraction results from the exchange of LDL cholesteryl ester for VLDL triglyceride. If so, a similar density shift of injected LDL from the hypertriglyceridemic donor (autologous LDL) would not be expected, since the core lipid composition of this LDL had presumably already equilibrated with that of VLDL prior to reinjection into the recipient rabbit. In fact, LDL from the hypertriglyceridemic donor remained in the LDL fraction when reinjected (Fig. 2, left panel). Neither LDL preparation showed any transfer of label to the IDL fraction when injected into a normal, cholesterol-fed rabbit (Fig. 2, right panel). Disappearance of total label from plasma was similar for both types of LDL in the hypertriglyceridemic rabbit as well as in the normal, cholesterol-fed rabbit. Not more than a total of 12% of the injected label appeared in HDL or VLDL.

In similar experiments, the percent of labeled LDL from normal, cholesterol-fed rabbits remaining in the LDL fraction 3 hr after injection decreased with increasing plasma triglyceride in recipient rabbits (**Fig. 3**). This decrease represents an increase in the appearance of labeled LDL in the IDL fraction (data not shown). It is also apparent from Fig. 3 that injected autologous LDL remained in the LDL fraction in rabbits with a wide range of plasma triglyceride levels. Neither LDL preparation left the LDL fraction when injected into normal, cholesterol-fed rabbits (data not shown). Whether the increasing percent of labeled LDL from normal, cholesterol-

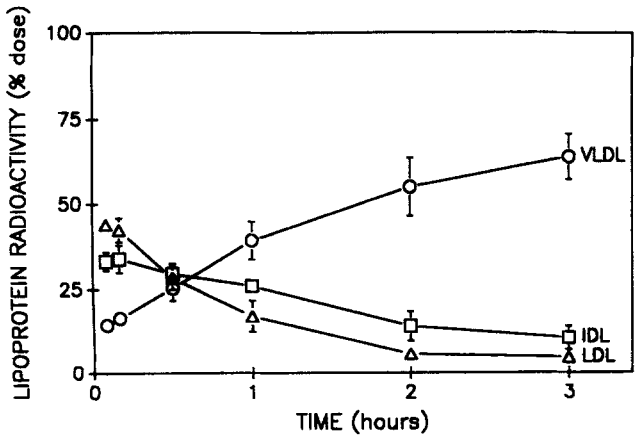

Fig. 1. In vivo cholesteryl ester transfer from IDL and LDL to VLDL versus time. IDL + LDL were obtained from a normal, cholesterol-fed rabbit, labeled with liposomes containing [^{3}H]cholesteryl ester, and injected into hypertriglyceridemic, cholesterol-fed rabbits (VLDL triglyceride 7693 ± 569 mg/dl, n = 3). Values are shown as means ± standard error.

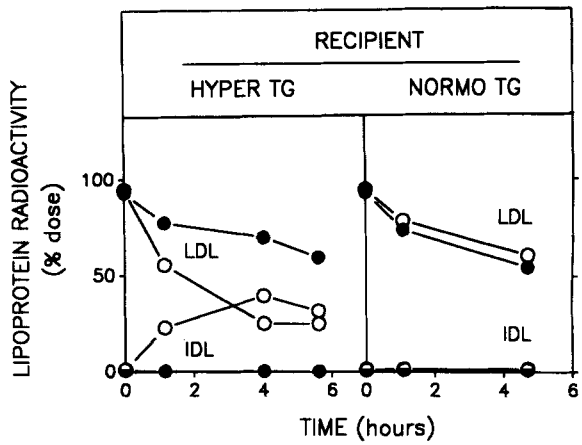

Fig. 2. Behavior of injected iodinated LDL in rabbit plasma. LDL from two cholesterol-fed rabbits: one hypertriglyceridemic (●) and one normal (○), were iodinated with ^{125}I and ^{131}I, respectively. A mixture of the iodinated LDL preparations was injected intravenously into the same two rabbits. Plasma triglyceride was 8,800 and 80 mg/dl in the hypertriglyceridemic (HYPER TG) and normal rabbit (NORMO TG), respectively.

fed rabbits that appeared in lower density fractions was attributable to increments in the recipients' plasma triglyceride, per se, or to other factors that change in parallel with plasma triglyceride, cannot be concluded from these in vivo studies. Two such potential factors are the ratio of triglyceride to cholesteryl ester in VLDL, since the ratio of triglyceride to total cholesterol increased in rabbits with increasing hypertriglyceridemia (data not shown), and VLDL particle number. These possibilities were studied with an in vitro system, as described below.

In vitro experiments

In order to identify factors that influence the density shift of LDL, the in vivo experiments were modelled with an in vitro system (**Fig. 4**). When LDL from normal, cholesterol-fed rabbits was incubated with VLDL from hypertriglyceridemic rabbits, 90% of labeled cholesteryl ester left the LDL fraction and 75% appeared in the VLDL fraction (upper panel). Similarly, during 6 hr, 70% of the protein label, which traces the behavior of whole LDL particles, left the LDL fraction, but in contrast to labeled cholesteryl ester, appeared mainly in the IDL fraction (lower panel).

Four similar incubations in which doubly labeled IDL from normotriglyceridemic rabbits was incubated with triglyceride-rich VLDL were also performed. In a representative 6-hr incubation (VLDL triglyceride, 23.7 mg/ml, IDL esterified cholesterol, 0.95 mg/ml, and VLDL triglyceride to cholesteryl ester molar ratio, 3.2), 48% of the protein label and 86% of the cholesteryl ester label appeared in the VLDL density range.

Table 2 shows that movement of cholesteryl ester from LDL to VLDL was enhanced threefold, and appearance of LDL in the IDL fraction was enhanced fourfold by addition of plasma lipid transfer protein to the incubation mixture. However, even without the addition of lipid transfer protein, both these processes occurred, although to a much smaller extent. This could possibly be due to contamination of lipoproteins with lipid transfer protein.

In order to assess the effect of VLDL concentration on the LDL density shift, four experiments were performed (two to four incubations in each), within which the VLDL concentration was varied by dilution of the same VLDL preparation (**Fig. 5**). These data reveal no particular dependence of the appearance of LDL in the IDL fraction on VLDL concentration when VLDL triglyceride to cholesteryl ester molar ratio was high (3.6 and 5.7). It appears that a maximum of 70% of LDL undergoes a density shift under these conditions. In most experiments, the labeled LDL that had left the LDL density range appeared in the IDL density range. However, results of one experiment (VLDL TG/CE = 5.7, Fig. 5) showed that as much as 28% of labeled LDL was present in the VLDL density range after a 6-hr incubation period. Fig. 5 also suggests that the percent of LDL that appears in the IDL fraction increases with increasing molar ratios of triglyceride to cholesteryl ester in VLDL.

The effect of VLDL core lipid composition on LDL density shift was tested directly with different sources of VLDL, in order to eliminate potential variability due to

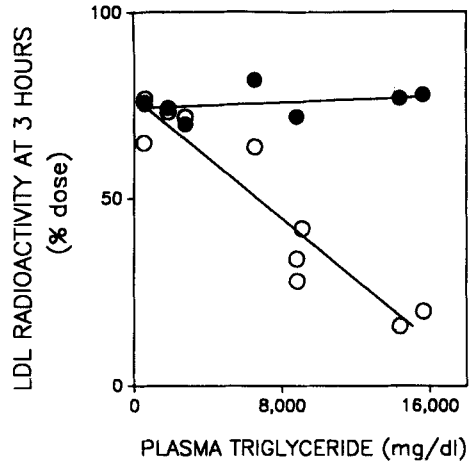

Fig. 3. Radioactivity remaining in the LDL fraction 3 hr after injection of labeled LDL as a function of recipient plasma triglyceride. Data were generated from eight hypertriglyceridemic, cholesterol-fed rabbits injected simultaneously with iodinated autologous LDL (●) and LDL from normal, cholesterol-fed rabbits (○), and from two additional hypertriglyceridemic cholesterol-fed rabbits injected only with iodinated LDL from a normal, cholesterol-fed rabbit. The regression lines for autologous LDL as well as for LDL from normal, cholesterol-fed rabbits ($r = -0.94$, $P < 0.001$) are shown.

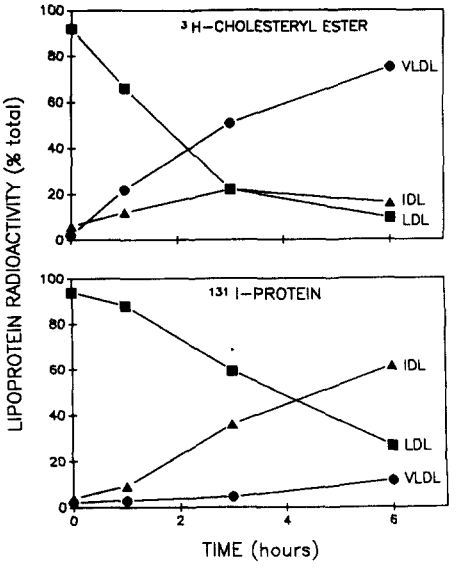

Fig. 4. Redistribution of LDL [^{3}H]cholesteryl ester and ^{131}I-labeled protein versus time of incubation with triglyceride-rich VLDL. LDL from a normal, cholesterol-fed rabbit, labeled in vivo with [^{3}H]cholesteryl ester and subsequently iodinated, were incubated in the presence of plasma lipid transfer protein (d > 1.21 g/ml from 1.3 ml plasma) with VLDL from a hypertriglyceridemic, hypercholesterolemic rabbit, in a total volume of 2.6 ml. VLDL triglyceride concentration in the assay was 23.1 mg/ml, LDL esterified cholesterol was 0.56 mg/ml, and VLDL triglyceride to cholesteryl ester molar ratio was 3.5.

interexperimental comparisons. In three experiments (three to five incubation in each), the molar ratio of triglyceride to cholesteryl ester in VLDL varied while VLDL triglyceride concentration was held approximately constant. **Fig. 6,** which shows the results of one such experiment, indicates that with increasing molar ratios of triglyceride to cholesteryl ester in VLDL, the percent of LDL that appeared in the IDL fraction increased. Results of the two other experiments, performed in the presence of different VLDL triglyceride concentrations, showed a similar trend (data not shown).

Since the incubations in the experiment shown in Fig. 6 contained similar amounts of VLDL triglyceride but VLDL with different composition, the possibility for variation in the number of VLDL particles among incubations existed. To determine whether differences in the extent of LDL density shift could be explained by variation in the number of VLDL particles, VLDL apolipoprotein B was measured for each incubation (Fig. 6, lower x-axis scale). Increasing molar ratios of triglyceride to cholesteryl ester in VLDL, associated with increasing percent of labeled LDL appearing in the IDL fraction, were also associated with decreasing VLDL particle numbers in the incubations.

Fig. 7 shows a more detailed characterization of the density distribution of ^{131}I-labeled LDL as determined by sequential ultracentrifugation. The mean density of ^{131}I-labeled LDL after incubation with triglyceride-rich VLDL was 1.030 and 1.023 g/ml in the absence and presence, respectively, of plasma lipid transfer protein. The approximately 20% of labeled LDL that appeared as d > 1.05 g/ml was not included in the calculation of mean density. Density gradient ultracentrifugation of ^{131}I-labeled LDL from a similar incubation showed a similar density shift of 0.008 g/ml. Results of density gradient ultracentrifugation also showed that the density distribution of ^{131}I-labeled LDL in the absence of VLDL was virtually identical to that of ^{131}I-labeled LDL incubated with VLDL in the absence of plasma lipid transfer protein.

DISCUSSION

The density shift of LDL reported in the present paper is likely to occur as a consequence of core lipid exchange between LDL and VLDL. This is supported by the in vivo observation that cholesteryl ester moves rapidly from injected LDL to triglyceride-rich VLDL under experimental conditions similar to those used to demonstrate the in vivo density shift of LDL. More direct evidence is

TABLE 2. Effect of plasma lipid transfer protein on density shift of LDL

Incubation	Label	Percent of ^{131}I-Labeled LDL in d < 1.019 g/ml Fraction at 6 hr
		% total
LDL + VLDL	[^{3}H]cholesteryl ester (n = 2)	24
LDL + VLDL	^{131}I-labeled protein (n = 4)	16 ± 3.2[a]
LDL + VLDL + transfer protein	[^{3}H]cholesteryl ester (n = 2)	76
LDL + VLDL + transfer protein	^{131}I-labeled protein (n = 4)	57 ± 5.8[a]

Incubations contained VLDL from hypertriglyceridemic, cholesterol-fed rabbits, LDL from normal, cholesterol-fed rabbits, and a source of plasma lipid transfer protein (d > 1.21 g/ml from 0.7–1.3 ml plasma) or an equal volume of saline, in a total volume of 1.0–2.6 ml. LDL were iodinated and, in two experiments, also labeled in vivo with ^{3}H cholesteryl ester. VLDL triglyceride was 47.0 ± 8.9 mg/ml. VLDL triglyceride to cholesteryl ester molar ratio was 3.4 ± 0.87. LDL esterified cholesterol was 0.64 ± 0.12 mg/ml.

[a]Mean ± standard error.

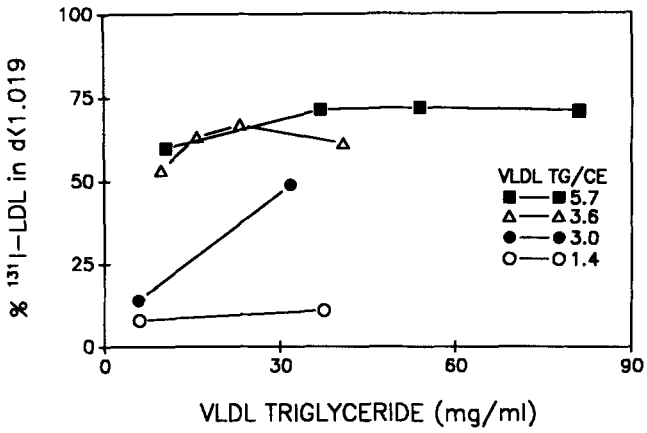

Fig. 5. Effect of VLDL concentration on density shift of LDL. ^{131}I-labeled LDL from a normal, cholesterol-fed rabbit was incubated with VLDL from a hypertriglyceridemic, cholesterol-fed rabbit at 37°C. Each assay contained similar amounts of LDL esterified cholesterol (average 0.98 mg/ml) and of plasma lipid transfer protein (from 0.7–1.3 ml plasma) in a volume of 1.3–2.6 ml. Each set of symbols represents a different experiment. Within an experiment, different concentrations of VLDL were achieved by dilution of the same source of VLDL. VLDL triglyceride to cholesteryl ester molar ratio (VLDL TG/CE) varied for the different experiments, as indicated in the figure. ^{131}I-Labeled LDL that appeared in the d < 1.019 g/ml fraction after 6 hr of incubation is expressed as a percent of that in LDL + IDL + VLDL.

obtained from the in vitro observations that the appearance of LDL in the IDL fraction coincided with the transfer of LDL cholesteryl ester to VLDL and was dependent on the activity of plasma lipid transfer protein. Morton and Zilversmit (26) have shown that triglyceride and cholesteryl ester are transferred in proportion to their content in donor lipoproteins, and that the exchange of core lipids between lipoproteins is equimolar. Deckelbaum et al. (13) have shown that LDL loses cholesteryl ester and acquires triglyceride when exposed to triglyceride-rich VLDL. Therefore, it is quite likely that transfer of cholesteryl ester out of LDL in the present experiments occurs in exchange for VLDL triglyceride. It can be calculated, for example, that a shift in density of LDL from 1.04 to 1.01 g/ml would result if 60% of LDL cholesteryl ester (d = 0.98 g/ml) were replaced with an equimolar amount of triglyceride (d = 0.92 g/ml). Thus, the observed exchange of LDL cholesteryl ester for VLDL triglyceride appears to be responsible for the observed magnitude of the LDL density shift.

Deckelbaum et al. (13) demonstrated a slight density shift of LDL after exposure to triglyceride-rich VLDL, but did not observe appearance of LDL in the IDL density range. This result can possibly be attributed to a relatively low molar ratio of triglyceride to cholesteryl ester in VLDL. We observed appearance of a greater percentage of LDL in the IDL fraction with higher molar ratios of triglyceride to cholesteryl ester in VLDL. It is probable, however, that another experimental factor affecting the potential for LDL to float in the IDL fraction is the starting density of the LDL. In one experiment in which the density distribution of LDL was characterized in detail, the mean density of the starting LDL was 1.03 g/ml. In this case, a mean shift in density of 0.007 g/ml resulted in the appearance of 23% of the LDL in the IDL fraction after incubation with VLDL containing a relatively low (1.4) molar ratio of triglyceride to cholesteryl ester. Theoretically, this mean density shift from 1.030 to 1.023 g/ml could be induced by replacement of 35% of cholesteryl ester with an equimolar amount of triglyceride (i.e., a triglyceride to cholesteryl ester molar ratio of 0.54).

Under our experimental conditions, increasing VLDL concentration per se did not appear to increase the appearance of LDL in the IDL fraction at relatively high molar ratios of triglyceride to cholesteryl ester in VLDL. Based on kinetic studies of cholesteryl ester exchange (27, 28), an increase in the number of VLDL particles might be expected to increase the rate of core lipid equilibration between VLDL and LDL. However, this principle may not extend to our measurements of an LDL density shift, which results from the heteroexchange of core lipids, if the ratio of triglyceride to cholesteryl ester in VLDL is high

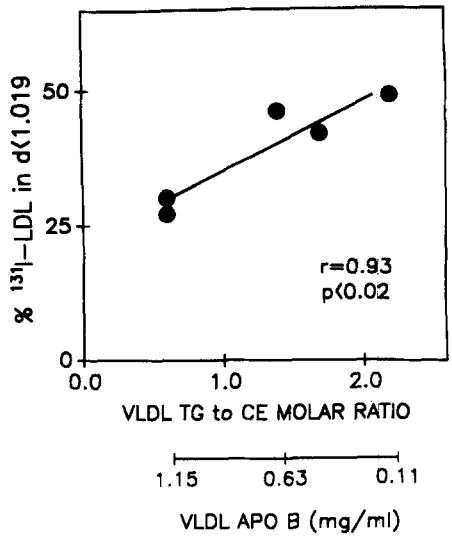

Fig. 6. Effect of VLDL core lipid composition on density shift of LDL. ^{131}I-Labeled LDL from a normal, cholesterol-fed rabbit was incubated at 37°C for 6 hr with VLDL from hypertriglyceridemic, cholesterol-fed rabbits. VLDL containing different molar ratios of triglyceride to cholesteryl ester were obtained by preparing VLDL from different rabbits. The concentration of VLDL triglyceride was similar for the different incubations (37.6 ± 4.3 mg/ml). LDL esterified cholesterol was 0.5 mg/ml. Transfer protein was from 0.7 ml plasma, and total assay volume was 1.0 ml. VLDL apolipoprotein B (apoB) was determined as in Methods for each incubation. The x-axis scale beneath the figure was derived from the equation for the regression of VLDL apoB on the molar ratio of triglyceride to cholesteryl ester molar ratio in VLDL (r = −0.85).

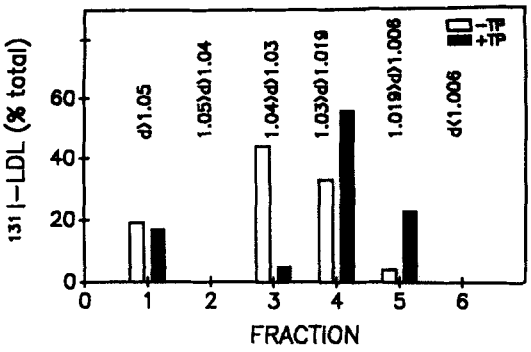

Fig. 7. Density distribution of ^{131}I-labeled LDL incubated with triglyceride-rich VLDL. ^{131}I-Labeled LDL (0.44 mg/ml esterified cholesterol) from a normal, cholesterol-fed rabbit was incubated with VLDL from a hypertriglyceridemic, cholesterol-fed rabbit (triglyceride 19.5 mg/ml, triglyceride to cholesteryl ester molar ratio 1.4) in the absence (−TP) or presence (+TP) of plasma lipid transfer protein from 0.9 ml plasma, in a final volume of 1.0 ml. Fractions were generated by ultracentrifugation of equal aliquots of the incubation mixtures at 1.054, 1.04, 1.03, 1.019, and 1.006 g/ml.

enough to induce the maximum percent of LDL to appear in the IDL fraction even at the minimum VLDL concentrations tested.

The association of appearance of LDL in the IDL density range with increasing VLDL triglyceride to cholesteryl ester molar ratios cannot be explained by increasing number of VLDL particles. The decreasing apolipoprotein B concentration with this increasing density shift (Fig. 6) merely reflects the higher triglyceride to protein ratio in VLDL with increasing ratio of triglyceride to cholesteryl ester. Also, it is unlikely that increasing movement of LDL to the IDL fraction is due to differences in apoprotein content among the different VLDL, since Granot et al. (14) have demonstrated that exchange of core lipids also occurs when LDL is incubated with Intralipid®. Under the present experimental conditions, increases in the amount of LDL appearing in the IDL fraction appear to be due mainly to increases in the molar ratio of triglyceride to cholesteryl ester in VLDL.

This influence on LDL density shift may also operate in vivo, since in vitro incubations contained proportions of plasma constituents which were designed to approximately reflect those in plasma of hypertriglyceridemic rabbits. Among hypertriglyceridemic rabbits injected with iodinated LDL, plasma triglyceride to cholesterol molar ratios increased in parallel with plasma triglyceride levels. It is therefore likely that the increase in the percent of injected labeled LDL that appeared in the IDL fraction in rabbits with increasing hypertriglyceridemia can be attributed to the increasing molar ratios of triglyceride to cholesteryl ester in VLDL.

Behavior of injected LDL from a normotriglyceridemic rabbit when exposed to VLDL from a hypertriglyceridemic rabbit illustrates the possibility for a similar mechanism in plasma of any type of hypertriglyceridemic rabbit: exchange of cholesteryl ester for VLDL triglyceride could induce the density shift of LDL subsequent to its formation. It is also conceivable, however, that core lipid exchange and density shift may occur continuously and concomitantly with lipolysis of VLDL in such a way as to diminish the production rate of lipoproteins that float in the LDL density range. Continual core lipid heteroexchange may also cause VLDL to become so enriched with cholesteryl ester that even complete lipolysis does not result in formation of lipoproteins in the LDL density range (10).

The extreme plasma triglyceride levels at which the density shift of injected LDL was observed in the hypertriglyceridemic rabbit (4000–16,000 mg/dl) are relatively rare in humans. However, the in vitro experiments demonstrated pronounced density shifts of LDL in the presence of 1000–4000 mg/dl VLDL triglyceride when the molar ratios of triglyceride to cholesteryl ester in VLDL were relatively high. Such ratios (approximately 2–6) are noted in humans with type I (2, 29, 30) and type V (6, 7, 31) hyperlipoproteinemia. Under these circumstances, LDL may shift density to the IDL fraction, or the unusually low density of lipolyzed VLDL might prevent their appearance in the LDL fraction. Observations of low LDL apolipoprotein B in some cases of human hypertriglyceridemia (2, 3, 32–35) are consistent with this hypothesis.

In conclusion, the present study reports a new observation which, together with cholesteryl ester exchange out of LDL, may account for low LDL concentrations associated with severe hypertriglyceridemia. Such loss of cholesteryl ester in exchange for VLDL triglyceride may induce a density shift in LDL or VLDL lipolytic products so as to reduce the presence of these lipoproteins in the LDL density fraction.

We thank Douglas Compton and Liliana O. de Furlong for skillful technical assistance and Patricia Curran for preparing the manuscript. Anne Minnich held a NIH training grant (#2T32-DK 07158) during these studies. Børge G. Nordestgaard was a Career Investigator Fellow of the American Heart Association and was also supported by the Danish Medical Research Council during these studies. Donald B. Zilversmit is a Career Investigator of the American Heart Association. These studies were supported by Research Grant HL-10933 from the National Heart, Lung, and Blood Institute of the U.S. Public Health Service.

Manuscript received 23 March 1988 and in revised form 22 August 1988.

REFERENCES

1. Havel, R. J., and R. S. Gordon, Jr. 1960. Idiopathic hyperlipidemia: metabolic studies in an affected family. *J. Clin. Invest.* **39:** 1777–1790.

2. Fellin, R., G. Baggio, A. Poli, J. Augustin, M. R. Baiocchi, G. Baldo, M. Sinigaglia, H. Greten, and G. Crepaldi. 1983. Familial lipoprotein lipase and apolipoprotein C-II deficiency. Lipoprotein and apoprotein analysis, adipose tissue and hepatic lipoprotein lipase levels in seven patients and their first degree relatives. *Atherosclerosis.* **49:** 55–68.
3. Nikkila, E. A. 1983. Familial lipoprotein lipase deficiency and related disorders of chylomicron metabolism. *In* The Metabolic Basis of Inherited Disease. 5th ed. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. McGraw-Hill, New York. 622–642.
4. Breckenridge, W. C., J. A. Little, G. Steiner, A. Chow, and M. Poapst. 1978. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. *N. Engl. J. Med.* **298:** 1265–1273.
5. Miller, N. E., S. N. Rao, P. Alaupovic, N. Noble, J. Slack, J. D. Brunzell, and B. Lewis. 1981. Familial apolipoprotein CII deficiency: plasma lipoproteins and apolipoproteins in heterozygous and homozygous subjects and the effects of plasma infusion. *Eur. J. Clin. Invest.* **11:** 69–76.
6. Manzato, E., A. Gasparotto, R. Marin, G. Baggio, G. Baldo, and G. Crepaldi. 1984. Characterization with zonal ultracentrifugation of low-density lipoproteins in type V hyperlipoproteinemia. *Biochim. Biophys. Acta.* **793:** 365–371.
7. Manzato, E., R. Marin, A. Gasparotto, G. Baggio, S. Martini, C. Gabelli, and G. Crepaldi. 1986. Lipoprotein modifications during dietary treatment in patients with primary type V hyperlipoproteinemia. *Eur. J. Clin. Invest.* **16:** 149–156.
8. Van Sickle, W. A., P. Brecher, A. Aspen, and A. V. Chobanian. 1985. Effect of fasting on the composition of plasma lipoproteins in cholesterol-fed diabetic rabbits. *J. Lipid Res.* **26:** 442–450.
9. Eisenberg, S., D. Gavish, Y. Oschry, M. Fainaru, and R. J. Deckelbaum. 1984. Abnormalities in very low, low, and high density lipoproteins in hypertriglyceridemia. Reversal toward normal with bezafibrate treatment. *J. Clin. Invest.* **74:** 470–482.
10. Eisenberg, S. 1987. Lipoprotein abnormalities in hypertriglyceridemia: significance in atherosclerosis. *Am. Heart J.* **113:** 555–561.
11. Barter, P. J., J. M. Gooden, and O. V. Rajaram. 1979. Species differences in the activity of a serum triglyceride transferring factor. *Atherosclerosis.* **33:** 165–169.
12. Zilversmit, D. B., L. B. Hughes, and J. Balmer. 1975. Stimulation of cholesteryl ester exchange by lipoprotein-free rabbit plasma. *Biochim. Biophys. Acta.* **409:** 393–398.
13. Deckelbaum, R. J., S. Eisenberg, Y. Oschry, E. Butbul, I. Sharon, and T. Olivecrona. 1982. Reversible modification of human plasma low density lipoproteins toward triglyceride-rich precursors. *J. Biol. Chem.* **257:** 6509–6517.
14. Granot, E., R. J. Deckelbaum, S. Eisenberg, Y. Oschry, and G. Bengtsson-Olivecrona. 1985. Core modification of human low-density lipoprotein by artificial triacylglycerol emulsion. *Biochim. Biophys. Acta.* **833:** 308–315.
15. Hough, J. L., and D. B. Zilversmit. 1984. Comparison of various methods for in vitro cholesteryl ester labeling of lipoproteins from hypercholesterolemic rabbits. *Biochim. Biophys. Acta.* **792:** 338–347.
16. Stender, S., and D. B. Zilversmit. 1982. Comparison of cholesteryl ester transfer from chylomicrons and other plasma lipoproteins to aortic intima media of cholesterol-fed rabbits. *Arteriosclerosis.* **2:** 493–499.
17. McFarlane, A. S. 1958. Efficient trace-labelling of proteins with iodine. *Nature.* **182:** 53–57.
18. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
19. Klein, R. L., and D. B. Zilversmit. 1984. Direct determination of human and rabbit apolipoprotein B selectively precipitated with butanol–isopropyl ether. *J. Lipid Res.* **25:** 1380–1386.
20. Thompson, J. N., P. Erdody, R. Brien, and T. K. Murray. 1971. Fluorometric determination of vitamin A in human blood and liver. *Biochem. Med.* **5:** 67–89.
21. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.
22. Zak, B., N. Moss, A. J. Boyle, and A. Zlatkis. 1954. Reactions of certain unsaturated steroids with acid iron reagent. *Anal. Chem.* **26:** 776–777.
23. 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.
24. Brattsand, R. 1976. Distribution of cholesterol and triglycerides among lipoprotein fractions in fat-fed rabbits at different levels of serum cholesterol. *Atherosclerosis.* **23:** 97–110.
25. Nordestgaard, B. G., and D. B. Zilversmit. 1988. Large lipoproteins are excluded from the arterial wall in diabetic cholesterol-fed rabbits. *J. Lipid Res.* **29:** 1491–1500.
26. Morton, R. E., and D. B. Zilversmit. 1983. Interrelationship of lipids transferred by the lipid-transfer protein isolated from human lipoprotein-deficient plasma. *J. Biol. Chem.* **258:** 11751–11757.
27. Ihm, J., D. M. Quinn, S. J. Busch, B. Chataing, and J. A. K. Harmony. 1982. Kinetics of plasma protein-catalyzed exchange of phosphatidylcholine and cholesteryl ester between plasma lipoproteins. *J. Lipid Res.* **23:** 1328–1341.
28. Barter, P. J., and M. E. Jones. 1980. Kinetic studies of the transfer of esterified cholesterol between human plasma low and high density lipoproteins. *J. Lipid Res.* **21:** 238–249.
29. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: in vivo and in vitro studies in man. *Eur. J. Clin. Invest.* **10:** 487–495.
30. Manzato, E., S. Zambon, R. Marin, G. Baggio, and G. Crepaldi. 1986. Modifications of plasma lipoproteins after lipase activation in patients with chylomicronemia. *J. Lipid Res.* **27:** 1248–1258.
31. Simons, L. A., and P. F. Williams. 1975. The biochemical composition and metabolism of lipoproteins in type V hyperlipoproteinemia. *Clin. Chim. Acta.* **61:** 341–352.
32. Packard, C. J., J. Shepherd, S. Joerns, A. M. Gotto, and O. D. Taunton. 1980. Apolipoprotein B metabolism in normal, type IV, and type V hyperlipoproteinemic subjects. *Metabolism.* **29:** 213–222.
33. Sigurdsson, G., A. Nicoll, and B. Lewis. 1976. The metabolism of low density lipoprotein in endogenous hypertriglyceridemia. *Eur. J. Clin. Invest.* **6:** 151–158.
34. Ginsberg, H. N., N.-A. Le, and J. C. Gibson. 1985. Regulation of the production and catabolism of plasma low density lipoproteins in hypertriglyceridemic subjects. *J. Clin. Invest.* **75:** 614–623.
35. Brook, J. G., H. Torsvik, R. S. Lees, M. A. McCluskey, and H. A. Feldman. 1979. Low density lipoprotein metabolism in type IV and type V hyperlipoproteinemia. *Metabolism.* **28:** 4–8.