Interactions of high density lipoproteins with very low and low density lipoproteins during lipolysis

Thomas A. Musliner,[1,*,†] H. Jakob Michenfelder,[*] and Ronald M. Krauss[†]

Clinical Investigation Center,[*] Naval Hospital, Oakland, CA 94627, and Lawrence Berkeley Laboratory,[†] University of California, Berkeley, CA 94720

Abstract Interactions of high density lipoproteins (HDL) with very low (VLDL) and low (LDL) density lipoproteins were investigated during in vitro lipolysis in the presence of limited free fatty acid acceptor. Previous studies had shown that lipid products accumulating on lipoproteins under these conditions promote the formation of physical complexes between apolipoprotein B-containing particles (*Biochim. Biophys. Acta*, 1987. **919:** 97–110). The presence of increasing concentrations of HDL or delipidated HDL progressively diminished VLDL-LDL complex formation. At the same time, association of HDL-derived apolipoprotein (apo) A-I with both VLDL and LDL could be demonstrated by autoradiography of gradient gel electrophoretic blots, immunoblotting, and apolipoprotein analyses of reisolated lipoproteins. The LDL increased in buoyancy and particle diameter, and became enriched in glycerides relative to cholesterol. Both HDL_2 and HDL_3 increased in particle diameter, buoyancy, and relative glyceride content, and small amounts of apoA-I appeared in newly formed particles of <75 Å diameter. Association of apoA-I with VLDL or LDL could be reproduced by addition of lipid extracts of lipolyzed VLDL or purified free fatty acids in the absence of lipolysis, and was progressively inhibited by the presence of increasing amounts of albumin. We conclude that lipolysis products promote multiple interactions at the surface of triglyceride-rich lipoproteins undergoing lipolysis, including physical complex formation with other lipoprotein particles and transfers of lipids and apolipoproteins. These processes may facilitate remodeling of lipoproteins in the course of their intravascular metabolism. — **Musliner, T. A., H. J. Michenfelder, and R. M. Krauss.** Interactions of high density lipoproteins with very low and low density lipoproteins during lipolysis. *J. Lipid Res.* 1988. **29:** 349–361.

Supplementary key words apolipoprotein A-I • fatty acids • HDL • lipid transfer • lipolysis • LDL • VLDL

Transfer of lipids and apolipoproteins between lipoproteins is known to accompany the intravascular lipolysis of triglyceride-rich lipoproteins by lipoprotein lipase. As the size of the triglyceride core of substrate lipoproteins decreases, surface phospholipids and soluble apolipoproteins move to the high density lipoproteins (HDL) (1–4). It recently has been shown that lipolysis also enhances the transfer of cholesteryl esters from HDL to very low density lipoproteins (VLDL) by plasma lipid transfer protein (LTP-1) (5). This enhancement is believed to result from the accumulation of lipolysis products (particularly fatty acids) on the surface of lipoproteins, leading to increased binding of lipid transfer protein to lipoprotein particles (6). However, the processes by which lipid and protein molecules move from one lipoprotein to another are not well understood. Possibilities include the shuttling of lipids by transfer proteins, movement of free soluble apolipoproteins in the plasma, and the transient formation of physical complexes between lipoprotein particles.

We have recently reported (7) that lipolysis products promote the formation of complexes between VLDL and other apolipoprotein (apo) B-containing lipoproteins in vitro. It was hypothesized that interactions of this nature might mediate molecular transfers between different lipoprotein particles. To further study this phenomenon, we have investigated interactions between HDL, VLDL, and LDL in the setting of partial lipolysis by bovine milk lipoprotein lipase. We report that HDL can inhibit complex formation between VLDL and LDL, through a process that results in the association of apoA-I with both VLDL and LDL.

METHODS

Isolation, subfractionation, and radiolabeling of lipoproteins

Blood was collected from normolipidemic or hyperlipidemic volunteers, after a 12–14 hr fast, in tubes containing disodium ethylene diaminetetraacetate (EDTA) at a final concentration of 1.5 mg/ml. The VLDL ($d < 1.006$

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

[1]To whom reprint requests should be addressed at: Donner Laboratory, Lawrence Berkeley Laboratory, 1 Cyclotron Road, Berkeley, CA 94720.

g/ml), LDL (d 1.019–1.063 g/ml), and HDL (1.063–1.21 g/ml) fractions were isolated by preparative ultracentrifugation under standard conditions (8). LDL was further subfractionated by density gradient ultracentrifugation as described by Shen et al. (9). Contaminating Lp[a] was removed from HDL preparations by gel chromatography over Sepharose 4B (Pharmacia) of the d 1.063–1.21 g/ml ultracentrifugation fraction as described by Ehnholm et al. (10). VLDL and IDL subfractions were isolated by nonequilibrium density gradient ultracentrifugation as previously described (11). Where necessary, lipoprotein solutions were concentrated by ultrafiltration using Amicon (Danvers, MA) XM 50 or PM 10 filters. Lipoproteins were iodinated using the iodine monochloride method (12, 13). Free iodine was removed by chromatography on Sephadex G25 (Pharmacia) followed by sequential dialysis against 0.1 M potassium iodide in 0.15 M sodium bicarbonate and then normal saline containing 0.01% EDTA, pH 7.4. Approximately 95% of the label remained bound to protein following delipidation. VLDL was radiolabeled with tri[^{3}H]oleoylglycerol using the method of Fielding (14).

Purification and use of lipoprotein lipase

Lipoprotein lipase was purified from bovine milk by the method of Bengtsson and Olivecrona (15), except that a gradient of 0.5 M to 2.0 M NaCl was employed for the second heparin-Sepharose affinity chromatography step. The specific activity of the purified enzyme ranged from 3,000 to 6,000 units/mg, where 1 unit = 1 μmol of fatty acid liberated per hour at 27°C, using the Triton X-100-stabilized triolein emulsion assay of Krauss, Levy, and Fredrickson (16). Lipoprotein incubations were carried out in covered polypropylene tubes in a shaking water bath at 37°C. Unless otherwise specified, incubation mixtures included 0.01% EDTA and 0.2 M Tris buffer, pH 7.5. Fatty acid-free bovine serum albumin was purchased from Sigma. In experiments in which hydrolysis of radiolabeled triacylglycerol was measured, released free fatty acids were extracted from incubation mixtures and quantitated as previously described (16). Release of free fatty acids from [^{3}H]triolein-labeled VLDL in the absence of added free fatty acid acceptor ranged from 12 to 25% of the total esterified fatty acids for the concentrations of lipase used in these experiments.

Lipoprotein and apolipoprotein analyses

Nonequilibrium density gradient ultracentrifugation for the separation of VLDL and VLDL-LDL complexes from LDL and HDL was performed using the following solutions layered from bottom to top in 12.5-ml Beckman Ultraclear tubes: 1.105 g/ml, 2 ml; 1.075 g/ml, 2 ml (containing the sample); 1.054 g/ml, 2 ml; 1.040 g/ml, 2 ml; 1.028 g/ml, 2 ml; and 1.0063 g/ml, 2 ml. Centrifugation was carried out for 16 hr at 40,000 rpm at 19°C in a Beckman SW-41 rotor.

Nondenaturing polyacrylamide gradient gel electrophoresis was performed using 2–16% or 4–30% gels (Pharmacia PAA 2/16 and 4/30) as described in detail elsewhere (17, 18). Gels were stained for protein or lipid, calibrated and scanned by densitometry as previously described (17–19). Electrophoretic blotting of gradient gels onto nitrocellulose paper, autoradiography of gel blots, and standardization and densitometric scanning of autoradiograms were also carried out using previously described methods (19). Gradient gel electrophoresis in the presence of sodium dodecyl sulfate (SDS) was performed using 4–30% polyacrylamide gradient gels (Pharmacia PAA 4/30) in a buffer system containing 0.04 M Tris, 0.02 M sodium acetate, 2 mM EDTA, and 0.2% SDS, pH 7.4. The sample buffer consisted of 10 mM Tris, 1 mM EDTA, pH 8.0, containing 2.5% SDS and 5% β-mercaptoethanol. Lipoproteins were delipidated prior to electrophoresis using methanol and diethylether as described by Herbert et al. (20), dissolved in the sample buffer, and heated for 5 min at 100°C prior to application to the gel. Electrophoresis was carried out for 4 hr at 150 V, 12°C. Electrophoretic blotting of SDS gels and immunostaining of apolipoproteins on nitrocellulose blots were performed as described by Towbin, Staehelin, and Gordon (21), using sheep antisera against apoA-I and apoA-II purchased from Boehringer Mannheim Corporation.

Radioactivity associated with total apolipoprotein fractions was measured following delipidation of lipoproteins with methanol–diethylether (20).

Extraction of neutral and acidic lipids from VLDL or lipolyzed VLDL was carried out as previously described (22). In brief, the lipoprotein solution in 1 ml was agitated with 4 ml of isopropanol, 2 ml of 0.05 M potassium hydroxide, and 5 ml of hexane. The hexane phase was removed and yielded the nonacidic fraction. The aqueous isopropanol phase was then immediately acidified by the addition of 0.15 ml of 1.5 M sulfuric acid and extracted again with hexane, yielding the acidic fraction. Aliquots of the lipid extracts (or of purified palmitic or lauric acids in heptane) were dried under nitrogen in glass tubes prior to incubation with lipoprotein fractions in buffer containing 0.01% EDTA and 0.2 M Tris, pH 7.5.

Protein and lipid measurements

Protein concentrations were determined by the Lowry procedure modified to include SDS (23). Phospholipid was determined by the method of Bartlett (24). Total cholesterol and triglyceride concentrations were measured by using enzymatic methods on a System 3500 Gilford Computer-Directed Analyzer (Gilford Instruments, Oberlin, OH). Assay reagents were purchased from Worthington Biochemical Corporation (Freehold, NJ). Free cholesterol and cholesteryl ester were determined by gas–liquid

chromatography with a Hewlett-Packard 5830A gas chromatograph (25).

RESULTS

Inhibition of VLDL-LDL complex formation by HDL

^{125}I-Labeled LDL (d 1.039–1.044 g/ml) (16 μg of protein) derived from normolipidemic plasma was incubated at a concentration of 0.27 mg of protein/ml with hypertriglyceridemic VLDL (46 μg of protein) and bovine milk lipoprotein lipase (7 units) for 2 hr at 37°C. In the absence of HDL, most of the labeled LDL associated with two species of VLDL-sized particles (**Fig. 1B**), as described previously (7). Inclusion of increasing amounts of normal HDL in otherwise identical incubations resulted in progressive diminution of the displacement of LDL-radioactivity into the VLDL-size range (Fig. 1C–1E) with complete inhibition of complex formation at a 2:1 ratio of HDL to VLDL protein. At a concentration of HDL that partially prevented complex formation, the size of the VLDL-^{125}I-labeled LDL complexes decreased somewhat (compare Fig. 1B and 1C), and label appeared in a third, intermediate-size band, up to 20 Å larger than the unincubated LDL. The remainder of the ^{125}I-labeled LDL was found in a separate band with peak particle diameter slightly less than that of the unincubated ^{125}I-labeled LDL. As the quantity of HDL in the incubation was increased, the amount of label in the intermediate-sized band decreased (Fig. 1D) and finally, at an HDL/VLDL protein ratio of 5:1, only one LDL band was observed, similar in size to the original LDL preparation. These changes were seen only when lipase was present in the incubation mixtures. Incubation of ^{125}I-labeled LDL with HDL in the absence of added VLDL, moreover, with or without lipase, resulted in no apparent changes (data not shown).

The presence of HDL in incubations with VLDL and lipase had a small stimulating effect on the rate of lipolysis. In experiments using VLDL labeled with [^{3}H]triolein to facilitate measurement of released fatty acids (see Methods), addition of HDL to HDL/VLDL protein ratios of up to 6:1 resulted in graded stimulation of lipolysis by as much as 1.6-fold. While this stimulation may have been due to the ability of HDL to serve as a fatty acid acceptor (26), stimulation of lipolysis by the presence of 6% bovine serum albumin was considerably greater (5-fold) under comparable conditions.

Transfer of HDL apolipoproteins

To determine whether HDL apolipoproteins redistribute under the conditions described above, incubations were performed using radioiodinated HDL and unlabeled VLDL and/or LDL in the presence or absence of lipase. Distribution of label was analyzed on both 2–16% (Fig. 2) and 4–30% (Fig. 3) gradient gels. In the experiment shown in **Fig. 2,** small quantities of radioactivity moved from the HDL- to the VLDL-size range in the absence of lipase (Fig. 2A), presumably reflecting transfer of exchangeable apolipoproteins or small quantities of labeled lipid. No detectable radioactivity appeared in association with the LDL band. When lipase was present, however, much larger quantities of label appeared in association with the VLDL (Fig. 2B). When unlabeled LDL was included in the incubation mixture (Fig. 2C), label associated with a band similar in size to the intermediate-sized species that was observed in the studies using ^{125}I-labeled

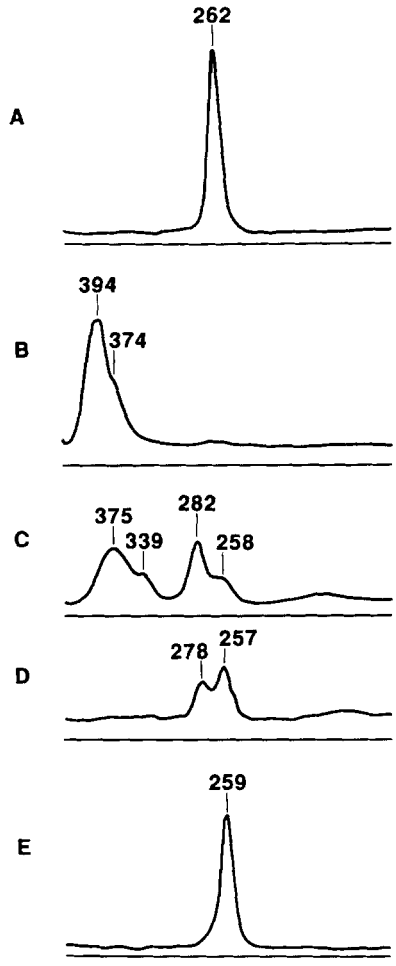

Fig. 1. Effects of HDL on VLDL-LDL complex formation. The curves represent densitometric scans of autoradiograms of nitrocellulose blots of 2–16% polyacrylamide gradient gels. The samples contained mixtures of ^{125}I-labeled LDL (d 1.039–1.044 g/ml, 16 μg of protein) and hypertriglyceridemic VLDL (46 μg of protein) in a final volume of 60 μl, with the following: (A) no lipase or HDL; (B) 7 units of lipoprotein lipase; (C) 7 units of lipoprotein lipase, normal HDL (43 μg of protein); (D) 7 units of lipoprotein lipase, normal HDL (86 μg of protein); (E) 7 units of lipoprotein lipase, normal HDL (215 μg of protein). Samples were incubated for 2 hr at 37°C prior to electrophoresis. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods.

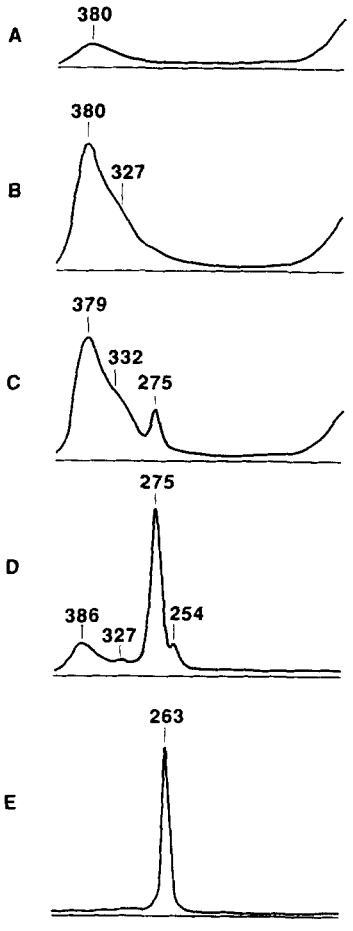

Fig. 2. Association of HDL-derived ^{125}I-label with VLDL or LDL upon incubation with lipase in the absence of added free fatty acid acceptor. The curves represent densitometric scans of autoradiograms of nitrocellulose blots of 2–16% polyacrylamide gradient gels. The samples contained the following in a final volume of 60 μl: (A) ^{125}I-labeled HDL (22 μg of protein), hypertriglyceridemic VLDL (46 μg of protein), LDL (d 1.039–1.044 g/ml, 10 μg of protein); (B) the same mixture, but without LDL and with 7 units of lipoprotein lipase; (C) the same mixture but including the LDL and with 7 units of lipoprotein lipase; (D) unlabeled HDL (24 μg of protein), hypertriglyceridemic VLDL (46 μg of protein), ^{125}I-labeled LDL (d 1.039–1.044 g/ml, 16 μg of protein), and 7 units of lipoprotein lipase; (E) the same mixture as in (D) but without lipoprotein lipase. Samples were incubated for 2 hr at 37°C prior to electrophoresis. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods.

LDL (Fig. 1). This is illustrated in Fig. 2D, showing the gradient gel profile of a radioiodinated preparation of the same LDL fraction incubated with unlabeled HDL and VLDL; the starting ^{125}I-labeled LDL (in the absence of lipase) is shown for comparison in Fig. 2E. HDL-derived ^{125}I-label, however, was also observed in association with the smaller LDL subspecies in some experiments. When VLDL was omitted from incubations of ^{125}I-labeled HDL and LDL, there was no transfer of label to the LDL band, regardless of whether lipase was present (data not shown).

The size distribution of the residual ^{125}I-labeled HDL in these incubations also changed. The major HDL_2 and HDL_3 subspecies both increased in diameter by up to 10 Å, and small amounts of label appeared in particles of 71 Å and smaller (**Fig. 3**). The observed increases in HDL size were less pronounced when the amount of HDL present in incubation mixtures was greater relative to VLDL. Immunostaining of the electrophoretic gradient gel blots shown in Figs. 2 and 3 confirmed the presence of increased amounts of apoA-I in the VLDL and LDL bands from the incubation with lipase (**Fig. 4**). Faint staining for apoA-I was also observed (not shown) in the region corresponding to the 71 Å and smaller radioactive particles formed in the presence of lipase (Fig. 3).

The association of HDL apolipoproteins with VLDL and LDL was quantitated following ultracentrifugal separations of lipoproteins. Hypertriglyceridemic VLDL (330 μg of protein) and normal ^{125}I-labeled HDL (600 μg of protein) were incubated with or without normal LDL (d 1.030–1.039 g/ml, 300 μg of protein) for 2 hr at 37°C in the presence or absence of lipase. The mixtures were then fractionated by nonequilibrium density gradient ultracentrifugation as described in Methods. The distribution of radioactivity in the fractions obtained is shown in **Fig. 5.** Autoradiograms of gradient gel blots of these fractions were used to identify the size of the lipoproteins they contained. Approximately 16% of the total radioactivity was

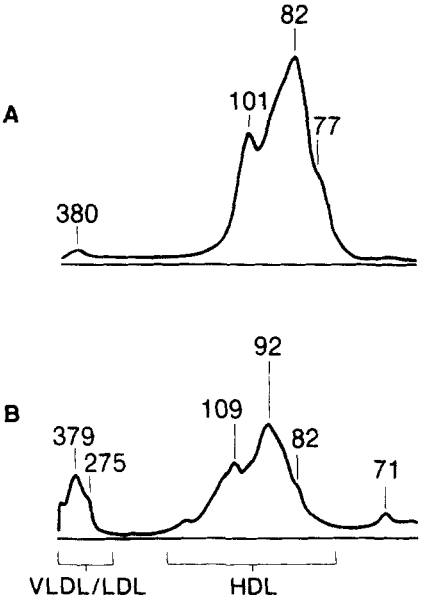

Fig. 3. Distribution of ^{125}I-labeled HDL (22 μg of protein) following incubation for 2 hr at 37°C with hypertriglyceridemic VLDL (46 μg of protein) and LDL (d 1.039–1.044 g/ml, 10 μg of protein) in the absence (A) or presence (B) of lipoprotein lipase (7 units). The curves represent densitometric scans of autoradiograms of nitrocellulose blots of 4–30% polyacrylamide gradient gels. Particle diameters of the major peaks (in Å) were determined from calibration curves as described in Methods.

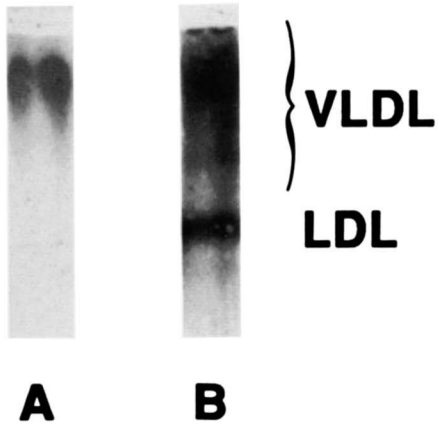

Fig. 4. Nitrocellulose blots of 2–16% gradient gels, immunostained using an antiserum directed against human apolipoprotein A-I, as described in Methods. The samples are of incubation mixtures (final volume 60 μl) containing ^{125}I-labeled HDL (22 μg of protein), hypertriglyceridemic VLDL (46 μg of protein), in the absence (A) or presence (B) of lipoprotein lipase (7 units).

found in VLDL-containing fractions 1 and 2 in the presence of lipase, compared to 1.6% in its absence. When LDL was included, ^{125}I in LDL-containing fractions 4 to 6 increased more than 20-fold upon incubation with VLDL and lipase and accounted for 7% of the total HDL-derived label. The amount of ^{125}I shifting from the HDL to the VLDL fraction changed little with the inclusion of LDL, while the amount of label associating with the combined VLDL plus LDL fractions increased from 16.4 to 23.1% of the total. The density gradient profiles (Fig. 5) also demonstrated significant decreases in buoyant density for both the HDL and the LDL following incubation with VLDL and lipase. As a result, the density of the leading shoulder of the product HDL overlapped that of the starting LDL preparation.

Autoradiograms of gradient gel blots of the LDL-containing fractions (4 through 6) reisolated by density gradient ultracentrifugation after incubation of VLDL, ^{125}I-labeled HDL, and lipase demonstrated that the label was specifically associated with the LDL band (data not shown). No detectable label was found in association with the LDL band in control incubations without lipase. Delipidated samples of the reisolated VLDL, LDL, and HDL were analyzed by SDS-gradient gel electrophoresis, followed by electrophoretic blotting onto nitrocellulose paper and autoradiography (**Fig. 6**). In the incubation without lipase (not shown), the trace quantities of labeled apolipoproteins found in association with the VLDL migrated predominantly in the size range of the C-apolipoproteins with smaller amounts of ^{125}I-labeled apoA-I. In the VLDL reisolated after incubation with lipase, apoA-I was the major iodinated component, with much smaller quantities of labeled peptides migrating in the size range of apoA-II and the C-apolipoproteins (Fig. 6). A similar predominance of iodinated apoA-I relative to apoA-II was seen in delipidated LDL from the incubation in the presence of lipase. Comparison with the distribution of labeled apoA-I and apoA-II in delipidated samples of the ^{125}I-labeled HDL used in these incubations (Fig. 6) clearly showed preferential transfer of A-I relative to A-II to both VLDL and LDL upon incubation with lipase. Densitometric scanning of Coomassie blue-stained SDS gradient gels of delipidated lipoprotein fractions from the above experiment revealed that apoA-I comprised up to 14% of the total VLDL protein and 17% of the LDL protein following

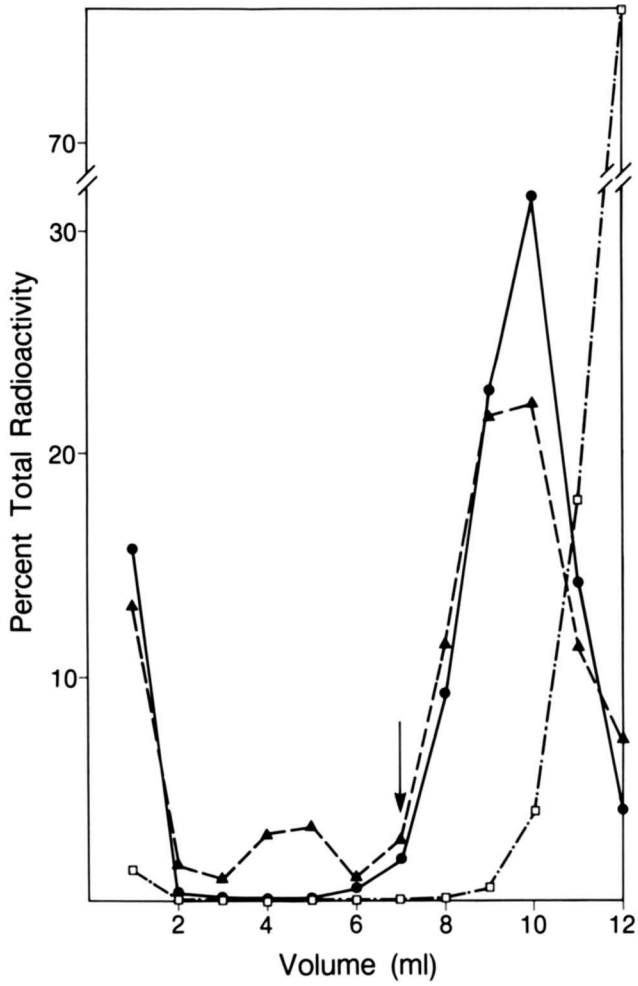

Fig. 5. Redistribution of radioactivity following incubation of normal ^{125}I-labeled HDL (600 μg of protein) with hypertriglyceridemic VLDL (330 μg of protein) in the absence of lipase (- · - · -), the presence of lipase (26 units) (——), or in the presence of lipase plus normal LDL (d 1.030–1.039 g/ml, 300 μg of protein) (- - -). The final incubation volume was 0.75 ml. The mixtures were fractionated by nonequilibrium density gradient ultracentrifugation, employing a gradient extending from d 1.006 to 1.120 g/ml as described in Methods. The arrow indicates the peak of unlabeled LDL incubated in the absence of VLDL. Autoradiograms of gradient gel blots of the ultracentrifugation fractions from the incubations that included lipase identified VLDL-sized lipoproteins in fractions 1 and 2, LDL-sized lipoprotein in fractions 4 to 6, and HDL-sized lipoproteins in fractions 7 to 12.

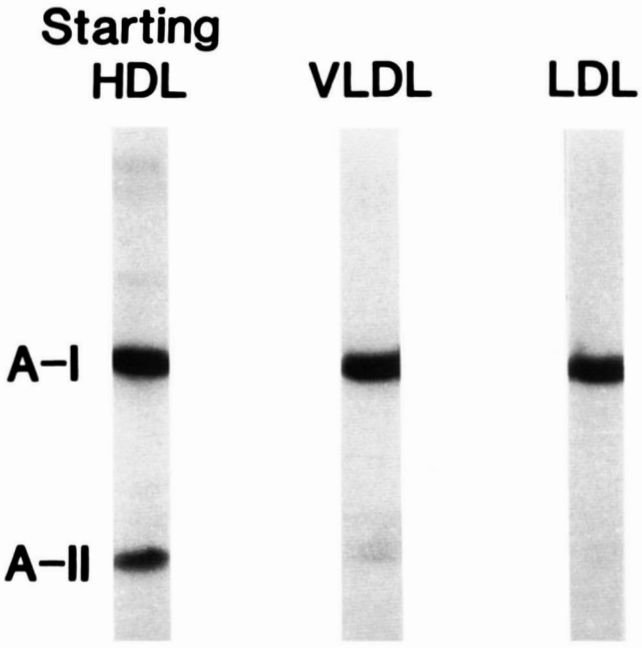

Fig. 6. Autoradiograms of nitrocellulose blots of 4–30% SDS polyacrylamide gradient gels of (A) delipidated ^{125}I-labeled HDL (starting HDL), (B) delipidated VLDL, and (C) LDL reisolated by nonequilibrium density gradient ultracentrifugation (see Methods) following incubation with ^{125}I-labeled HDL in the presence of bovine milk lipoprotein lipase. Conditions of the incubation were as described in the legend for Fig. 5.

such incubations with lipase. The reisolated LDL fractions also contained sizeable quantities of peptides migrating in the size range of the C-apolipoproteins (up to 14% of total protein) and much smaller amounts of apoE (up to 1.5% of total protein).

Effects of extracted products of lipolysis and purified unesterified fatty acids

Previous studies (7) have shown that VLDL-LDL complex formation can be reproduced in the absence of lipase by incubation of VLDL and LDL with solvent-extracted lipolysis products. To determine whether lipids generated during lipolysis mediate the observed transfers of HDL apolipoprotein to apoB-containing lipoproteins, lipids were extracted from unlipolyzed VLDL and VLDL which had been preincubated with lipoprotein lipase. Extractions were performed in two steps using an isopropanol–hexane system as described in Methods, initially under alkaline conditions to remove apolar and nonacidic lipids, and subsequently under acid conditions to extract the fatty acids. Lipids from each of these fractions (deriving from 34 μg of VLDL protein, 142 μg of triglycerides) were then incubated with ^{125}I-labeled HDL (75 μg of protein) and either VLDL (d < 1.003 g/ml, 85 μg of protein) or LDL (d 1.033–1.038 g/ml, 85 μg of protein) for 2 hr at 37°C. The amount of extracted lipid added approximated that which would have been generated by hydrolysis of 20 to 30% of the VLDL triglyceride in the mixture. The distribution of radioactivity was subsequently analyzed both by gradient gel electrophoresis and by separation of the VLDL or LDL from the HDL by ultracentrifugation at d 1.054 g/ml. Apolipoprotein-associated radioactivity was quantitated following delipidation of the ultracentrifugation fractions as described in Methods. The percent of the total ^{125}I-labeled HDL protein recovered with the VLDL or LDL is shown in **Table 1.** Small amounts of ^{125}I-labeled HDL protein were found in association with VLDL and, to a lesser extent, LDL, even in the absence of added lipid extract. Estimation of apolipoprotein distribution by SDS-gradient gel analysis with blotting and autoradiography revealed that 64% of the label in the d ≤ 1.054 g/ml fraction from the incubation with VLDL comigrated with apoA-I, while the remainder was found in the region of the C apolipoproteins and apoA-II monomer. Comparable low levels of association of ^{125}I-labeled HDL protein (59–70% apoA-I) were observed in incubations including both acidic and nonacidic lipid extracts from unlipolyzed VLDL, as well as the nonacidic fraction from lipolyzed VLDL. The acidic fraction from lipolyzed VLDL, however, stimulated binding of ^{125}I-labeled HDL apolipoprotein by VLDL nearly 4-fold, and binding by LDL more than 10-fold. In both cases, SDS-gradient gel autoradiograms demonstrated that greater than 90% of the bound label consisted of apoA-I. Quantitation of association of ^{125}I-labeled HDL protein with VLDL or LDL by densitometric scanning of gradient gel autoradiograms of the unspun incubates yielded comparable results.

Purified unesterified fatty acids had a similar effect in promoting transfer of labeled HDL apolipoprotein to LDL. In the experiment shown in **Fig. 7,** unlabeled normal LDL (d 1.033–1.038 g/ml, 44 μg of protein) was incubated with ^{125}I-labeled HDL (55 μg of protein) in the absence of added fatty acid (A) or in the presence of 0.05 μmol of sodium palmitate (B) or laurate (C). The mix-

TABLE 1. Association of ^{125}I-labeled HDL apolipoprotein with VLDL or LDL

Lipid Extract	Percent Total ^{125}I-Labeled HDL Apolipoprotein Associated with VLDL	LDL
None	4.3	1.5
Unlipolyzed VLDL (nonacidic fraction)	3.5	1.8
Unlipolyzed VLDL (acidic fraction)	4.0	2.0
Lipolyzed VLDL (nonacidic fraction)	4.4	1.8
Lipolyzed VLDL (acidic fraction)	12.0	16.4

^{125}I-Labeled HDL (75 μg of protein) was incubated with VLDL (d < 1.003 g/ml, 85 μg of protein) or LDL (d 1.033–1.038 g/ml, 85 μg of protein) in the presence of lipids extracted from lipolyzed or unlipolyzed VLDL as described in Methods. Incubation was for 2 hr at 37°C in a final volume of 110 μl.

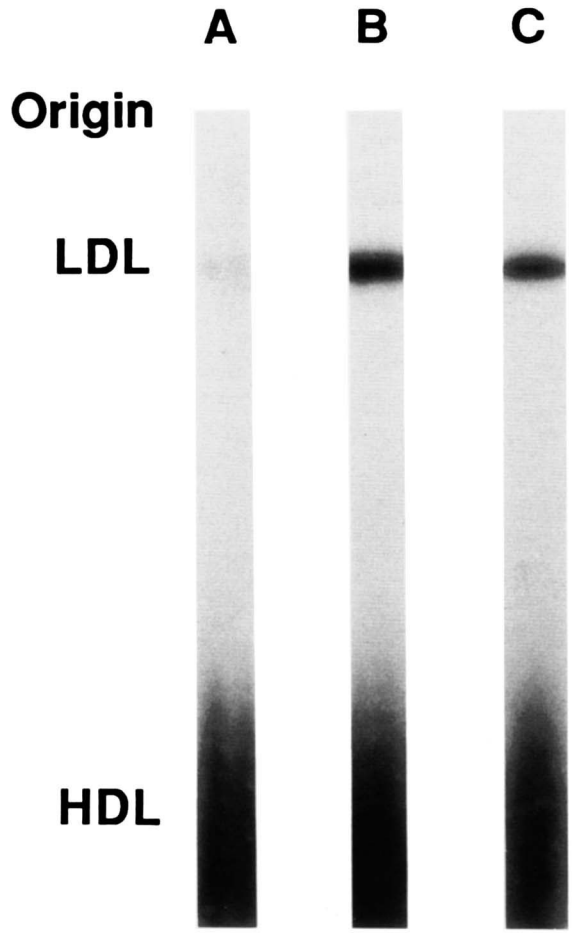

Fig. 7. Autoradiograms of nitrocellulose blots of 2–16% nondenaturing gradient gels, showing transfer of labeled HDL apolipoprotein to the LDL band following incubation with purified unesterified fatty acids. Normal LDL (d 1.033–1.038 g/ml, 44 μg of protein) were incubated for 2 hr at 37°C in the absence of added unesterified fatty acids (A), in the presence of 0.05 μmol of palmitate (B), or in the presence of 0.05 μmol of laurate (C).

tures were incubated for 2 hr at 37°C and then analyzed by 2–16% gradient gel electrophoresis followed by gel blotting onto nitrocellulose paper and autoradiography. Only minimal amounts of tracer associated with the LDL band in the absence of added fatty acids, while much larger amounts were found in their presence. Densitometric scanning of the autoradiograms shown in Fig. 7 showed approximately 20-fold stimulation of binding of HDL-derived ^{125}I-label to the LDL band at these concentrations of the two fatty acids.

Inhibition of apoA-I transfer by albumin

The association of labeled HDL apolipoprotein with VLDL and LDL was influenced by the amount of albumin available for binding of lipolysis products. Hypertriglyceridemic VLDL labeled with tri[^{3}H]oleoylglycerol (100 μg of triglyceride) was incubated with ^{125}I-labeled HDL (18 μg of protein), lipase (0.8 units), and increasing concentrations (up to 5%) of fatty acid-free bovine serum albumin. Association of HDL-derived ^{125}I-label with VLDL was measured by direct counting of VLDL and HDL bands sliced from 4–30% gradient gels. Triglyceride hydrolysis was monitored by measuring release of radiolabeled free fatty acids (see Methods) in parallel incubations using unlabeled HDL. The percent of the total HDL label associated with the VLDL band increased from 2.7% to 3.3% to 8.3% as the molar ratio of released free fatty acid to albumin rose from 1.2 to 2.6 to 5.1, respectively.

Effects of delipidated HDL

In the experiment summarized in **Table 2,** normal HDL and equivalent quantities of HDL that had been solvent-delipidated (see Methods) were compared for their capacity to inhibit high molecular weight complex formation in incubations with VLDL, ^{125}I-labeled LDL, and lipase. Complex formation was quantified by direct counting of bands sliced from gradient gels. HDL apolipoprotein effectively inhibited association of ^{125}I-labeled LDL with the VLDL band, although less completely than native HDL at higher concentrations.

In a separate experiment, ^{125}I-labeled HDL protein (10–30 μg) was incubated with VLDL (44 μg of protein) and LDL (d 1.039–1.044 mg/dl, 10 μg of protein) in the presence or absence of lipase (4 units). The mixtures were analyzed on 2–16% gradient gels stained for protein (**Fig. 8**). The distribution of radiolabel was assessed by autoradiography of nitrocellulose blots of duplicate 2–16% gradient gels (**Fig. 9**). The protein-stained gradient gels

TABLE 2. Inhibition of VLDL-LDL complex formation by HDL apolipoprotein

Incubation Contained	% Total ^{125}I-Labeled LDL complexed in Particles of Diameter > 300 Å
No LPL	5.2
LPL	57.3
LPL + HDL (15 μg protein)	
Native	21.0
Delipidated	19.4
LPL + HDL (30 μg protein)	
Native	16.9
Delipidated	16.9
LPL + HDL (60 μg protein)	
Native	9.0
Delipidated	13.6
LPL + HDL (150 μg protein)	
Native	4.9
Delipidated	11.9

VLDL (44 μg protein) was incubated for 2 hr at 37°C with ^{125}I-labeled LDL (d 1.039–1.044 g/ml, 10 μg of protein), lipase (LPL, 2 units), and either HDL or delipidated HDL in a final volume of 80 μl. Complex formation was quantified by directly counting bands sliced from gradient gels.

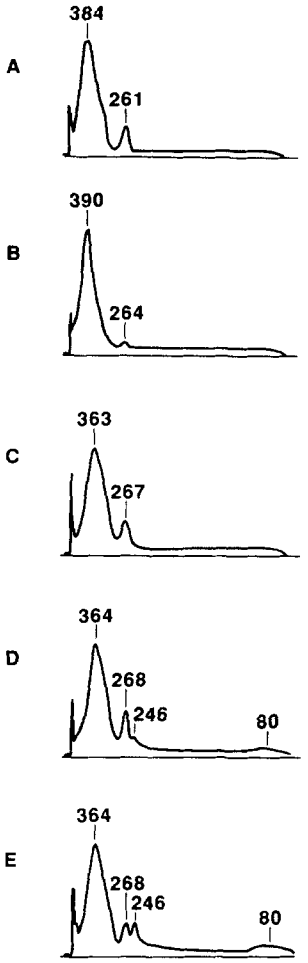

Fig. 8. Effects of HDL apolipoprotein on LDL size distribution following incubation with VLDL and lipoprotein lipase. The curves represent densitometric scans of 2-16% polyacrylamide gradient gels stained for protein with Coomassie Blue R-250. The samples contained LDL (d 1.039-1.044 g/ml, 10 μg of protein) and VLDL (44 μg of protein) with: (A) no added lipase; (B) lipase (4 units); (C) lipase (4 units) and 10 μg of HDL apolipoprotein; (D) lipase (4 units) and 20 μg of HDL apolipoprotein; and (E) lipase (4 units) and 30 μg of HDL apolipoprotein. Incubation was for 2 hr at 37°C. Particle diameters are shown in Å.

revealed diminution in the size of the LDL peak in the incubation with lipase without added HDL apolipoprotein, resulting from complexing of LDL with VLDL. With addition of increasing quantities of HDL apolipoprotein, the amount of protein staining in the LDL region again increased and changes were seen in LDL size similar to those produced by native HDL (Fig. 1). The LDL was now distributed in two overlapping peaks (Fig. 8), one smaller and the other somewhat larger than the starting LDL. In the presence of lipase, autoradiography of gel glots demonstrated association of ^{125}I-labeled HDL apolipoprotein with both LDL bands, as well as with the VLDL. In the absence of lipase, smaller amounts of label were found with the VLDL band, but none with the LDL. Increasing quantities of ^{125}I-labeled HDL apolipoprotein also appeared in a band near the bottom of the gels, with estimated peak diameter in the range of 80 Å. Small amounts of staining in the same size range could be seen on corresponding 2-16% gradient gels stained for protein with Coomassie blue (Fig. 8). This material has not yet been further characterized.

Changes in lipoprotein composition

Chemical composition analyses were performed on VLDL, LDL, and HDL fractions reisolated by density gradient ultracentrifugation following incubation in the presence or absence of lipase (**Table 3**). The compositions of the corresponding unincubated fractions are included for comparison. Small increases in glyceride content of both LDL and HDL were seen following incubation in the absence of lipase, with still larger increases when lipase was included, in association with a fall in VLDL triglyceride from 61 to 55%. The VLDL became slightly cholesterol-enriched and the LDL and HDL slightly cholesterol-depleted, although the absolute amounts of cholesterol in these fractions changed minimally. Slight protein enrichment was seen in both LDL and VLDL, with a decrease in the percent protein in HDL. The absolute amount of protein in the LDL increased by 14% in the incubation with lipase.

DISCUSSION

Previous studies have shown that lipids generated by lipoprotein lipase-mediated hydrolysis of triglyceride-rich lipoproteins, if allowed to accumulate on VLDL or LDL, promote the formation of physical complexes between these lipoprotein particles (7). In the experiments reported here, we found that increasing amounts of HDL diminished and eventually completely inhibited VLDL-LDL complex formation (Fig. 1). The presence of HDL (together with albumin) probably explains why complex formation was not observed upon incubation of normotriglyceridemic plasma with lipoprotein lipase, unless the plasma was supplemented with VLDL to bring the triglyceride concentration to greater than 300 mg/dl (7).

Despite the inhibition of VLDL-LDL complex formation by HDL, a variety of changes in the lipoproteins present in these incubations suggest that interactions continue to occur between them. When radioiodinated HDL was incubated with VLDL, LDL, and lipase in the absence of free fatty acid acceptor, association of HDL-derived apolipoprotein with VLDL remnants as well as with LDL was observed. The transferred apolipoprotein consisted predominantly of apoA-I. The amount of apoA-I that associated with VLDL or LDL under these conditions was

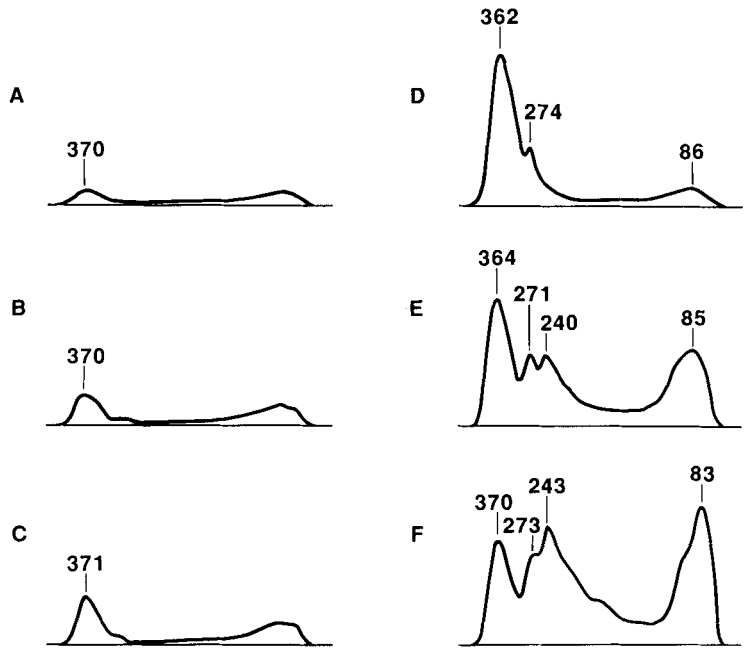

Fig. 9. Redistribution of ^{125}I-labeled HDL apolipoprotein following incubation with VLDL and LDL in the presence or absence of lipoprotein lipase. Conditions were as described for Fig. 8. The curves represent densitometric scans of autoradiograms of 2–16% gradient gel nitrocellulose blots. ^{125}I-Labeled HDL apolipoprotein was present in amounts of 10 μg (A and D), 20 μg (B and E), or 30 μg (C and F) of protein. Lipoprotein lipase (4 units) was absent in A, B, and C, and present in D, E, and F. Particle diameters are shown in Å.

sizable, in the range of 15% of their total protein. For LDL, this represented a molar ratio of apoA-I to B of nearly 4:1. The presence of apoA-I on these particles may be responsible for inhibition of VLDL-LDL complex formation. Parks et al. (27) have recently shown that apoA-I prevents fusion or association of LDL with cholesteryl ester microemulsions. While it is still unknown whether lipolysis-induced VLDL-LDL complex formation involves particle fusion or aggregation without loss of particle integrity (7), the properties of apoA-I at the surface of lipoproteins suggested by the studies of Parks et al. (27) could be expected to oppose either of these processes.

Significant amounts of C apolipoproteins and smaller quantities of apoE were also found in reisolated LDL fractions following incubation with VLDL, ^{125}I-labeled HDL, and lipase. These apolipoproteins presumably originated

TABLE 3. Chemical composition of VLDL, LDL, and HDL reisolated by density gradient ultracentrifugation following incubation together for 2 hr at 37°C in the presence or absence of bovine milk lipoprotein lipase (26 units)

	Chemical Composition (% Dry Weight)				
Lipoprotein	Protein	Glycerides	Free Cholesterol	Cholesteryl Esters	Phospholipid
VLDL					
Unincubated	6.1	62	6.2	10	15
No lipase	6.2	61	5.5	10	16
Plus lipase	8.3	55	6.4	12	18
LDL					
Unincubated	21	3.6	10.0	44	21
No lipase	21	5.0	9.4	45	20
Plus lipase	22	8.6	8.2	41	20
HDL					
Unincubated	51	2.0	2.6	24	20
No lipase	53	4.5	2.7	20	21
Plus lipase	45	14	3.2	17	21

VLDL, LDL, and HDL protein concentrations in the incubations were 1.2, 1.0, and 2.5 mg/ml, respectively.

from the VLDL, as they were not detected as labeled HDL components by autoradiography (Fig. 6). The presence of small VLDL remnants carrying these unlabeled apolipoproteins into the LDL density range cannot be excluded, although most of the protein in the reisolated LDL fraction was confined to discrete LDL bands. The possibility that lipolysis products promote association of apoC and E as well as A-I with LDL merits further study.

The association of HDL-derived apoA-I with VLDL or LDL appears to be mediated by lipolysis products, as it can be reproduced in the absence of active lipolysis by the addition of acidic lipids previously solvent-extracted from lipolyzed VLDL, as well as by purified free fatty acids. We envisage three possible mechanisms for this process: *1*) initial dissociation of apoA-I from HDL followed by migration in aqueous or partially lipidated form and reassociation with VLDL or LDL; *2*) fusion or aggregation of an apoA-I-rich subspecies of HDL with VLDL or LDL; or *3*) transient complex formation of HDL with VLDL or LDL, facilitating transfer of apoA-I and probably other components.

The fact that apoA-I from delipidated HDL associates with VLDL or LDL when lipolysis products accumulate is consistent with the first mechanism but does not exclude the others. Derksen, Ekman, and Small (28) have shown that the capacity of large triglyceride–phospholipid emulsions to bind apoA-I is enhanced by increasing fatty acid content. Differential effects of fatty acids on binding affinity of apoA-I for different lipoproteins could result in net movement of A-I from HDL to VLDL and LDL. The 71 Å and smaller apoA-I-containing particles that we observed in small amounts in incubates of HDL, VLDL, and lipase (Fig. 3) could represent an intermediate that dissociates from HDL under these conditions and subsequently reassociates with VLDL or LDL. The possible relationship of this species to populations of very small HDL described by others (29, 30) is presently uncertain. There remains disagreement as to the extent to which these species or "free" apoA-I occur in vivo in plasma (29–32), but they provide a potential pool for apoA-I binding to apoB-containing lipoproteins carrying lipolysis products.

A mechanism involving direct physical association of HDL with VLDL is suggested by other aspects of our data. Transient complex formation, by facilitating lipid transfer, could provide a mechanism for the observed changes in chemical composition (Table 3) and buoyant density of both apoA-I-enriched LDL and HDL (Fig. 5) under these conditions. The glyceride enrichment of LDL and HDL occurred in the presumed absence of lipid transfer protein, as the ultracentrifugation procedures used to isolate the lipoprotein fractions in these studies are reported to completely dissociate lipid transfer protein from lipoproteins (33), suggesting that the lipoprotein interactions promoted by lipolysis products directly enhance lipid as well as apolipoprotein transfer.

On the other hand, the apparent "quantum" jumps in the size of a subpopulation of LDL observed in incubations of VLDL, lipase and HDL (or delipidated HDL) (Figures 1, 2, 8, 9) would be consistent with a mechanism involving LDL-HDL fusion. Fusion of an LDL particle of 260 Å diameter with one, two, or three HDL particles of 100 Å diameter would theoretically give rise to new particles (assuming sphericity) of 265, 270, and 274 Å, respectively, within the range of the LDL size increases observed for components of the apoA-I-containing LDL in these experiments. This alone, however, would not account for the observed triglyceride enrichment of LDL (Table 3), which would also be likely to contribute to the LDL particle size changes. The fact that apoA-I associates with both the smaller and larger LDL subspecies observed under some conditions (Fig. 9) argues that apoA-I content is not the major determinant of the observed size changes. In agreement with this, the smaller of the two observed LDL components predominated in incubations containing higher HDL concentrations (Fig. 1), probably because HDL became the predominant acceptor for lipolysis products and other transferred lipids. Quantum shifts in LDL particle diameter as measured by gradient gel electrophoresis might also reflect conformational changes in apoB resulting from altered LDL lipid or apolipoprotein content. Deviations from sphericity resulting from such conformational changes could also produce the apparent changes in particle diameter measured by this technique.

When delipidated ^{125}I-labeled HDL was present in incubations of VLDL, LDL, and lipase, not only did ^{125}I-labeled apoA-I associate with VLDL and LDL, but newly formed particles in the HDL size range accumulated (Fig. 9). Although these lipoproteins have not yet been characterized, they presumably represent apoA-I (and possibly other apolipoproteins) associated with lipids derived from VLDL undergoing lipolysis. Generation of such particles may also have occurred in incubations with native ^{125}I-labeled HDL, where their appearance would go unnoticed because of overlap with pre-existing ^{125}I-labeled HDL. The transfer of VLDL surface lipids and apolipoproteins to HDL in the course of lipolysis in vitro and in vivo is well established (1–4). Our observations suggest that this might occur through formation of an apoA-I-VLDL intermediary complex and subsequent release of a newly formed HDL particle.

Association of apoA-I with LDL has been observed by others in a variety of settings. Campos and McConathy (34) recently reported that apoA-I comprised as much as 8–10% of the apolipoprotein in both the $d < 1.03$ and 1.03–1.063 g/ml fractions from fasting normolipidemic plasma. Further subfractionation by vertical spin ultracentrifugation indicated a maximum in apoA-I composition

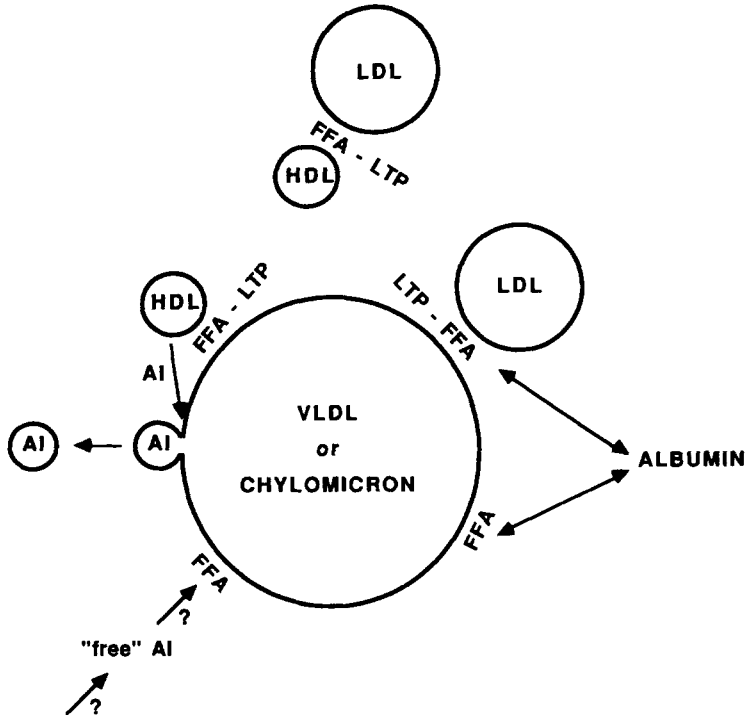

Fig. 10. Model of hypothetical lipoprotein interactions mediated by free fatty acids (FFA) or other lipolysis products; LTP, lipid transfer protein; A-I, apolipoprotein A-I. LTP is shown in association with fatty acid-lipoprotein complexes based on previous observations (6). See text for details.

(10–14% of total apolipoprotein measured) in the IDL region. Deckelbaum et al. (35) observed association of apoA-I as well as apoC and apoE with triglyceride-enriched LDL formed upon incubation with hypertriglyceridemic VLDL in the presence of d > 1.21 g/ml plasma fraction, but in the absence of lipase or HDL. It was speculated that the modified core lipid composition or size of the triglyceride-enriched LDL might have altered the binding properties of the LDL surface. However, as the incubations in that study were prolonged and included large amounts of VLDL that may contain endogenous-bound lipase activity (36, 37), it is possible that lipolysis products were generated which mediated the apoA-I binding observed. Although we cannot exclude the possibility that the triglyceride enrichment of LDL observed in our experiments (Table 3) fostered the apoA-I binding observed, the fact that fatty acids induced apoA-I binding to LDL in the absence of lipolysis and the fact that the affinity of partially lipolyzed VLDL for apoA-I increased despite depletion of core triglyceride argue that lipolysis products are the major determinants of apoA-I binding by LDL and VLDL in our experiments.

While these in vitro observations cannot be directly extrapolated to in vivo conditions, they suggest a hypothetical model for lipolysis product-mediated lipoprotein interactions that may occur in vivo (**Fig. 10**). It is proposed that free fatty acids and possibly other lipolysis products foster a variety of interactions at the surface of triglyceride-rich lipoproteins undergoing lipolysis. These would include complex formation with LDL or other VLDL (7), binding of lipid transfer protein (6), and binding of apoA-I or apoA-I-containing HDL subspecies. Physical complex formation between different lipoproteins fosters transfer of both lipid and apolipoprotein components (7). HDL and/or other forms of apoA-I as well as albumin compete with apoB-containing particles for lipolysis products and binding to the triglyceride-rich particle. The degree of complex formation and consequent lipid transfers, therefore, will be a function of the relative availability of these proteins in plasma. ApoA-I that has been transferred to the VLDL surface may be subsequently incorporated into new particles that dissociate from the triglyceride-rich lipoprotein in the course of lipolysis, perhaps as lipolysis products are depleted through equilibration with albumin or other binding sites. Lipolysis products accumulating on LDL may facilitate binding of apoA-I (and possibly other non-B apolipoproteins) or transient complex formation and lipid transfers with HDL. ApoA-I on VLDL and LDL may in turn inhibit or disrupt LDL-VLDL complex formation or influence other metabolic processes to which these lipoproteins are subject (i.e., lecithin:cholesterol acyltransferase reaction, receptor binding). Furthermore, rapid clearance of apoA-I bound to VLDL or chylomicron remnants could contribute to the low levels of HDL and the increased turnover of apoA-I (38, 39) observed in hypertriglyceridemia.

We wish to thank Kathleen M. McVicker, Joe F. Iosefa, Janet Selmek-Halsey, and Christine Giotas for excellent technical assistance, and Mary Lou Kurtz for preparing the manuscript. This work was performed under the United States Navy Clinical Investigation Program Study Number 83-48-1836, and was also supported by NIH Program Project Grant 18574 from the National Heart, Lung, and Blood Institute of the National Institutes of Health. All opinions are those of the authors and not necessarily those of the United States Navy.

Manuscript received 22 June 1987 and in revised form 21 September 1987.

REFERENCES

1. Chajek, T., and S. Eisenberg. 1978. Very low density lipoprotein: metabolism of phospholipid, cholesterol, and apolipoprotein C in the isolated perfused rat heart. *J. Clin. Invest.* **61:** 1654-1665.
2. Patsch, J. M., A. M. Gotto, T. Olivecrona, and S. Eisenberg. 1978. Formation of high density lipoprotein$_2$-like particles during lipolysis of very low density lipoproteins in vitro. *Proc. Natl. Acad. Sci. USA.* **75:** 4519-4523.
3. Tall, A. R., P. H. 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.
4. 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.
5. Tall, A. R., D. Sammett, G. M. Vita, R. Deckelbaum, and T. Olivecrona. 1984. Lipoprotein lipase enhances the cholesteryl ester transfer protein-mediated transfer of cholesteryl ester from high density lipoproteins to very low density lipoproteins. *J. Biol. Chem.* **259:** 9587-9594.
6. Sammett, D., and A. R. Tall. 1985. Mechanisms of enhancement of cholesteryl ester transfer protein activity by lipolysis. *J. Biol. Chem.* **260:** 6687-6697.
7. Musliner, T. A., K. M. McVicker, J. F. Iosefa, and R. M. Krauss. 1987. Lipolysis products promote the formation of complexes of very low density and low density lipoproteins. *Biochim. Biophys. Acta.* **919:** 97-110.
8. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. John Wiley & Sons, New York. 181-274.
9. Shen, M. S., R. M. Krauss, F. T. Lindgren, and T. M. Forte. 1981. Heterogeneity of serum low density lipoproteins in normal human subjects. *J. Lipid Res.* **22:** 236-244.
10. Ehnholm, C., H. Garoff, K. Simons, and H. Aro. 1971. Purification and quantitation of the human plasma lipoprotein carrying the Lp[a] antigen. *Biochim. Biophys. Acta.* **236:** 431-439.
11. Musliner, T. A., C. Giotas, and R. M. Krauss. 1986. Presence of multiple subpopulations of lipoproteins of intermediate density in normal subjects. *Arteriosclerosis.* **6:** 79-87.
12. MacFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature (London)* **182:** 53-57.
13. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. 1. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212-221.
14. Fielding, C. J. 1979. Validation of a procedure for exogenous isotopic labeling of lipoprotein triglyceride with radioactive triolein. *Biochim. Biophys. Acta.* **573:** 255-265.
15. Bengtsson, G., and T. Olivecrona. 1977. Interaction of lipoprotein lipase with heparin-Sepharose. *Biochem. J.* **167:** 109-119.
16. Krauss, R. M., R. I. Levy, and D. S. Fredrickson. 1974. Selective measurement of two triglyceride lipase activities in post-heparin plasma from normal subjects and patients with hyperlipoproteinemia. *J. Clin. Invest.* **54:** 1107-1124.
17. Krauss, R. M., and D. J. Burke. 1982. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. *J. Lipid Res.* **23:** 97-104.
18. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408-419.
19. Nichols, A. V., R. M. Krauss, and T. A. Musliner. 1986. Non-denaturing polyacrylamide gradient gel electrophoresis. *Methods Enzymol.* **128:** 417-431.
20. Herbert, P. N., L. L. Bausserman, L. O. Henderson, R. J. Heinen, M. J. LaPiana, E. C. Church, and R. S. Shulman. 1978. Apolipoprotein quantitation. *In* The Lipoprotein Molecule. H. Peeters, editor. Plenum Publishing Co., London. 35-56.
21. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* **76:** 4350-4354.
22. Musliner, T. A., P. N. Herbert, and M. J. Kingston. 1979. Lipoprotein substrates of lipoprotein lipase and hepatic triglyceride lipase from human post-heparin plasma. *Biochim. Biophys. Acta.* **557:** 277-288.
23. Markwell, M. A. K., S. M. Hass, L. L. Bieber, and N. E. Tolbert. 1978. A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Anal. Biochem.* **87:** 206-210.
24. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466-468.
25. Hindriks, F. R., B. G. Wolthers, and A. Groen. 1977. The determination of total cholesterol in serum by gas-liquid chromatography compared with two other methods. *Clin. Chim. Acta.* **74:** 207-215.
26. Shafrir, E. 1958. Partition of unesterified fatty acids in normal and nephrotic syndrome serum and its effect on serum electrophoretic pattern. *J. Clin. Invest.* **37:** 1775-1782.
27. Parks, J. S., J. A. Martin, F. L. Johnson, and L. L. Rudel. 1985. Fusion of low density lipoproteins with cholesteryl ester-phospholipid microemulsions. *J. Biol. Chem.* **260:** 3155-3163.
28. Derksen, A., S. Ekman, and D. M. Small. 1986. Opposing effects of fatty acids and cholesterol on apoprotein binding to large emulsion particles. *Circulation.* **74:** II-1.
29. Kunitake, S. T., K. J. LaSala, and J. P. Kane. 1985. Apolipoprotein A-I-containing lipoproteins with pre-beta electrophoretic mobility. *J. Lipid Res.* **26:** 549-555.
30. Hopkins, G. J., L. B. F. Chang, and P. J. Barter. 1985. Role of lipid transfers in the formation of a subpopulation of small high density lipoproteins. *J. Lipid Res.* **26:** 218-229.
31. Bøtreson, A-L., and K. Berg. 1980. Presence of "free" apoA-I in serum: implications for immunological quantification of HDL and its apoproteins. *Artery.* **7:** 139-160.
32. Daerr, W. H., U. Minzlaff, and H. Greten. 1986. Quantitative determination of apolipoprotein A-I in high density lipoproteins and 'free' apolipoprotein A-I by two-dimensional agarose gel lipoprotein-'rocket' immunoelectrophoresis of human serum. *Biochim. Biophys. Acta.* **879:** 134-139.

33. Pattnaik, N. M., and D. B. Zilversmit. 1979. Interaction of cholesteryl ester exchange protein with human plasma lipoproteins and phospholipid vesicles. *J. Biol. Chem.* **254:** 2782–2786.

34. Campos, E., and W. J. McConathy. 1986. Distribution of lipids and apolipoproteins in human plasma by vertical spin ultracentrifugation. *Arch. Biochem. Biophys.* **249:** 455–463.

35. Deckelbaum, R. J., S. Eisenberg, Y. Oschry, E. Butbul, I. Sharon, and T. Olivecrona. 1982. Reversible modification of human plasma low density lipoprotein toward triglyceride-rich precursors. *J. Biol. Chem.* **257:** 6509–6517.

36. Felts, J. M., H. Itakura, and R. T. Crane. 1975. The mechanism of assimilation of constituents of chylomicrons, very low density lipoproteins, and remnants: a new theory. *Biochem. Biophys. Res. Commun.* **66:** 1467–1475.

37. Goldberg, I. J., J. J. Kandel, C. B. Blum, and H. N. Ginsberg. 1986. Association of plasma lipoproteins with post-heparin lipase activities. *J. Clin. Invest.* **78:** 1523–1528.

38. Fidge, N., P. Nestel, T. Ishikawa, M. Reardon, and T. Billington. 1980. Turnover of apoproteins A-I and A-II of high density lipoprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. *Metabolism.* **29:** 643–653.

39. Schaefer, E. J., L. A. Zech, L. L. Jenkins, T. J. Bronzert, E. A. Rubalcaba, F. T. Lindgren, R. L. Aamodt, and H. B. Brewer, Jr. 1982. Human apolipoprotein A-I and A-II metabolism. *J. Lipid Res.* **23:** 850–862.