ApoE is necessary and sufficient for the binding of large triglyceride-rich lipoproteins to the LDL receptor; apoB is unnecessary[1]

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Abstract Large triglyceride-rich very low density lipoproteins (VLDL) S_f 60-400 from hypertriglyceridemic (HTG) patients, but not VLDL from normal subjects, bind to the LDL receptor of human skin fibroblasts because they contain apolipoprotein E (apoE) of the correct conformation, accessible both to the LDL receptor and to specific proteolysis by α-thrombin. Trypsin treatment of HTG-VLDL S_f 60-400 causes extensive apoB hydrolysis (fragments less than 100,000 mol wt), total degradation of apoE, and thus complete loss of LDL receptor binding. The reincorporation of apoE (1 mol/mol VLDL) into trypsin-treated HTG-VLDL completely restored the ability of HTG-VLDL to interact with the LDL receptor, suggesting that apoE probably does not induce a conformational change in apoB which results in receptor recognition, nor is intact apoB necessary to maintain the appropriate conformation of apoE for LDL receptor binding. As a model of large triglyceride-rich VLDL $S_f > 60$, we fractionated Intralipid by the Lindgren method of cumulative flotation and prepared apoE-Intralipid complexes. Competitive binding studies demonstrated that apoE-Intralipid is at least as effective as LDL for uptake and degradation of ^{125}I-labeled LDL. Control Intralipid complexes containing apoA-I instead of apoE do not compete with iodinated LDL. Since these TG-rich complexes contain no apoB, apoB is, therefore, not only not sufficient for receptor-mediated uptake of large particles, it is not necessary. ApoE of the correct conformation is not only necessary but is sufficient to mediate receptor binding of large triglyceride-rich particles to the LDL receptor. — **Bradley, W. A., and S. H. Gianturco.** ApoE is necessary and sufficient for the binding of large triglyceride-rich lipoproteins to the LDL receptor; apoB is unnecessary. *J. Lipid Res.* 1986. **27:** 40-48.

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We have investigated the nature of the LDL receptor binding determinants of large triglyceride-rich very low density lipoproteins (VLDL). In a series of studies (1-3) we reported that the binding of VLDL $S_f > 60$ from patients with hypertriglyceridemia (HTG) to the LDL receptor is mediated primarily by a specific conformation of apolipoprotein E (apoE). Furthermore, we have determined that this conformation of apoE is not generally found on the surface of the same subclass of VLDL from normolipemic (N) individuals (3), even though amounts of apoE are found in this VLDL fraction sufficient for each particle to have 1-2 mol of apoE per mol of VLDL (4-6). This difference between HTG-VLDL and N-VLDL is the reason for the original observation that HTG-VLDL bind to the LDL receptor whereas N-VLDL do not (5); that is, only the HTG-VLDL contain apoE of the appropriate conformation required for binding (2, 3). Using thrombin and trypsin as probes (3, 4), we demonstrated that apoE mediates uptake of large VLDL $S_f > 60$, while apoB is the primary ligand of smaller VLDL S_f 20-60, IDL, and LDL. This conclusion has recently been confirmed by Krul et al. (7) using monoclonal antibodies to apoB and apoE.

We conferred upon N-VLDL S_f 100-400 the ability to interact with the LDL receptor by incorporating an additional 1 mol of apoE per mol of VLDL (4). This was a specific function of apoE since the incorporation of an equivalent molar amount of apoA-I did not generate a VLDL that bound to the receptor. Similarly, reincorporation of apoE into thrombin-inactivated HTG-VLDL restored LDL receptor recognition. This led us to conclude that apoE was necessary and apoB not sufficient for receptor-mediated uptake of large VLDL $S_f > 60$. The in-

Abbreviations: HTG, hypertriglyceridemia; VLDL, very low density lipoprotein; $VLDL_1$, = S_f 100-400; $VLDL_2$, = S_f 60-100; $VLDL_3$ = S_f 20-60; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; apo, apolipoprotein; TG, triglyceride; TGRP, triglyceride-rich particles; apoB, apolipoprotein B; apoE, apolipoprotein E; N-VLDL, very low density lipoproteins from a normolipemic subject; PMSF, phenylmethylsulfonyl fluoride; PPACK, D-phenylalanyl-L-phenylalanyl-L-arginine-chloromethyl ketone; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl CoA reductase; LPDS, lipoprotein-deficient serum.

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effectiveness of apoA-I incorporation suggested that apoprotein (generic) binding to the VLDL does not induce apoB conformational changes, which then allowed apoB-mediated binding. However, we had not established whether apoB was necessary to maintain the specific conformation of apoE required to mediate uptake by the receptor nor had we completely eliminated the possibility that apoE specifically did not induce an allosteric change in apoB which allowed it to bind to the receptor. The monoclonal antibodies studies (7–9) also did not eliminate these possibilities. Therefore, the studies reported here are designed to determine whether model triglyceride-rich lipoproteins (TGLP), made from fractionated Intralipid, which contain only apoE and no apoB, are competent to interact with the LDL receptor. Furthermore, using native HTG-VLDL in which trypsin has totally degraded all the apoE and hydrolyzed apoB to fragments of apparent molecular weight less than 100,000, the addition of 1 mole of intact apoE per mole of VLDL restored receptor binding to this particle. These studies further indicate that apoE alone is absolutely necessary for the receptor-mediated uptake of triglyceride-rich lipoproteins $S_f > 60$, and show for the first time that apoB is not even required.

MATERIALS AND METHODS

Lipoprotein preparations

Lipoproteins were obtained from the plasma of fasted individuals with normal lipid values for LDL or from individuals with type 2b or type 4 hypertriglyceridemia. The diagnoses for these disorders were based on commonly used criteria (10). Lipoproteins were isolated by the method of Havel, Eder, and Bragdon (11) and the VLDL were subsequently fractionated by the cumulative flotation method of Lindgren, Jensen, and Hatch (12) as previously described (6).

Lipoprotein-apoproteins were protected from degradation by the inclusion of 1 mM NaN_3, 10 μM PMSF, 1 mM EDTA, and Trasylol (50 units/ml) and occasionally hirudin (Sigma, St. Louis, MO) and PPACK (Calbiochem) in plasma. Total protein was determined either by a modification (13) of the method of Lowry et al. (14) or by amino acid analysis.

Apolipoprotein E

Apo-E was isolated from VLDL essentially as described previously (1). Apoproteins were identified by SDS-polyacrylamide gel electrophoresis and by the immunoblot electrophoretic method using horseradish peroxidase conjugated to the second antibody (2, 3).

Preparation of Intralipid and Intralipid complexes

Intralipid (Cutters, Miles Laboratories, Berkeley, CA) was fractionated by Lindgren gradient ultracentrifugation (12) in the manner used to isolate the VLDL S_f 100–400 and VLDL S_f 60–100 (large triglyceride-rich lipoproteins) both in the presence and absence of glycerol (2.5%) at 23°C to remove phospholipid-rich (denser) vesicles from the preparations (15).

ApoE–Intralipid complexes were prepared by incubation of apoE with fractionated Intralipid at a ratio of 1:15 (wt/wt) (protein–phospholipid) for 2 hr at either 4°C or room temperature (initial studies at 37°C resulted in instability of the complexes), and then for 0–16 hr at 4°C. Complexes were either used directly in cell binding experiments or refractionated by the Lindgren method described above, as indicated.

Phospholipid content was determined by the Bartlett method (16); triglyceride content was determined using a Boehringer-Mannheim reagent-set (GPO #701912) based on the procedure of Wahlefeld (17).

Iodination of lipoproteins and apoproteins

Lipoproteins and apoE were iodinated by a modified iodine monochloride method (18) originally described by McFarlane (19). The excess noncovalently bound iodine was removed by gel filtration and extensive dialysis of the pooled column fractions. Lipoproteins were filtered (0.45 μM Millex) prior to use on the cells. Specific activities ranged from 40 to 200 cpm/ng of protein. Less than 2% of the label in LDL was extractable into organic solvents.

Protease treatment of VLDL

HTG-VLDL were incubated with trypsin (Worthington 3 × crystallized, 1% by weight) for 2 hr at 37°C or with thrombin (200 units thrombin/mg of apoprotein) in 0.15 M NaCl, 20 mM Tris, pH 7.4, 10 mM $CaCl_2$ (buffer), or with buffer alone (control). After incubation at 37°C for 2 hr, aliquots of the protease-treated and control lipoproteins were then recentrifuged through a discontinuous salt gradient (d 1.05–1.006 g/ml) to reisolate the HTG-$VLDL_1$. The lipoproteins were then tested for their effects on HMG-CoA reductase activity in normal fibroblasts as a sensitive intracellular indicator of receptor-mediated uptake.

Cells

Monolayer cultures of normal human newborn foreskin fibroblasts were grown as previously described (4, 5). In these experiments, 5×10^4 cells were seeded into dishes (60 × 15 mm) with 5 ml of complete medium containing 10% fetal calf serum. Within 2 to 3 days the cells grew to 75% confluency, at which time they were placed on 5% lipoprotein-deficient serum (LPDS) for 24 hr to derepress the LDL pathway. For measurement of HMG-CoA reductase activities, indicated quantities of lipoproteins were added to duplicate dishes and incubated at 37°C for 16 hr. Reductase activities were measured by previously

described procedures (6) modified from published methods (20, 21).

For competitive binding, uptake, and degradation experiments, cells were preincubated with LPDS for 36 hr prior to studies. Both duplicate dishes with cells as well as no-cell controls (for blanks) were incubated with ^{125}I-labeled LDL alone and in the presence of indicated amounts of the nonlabeled test lipoprotein at 4° or 37°C for 3 hr as indicated. Total cell-associated radioactivity was determined. The amount of noniodine, nonlipid, trichloroacetic acid-soluble radioactivity found in the medium was used to indicate the amount of ^{125}I-labeled LDL degraded (22, 23). All values were corrected for background "uptake" and degradation by subtraction of the amount bound and degraded in no-cell controls (24).

RESULTS

ApoE-mediated uptake of trypsin-inactivated HTG-VLDL

As we had shown earlier, both thrombin (2, 3) and trypsin (3) inactivated HTG-VLDL S_f 60–400 by partial or complete degradation of apoE, which has little or no effect on apoB-mediated uptake of VLDL S_f 20–60, IDL, or LDL (3). In the experiment shown in **Fig. 1,** we used HMG-CoA reductase activity as a sensitive intracellular endpoint of receptor-mediated uptake of trypsin-treated and thrombin-treated HTG-VLDL S_f 100–400. The upper curve in Fig. 1 shows that trypsin-inactivated HTG-VLDL S_f 100–400, like thrombin-inactivated HTG-VLDL S_f 100–400, fail to effectively suppress HMG-CoA reductase due to loss of their binding determinants. As demonstrated previously, in trypsin-treated HTG-VLDL S_f 100–400, the apoE is completely degraded as judged by immunochemical electrophoretic blots of apoVLDL (3) and by analysis using a radioimmunoassay (RIA) for apoE, while the apoB is degraded to species of less than 100,000 mol wt (3). When apoE is reincorporated into the trypsin-treated HTG-VLDL or into the thrombin-treated HTG-VLDL S_f 100–400 (bottom curve, Fig. 1), prior to reisolation by ultracentrifugation, both reconstituted particles are more effective than native HTG-VLDL in their ability to suppress HMG-CoA reductase. However, with reisolation, both particles have equivalent suppressive abilities compared to the starting HTG-VLDL. This result supported our contention that native apoB species need not be present on the surface of VLDL for the interaction of apoE with the LDL receptor or to induce the appropriate conformation of apoE to bind to the receptor. This experiment further demonstrates that only 1 mol of intact apoE/mol of $S_f > 60$ VLDL is required for binding, since that is the amount determined in the trypsin-

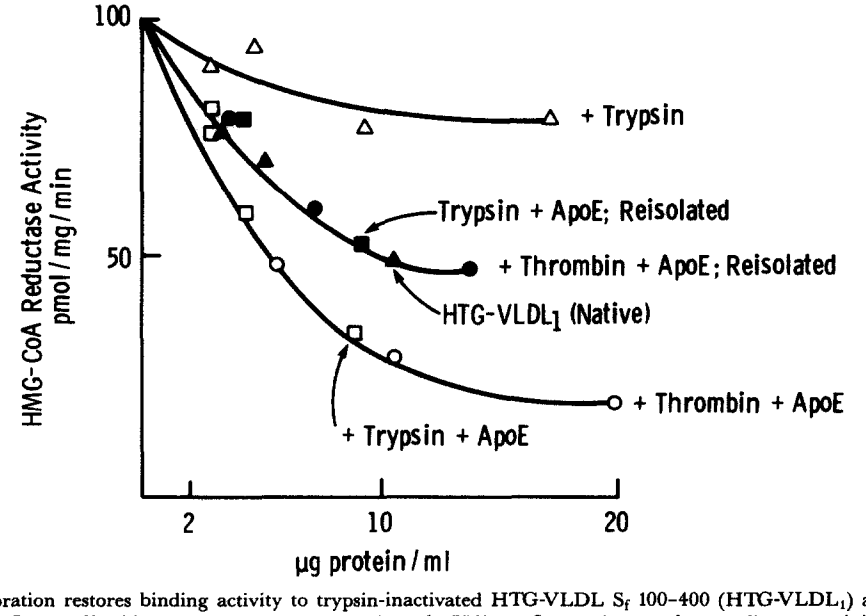

Fig. 1. ApoE reincorporation restores binding activity to trypsin-inactivated HTG-VLDL S_f 100–400 (HTG-VLDL$_1$) and thrombin-inactivated HTG-VLDL S_f 100–400. Human fibroblasts were grown to approximately 75% confluency in complete medium containing 10% fetal calf serum, washed, and placed on 2 ml of medium containing 5% LPDS for 24 hr. Indicated amounts of lipoproteins or reconstituted lipoproteins, in 0.2 ml, were added to duplicate dishes for 16 hr before cells were washed and harvested. Each data point is the average of duplicate determinations. The average variation between dishes was < 5%. Hypertriglyceridemic VLDL S_f 100–400, HTG-VLDL$_1$, were isolated from a patient with type 4 hyperlipoproteinemia. Protease inactivations were performed as described (2, 3). Trypsin- and thrombin-inactivated HTG-VLDL$_1$ (450 μg of protein) were incubated in the presence and absence of ^{125}I-labeled apoE (100 μg) for 2 hr at 37°C, then for 16 hr at 4°C. A portion of each incubation mixture was reisolated by flotation as described previously (2, 3) before testing in fibroblasts. Starting HTG-VLDL$_1$ (▲); trypsin-treated HTG-VLDL$_1$ (△); trypsin-treated HTG-VLDL$_1$ + apoE before (□), and after reisolation (■); thrombin-treated HTG-VLDL$_1$ + apoE before (○), and after reisolation (●).

TABLE 1. Compositions of isolated apoE-Intralipid complexes[a]

Preparation	Triglyceride	Phospholipid	Protein
		% *wt*	
1	93.6	6.2	0.12
2	94.1	5.8	0.12
3	90.6	9.2	0.20

[a] ApoE-Intralipid complexes were prepared as described in Methods, then isolated by density gradient ultracentrifugation.

ized-reconstituted particle based upon the incorporated radioiodinated apoE (Fig. 1). Therefore, apoE-apoE interaction also is not required for the appropriate apoE receptor binding conformation.

The following set of model studies was designed to determine the need for the presence of apoB in the phospholipid matrix for the interaction of apoE in large triglyceride-rich lipoproteins with the LDL receptor.

Isolation and characterization of Intralipid-apoE complexes

Intralipid was fractionated to obtain large triglyceride-rich particles by the method of cumulative flotation. This allowed the removal of phospholipid-rich denser particles (15). Only Intralipid, which floated in the upper 1.6 ml of the density gradient, was used for model studies to incubate with the apoE. Depending upon the batch of Intralipid used, the stability of the apoE-Intralipid complexes varied and could be enhanced by the inclusion of 2.5% glycerol and/or keeping the temperature of the complexes below 25°C. Three separate preparations are shown in **Table 1.** The average weight ratio of apoE to phospholipid in the reisolated apoE-Intralipid complex was approximately 2.0%; the ratio of apoE to triglyceride was 0.16%. Approximately 16% of the total apoE added to Intralipid was recovered in the top 1.6 ml.

The Intralipid-apoE complexes were further characterized according to their size, using gel permeation chromatography on a 1.6 × 30 cm column of S-1000 (**Fig. 2**). This column demonstrates that the TG-rich complexes are larger than HTG-VLDL S_f 100-400, and it demonstrates the necessity of separating the large particles from the free apoE and smaller phospholipid-rich complexes,

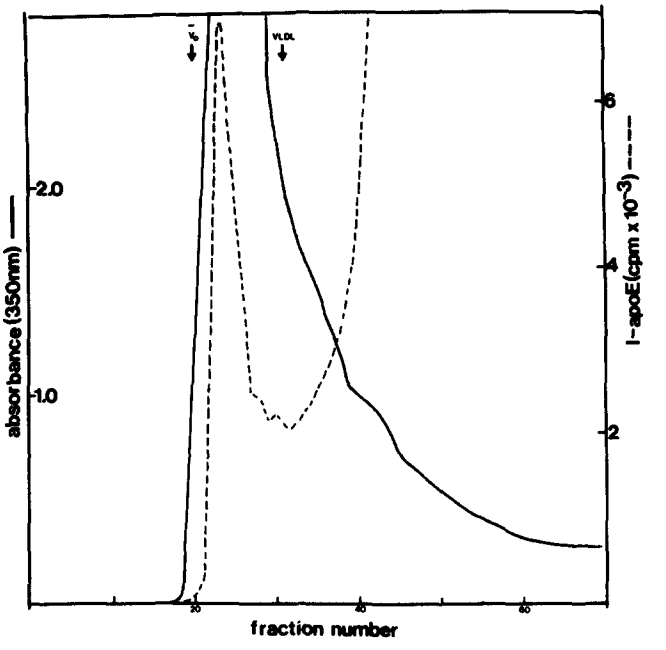

Fig. 2. Isolation of apoE-Intralipid complexes by column chromatography on S-1000. ^{125}I-Labeled apoE and Intralipid were incubated as described in Methods in the presence of 2.5% glycerol and placed on a 1.6 × 30 cm column. The column was allowed to develop and light scattering was monitored at 350 nm. Fractions (1 ml) were collected and radioactivity was monitored for the presence of ^{125}I-labeled apoE. The large apoE-Intralipid complex eluted just after the void volume (V_o) of the column. Smaller phospholipid-rich complexes (these complexes were not further characterized) and "free apoE" eluted later from the column. The large apoE-Intralipid complex is representative of the complex isolated by the gradient density ultracentrifugation and used in the competitive binding experiments.

which was accomplished by the gradient ultracentrifugation method.

Using the compositional data from Table 1 for the apoE-Intralipid complex, we calculated the size and average molecular weight of this complex according to the model of Shen, Scanu, and Kezdy (25). **Table 2** compares the wt% ratios of the reisolated complexes prepared in this study with those of large VLDL and chylomicrons. The apoE-Intralipid complex is larger than VLDL S_f 100-400 and has a triglyceride content and size similar to chylomicrons. Since it has less apoprotein in the surface than a chylomicron and little, if any, cholesterol, there is an increased amount of phospholipid in the

TABLE 2. Comparison of compositions of triglyceride-rich lipoproteins

	Weight % of Total Lipid Protein[a]				
	Triacylglycerol	Phospholipid	Protein	Cholesterol	Cholesteryl Ester
ApoE-Intralipid (n = 3)	92.7 ± 1.9	7.1 ± 1.9	0.15 ± 0.05		
N-VLDL S_f 100-400 (n = 5)	63.6 ± 2.6	18.5 ± 1.9	5.9 ± 0.7	5.9 ± 1.4	6.1 ± 1.6
HTG-VLDL S_f 100-400 (n = 7)	65.0 ± 5.0	15.0 ± 2.4	5.9 ± 0.9	5.0 ± 0.7	8.9 ± 2.4
Chylomicrons[b]	84.0	7.0	2.0	5.2	11.7

[a] Values given as mean ± SD.
[b] Values taken from reference 25.

TABLE 3. Molecules per particle of triglyceride-rich lipoprotein calculated from data in Table 2[a]

	Amino Acid	Phospholipid	Cholesterol	Cholesteryl Ester	Triacylglycerol	Radius (nm)
ApoE-Intralipid	9,834	69,521			627,104	60
N-VLDL S_f 100-400	18,290	7,400	4,727	2,909	23,195	24
HTG-VLDL S_f 100-400	18,290	6,000	4,005	4,245	23,706	23
Chylomicrons	102,000	45,160	25,840	27,700	507,000	60

[a]All values calculated (from data in Table 2) as described in reference 25.

surface monolayer. **Table 3** compares the component compositions of the reisolated apoE-Intralipid complex with other large TG-rich lipoproteins.

ApoE-containing TG-rich particles (from Intralipid) compete as effectively as LDL for the LDL receptor

When apoE was incubated with Intralipid from which phospholipid-rich particles had been previously removed, for 2 hr at either 4°C or at room temperature and then for 16 hr at 4°C, the particles formed were more effective than LDL itself in competing for binding of ^{125}I-labeled LDL (**Fig. 3**). Neither Intralipid alone nor apoE alone were effective competitors of binding, uptake, or degradation of ^{125}I-labeled LDL (**Fig. 4** and **Table 4**).

Since no apoB is present in large TG-rich Intralipid particles, the data demonstrate that, on these large particles with surfaces of low radius of curvature, the apoE can properly fold into the correct conformation in the phospholipid matrix to enable the particle to interact with the LDL receptor even in the absence of other apoproteins, particularly apoB. A control using apoE alone (see Fig. 4 and Table 4) again demonstrated that apoE alone is incapable of interacting with the receptor, as was previously reported (26).

Furthermore, Table 4 shows that the Intralipid-apoE complexes are better competitors on a particle basis for the LDL receptor than LDL itself. ApoE alone or apoA-I, on the other hand, is ineffective as a competitor of LDL at equal protein concentration and at far greater molar amounts. In fact, a slight increase in binding, uptake, and degradation of ^{125}I-labeled LDL was observed with apoE alone as the competitor of ^{125}I-labeled LDL for the LDL receptor. This observation suggests that free apoE can associate with the surface of a subpopulation of LDL, enhancing its interaction with the LDL receptor. This amount of transfer of apoE would be minimal and only loosely associated, however, since we have measured the amount of apoE that can be bound to LDL both in plas-

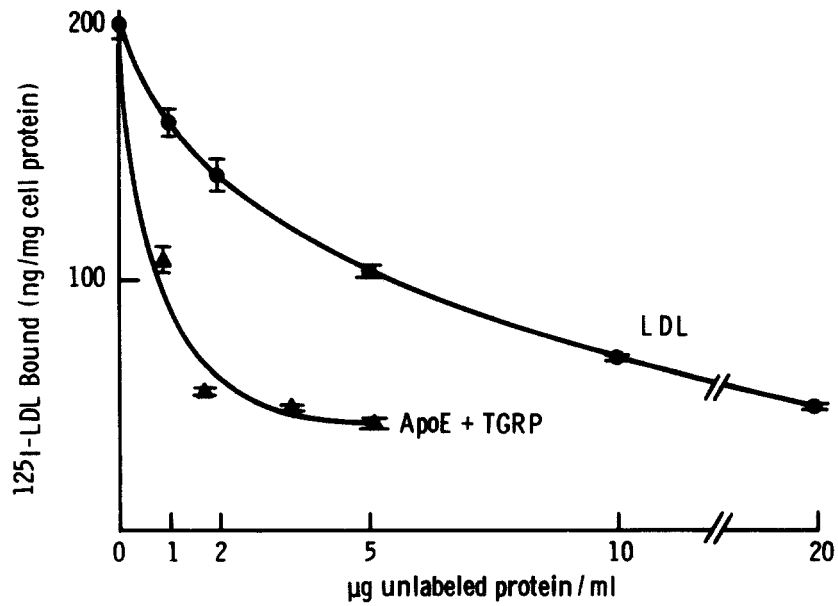

Fig. 3. Competition for ^{125}I-labeled LDL binding to the LDL receptor by apoE-Intralipid complexes. Cells were grown as described in the legend for Fig. 1, except that the cells remained in 5% LPDS for 36 hr prior to addition of lipoprotein. LDL and apoE-Intralipid complexes were prepared as described in Methods and the text. Indicated amounts of the unlabeled lipoprotein or complex (without reisolation), in 0.2 ml, in addition to ^{125}I-labeled LDL (d 1.025-1.050 g/ml, 5 µg of protein/ml; 113 cpm/ng) were added to each dish and incubated for 3 hr at 4°C. Cell-associated radioactivity after the cells were washed exhaustively with albumin-containing buffer at 4°C represents ^{125}I-labeled LDL bound to the fibroblasts. Each point represents the average value from duplicates with the average variation in duplicates of 8%. LDL (●); apoE-Intralipid (TGRP) (▲).

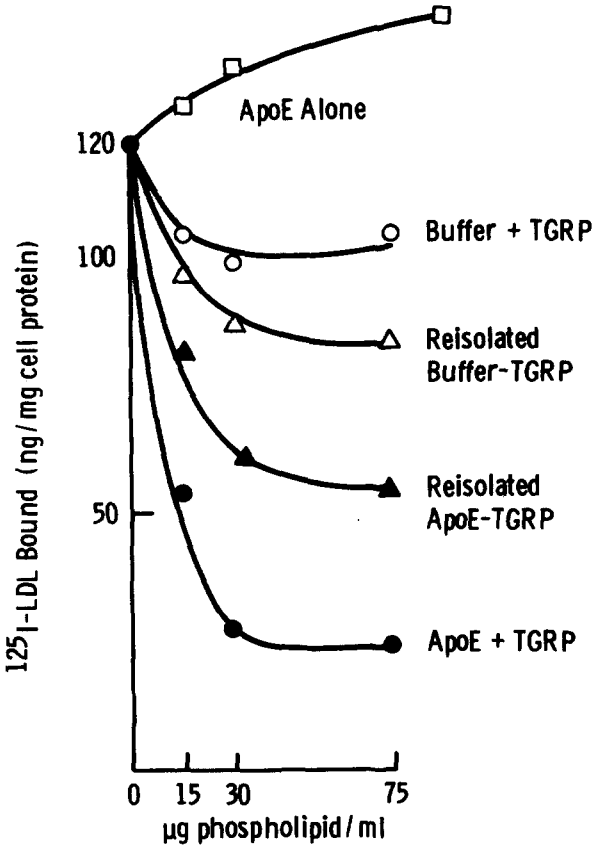

Fig. 4. Competitive binding of ^{125}I-labeled LDL by apoE, and apoE-Intralipid (TGRP) before and after reisolation. Cells were grown as described in the legend for Fig. 3. Unlabeled apoE-Intralipid complexes, before and after reisolation by flotation (see Methods), or apoE alone were added, in 0.2 ml, in the presence of ^{125}I-labeled LDL (d 1.025–1.050 g/ml, 5 μg of protein/ml) to duplicate dishes and incubated for 2 hr at 4°C. Since the TGRP contain no protein, the indicated amounts added of both complexes and TGRP are based on phospholipid which is an equivalent particle marker. The apoE alone (□) did not compete over a protein concentration range equivalent to the apoE concentration found associated with the TGRP complexes. The weight ratio of phospholipid/apoE was 50 in reisolated apoE-TGRP. Each point represents the average from duplicates with an average variation in duplicate of 8%. ApoE-TGRP (●); apoE-TGRP reisolated as described in Methods (▲); TGRP incubated with buffer alone (○); TGRP incubated with buffer alone and reisolated by flotation (△).

ma and by direct addition and reisolation of the LDL. Approximately only 1 in 20 LDL molecules has any apoE bound to its surface either when isolated from normal plasma (d 1.03–1.05 g/ml) or when direct addition is attempted (unpublished observations).

When the Intralipid-apoE complexes were reisolated by cumulative centrifugation, a decrease in the ability to bind to the LDL receptor was observed, as seen in Fig. 4. This decreased binding ability is similar to that observed in the studies concerning incorporation of apoE into N-VLDL (4) or reincorporation of apoE into thrombin- or trypsin-treated HTG-VLDL (Fig. 1 and refs. 2, 3). This occurs whenever we reisolate any particle that uses the apoE moiety as the ligand for LDL receptor binding, even though we employ a relatively gentle isolation method. Two possibilities exist to explain this observation: *1*) the amount of apoE per particle may be decreased upon flotation; and/or *2*) the conformation of apoE on the surface of the particle changes under these reisolation conditions. The ability of the control TG-rich particles without apoE to compete slightly for the receptor (Fig. 4) is a property of large lipid particles and is a function of nonspecific interaction of these enormous particles with cell surfaces. This is occasionally seen even with N-VLDL S_f 100–400 (4) and thrombin-inactivated HTG-VLDL S_f 60–400 (3), which can sterically inhibit LDL binding without themselves binding with high affinity to the LDL receptor. It is interesting to note that, upon recentrifugation of these control TG-rich particles, there is an increase in their ability to nonspecifically compete with LDL binding.

DISCUSSION

It was once thought that either apoE or apoB could serve in VLDL as the primary ligand for LDL receptor binding, since both are present and both, in small particles, bind to the LDL receptor. Identification of the apoprotein actually involved stems from the original observation that large triglyceride-rich HTG-VLDL S_f 60–400 and not N-VLDL of the same subclass have the ability to bind to the LDL receptor (4–6). Since both N- and HTG-lipoproteins contain at least 1 mole of apoprotein/mole of lipoprotein of each of these apoproteins, it was obvious that the mere presence of either or both apoproteins was not sufficient for receptor-mediated uptake. Furthermore, we demonstrated subsequently that the susceptibility of apoE to thrombin cleavage of HTG-VLDL subclasses ($S_f > 60$) paralleled the ability of these lipoproteins to be taken up by the LDL receptor (2, 3). That is, if one found thrombin-accessible apoE in the VLDL of a particular subclass, then it would bind to the LDL receptor and this binding could be abolished by thrombin cleavage. This demonstrated that a specific conformation of apoE existed on the surface of HTG-VLDL and mediated receptor recognition; this conformation was not detected in N-VLDL (3).

ApoB also appears to have different conformations on the surface of different lipoprotein classes and this is supported by several lines of evidence from a number of laboratories. N-VLDL S_f 60–400, for example, are unable to bind to the LDL receptor (4–6) indicating that the apoB receptor binding domains found in LDL, IDL, and VLDL S_f 20–60 are not expressed on the large VLDL $S_f > 60$ surface. That apoB of N-VLDL is potentially competent to bind the LDL receptor is demonstrated by its ability to be taken up by the receptor after lipolysis to a

TABLE 4. Competition for binding and degradation of ^{125}I-labeled LDL by Intralipid-apoprotein complexes

Unlabeled Lipoprotein Added	Concentration			^{124}I-labeled LDL (ng protein/mg cell protein)		
				Cell Associated		Degraded
	Phospholipid (μg/ml)	Protein (μg/ml)	Pmol/ml	(4°C)	(37°C)	(37°C)
None				204[a]	1280	1899
LDL	11	10	21[b]	153	880	1382
	52	50	105	44	317	450
Intralipid + buffer	29		1.0[b]	172	1121	1910
	116		4.0	224	900	1271
Intralipid + ApoE	29	1.9	1.0[b]	160	1006	1465
	116	7.8	4.0	73	523	821
Intralipid + ApoA-I	29	1.9	1.0[b]	190	1056	1830
	116	7.8	4.0	196	899	1216
ApoE		1.9	55[c]	256	1275	2155
		7.8	226	235	1438	2143
ApoA-I		1.9	60		1323	1980
		7.8	279	220	1395	1926

[a] ^{125}I-Labeled LDL, 5 μg of protein/ml, were incubated in the presence and absence of the indicated levels of unlabeled lipoproteins for 3 hr at 4°C or 37°C; the cells were washed extensively, cell-associated, and degraded. ^{125}I-Labeled LDL were determined as described in Methods. Triglyceride-rich Intralipid was prepared and incubated for 2 hr at room temperature with buffer, apoE, or apoA-I at the indicated levels immediately prior to the cell experiment. Each value is the average from duplicate dishes; the average variation between dishes was 8%.

[b] Based on values described in Table 2; for Intralipid and Intralipid complexes, the particle molar values were based on PL and TG content as found in Table 2; LDL was based on a particle weight of 2.2×10^6, 21% protein.

[c] ApoE and apoA-I molar concentrations were based on their known molecular weights, 34,500 and 28,000, respectively.

lipoprotein resembling LDL (27). The concept of different apoB conformation is further supported by the results of several investigators using poly- (28) and monoclonal (29–32) antibodies to apoB.

This present study supports our earlier conclusion that apoE is the primary ligand of large HTG-VLDL $S_f > 60$ (2–4). Fig. 1 shows that even when apoB is degraded by trypsin, which should allow fewer conformational constraints on apoprotein B, no apoB domain is expressed that allows interaction with the LDL receptor. On the other hand, in smaller cholesterol-rich lipoproteins $S_f < 60$, similar trypsin treatment (3) or other protease treatments (33) had little or no inhibitory effect on the ability of these lipoproteins to bind to the receptor. Only when intact apoE of the proper conformation (1 mol/mol VLDL) is returned to the VLDL surface (lower curve, Fig. 1) is the lipoprotein able to bind again. That apoE restores the binding of trypsinized HTG-VLDL, where all apoB is degraded to fragments < 100,000 molecular weight, again suggests that intact native apoB-100 or other large apoB species are not necessary for maintenance of the proper binding domain of apoE in the VLDL surface for receptor binding. There exists a native analog to this experiment since N-VLDL $S_f > 60$, which contain both intact apoproteins in stoichiometric amounts, do not bind to the receptor (4–6), again suggesting that there seems to be little protein-protein interaction (at least between apoB and apoE) that influences binding to the LDL receptor.

We fractionated Intralipid prior to interaction with apoE, buffer, or apoA-I to ensure that only large TG-rich particles were used in each experiment. As indicated in Fig. 4, reisolation affects the particles both with and without apoE. In these curves, since we are comparing similar particles, we have based the analyses on phospholipid content of the particles. With reisolation the buffer control competed better for the receptor than before reisolation. This probably reflects the fact that the fractionated Intralipid is somewhat unstable and that during the control incubations for incorporation of apoproteins there may be particle disintegration; upon reisolation there is further loss of phospholipid-rich species, which are more dense. Thus the reisolated large TGRP (per weight of phospholipid) may compete better nonspecifically for the LDL receptor because of increased average size of the particle. Increasing the temperature of the apoprotein incorporation incubation to 37°C resulted in major increases in the competitiveness of the control TGRP upon reisolation.

Upon reisolation of the apoE-Intralipid complexes there may be a loss of loosely associated apoE from the particles, thus causing diminished binding as discussed earlier. Alternatively, there may be an alteration of the conformation of apoE under the recentrifugation condi-

tions, which would also cause diminished binding to the receptor. Similar changes can be seen with column-isolated HTG-VLDL (unpublished observations), only the mechanism of conformational perturbation is different. Therefore, with the loss of the binding domain either through mass loss or change in conformation, the reisolated apoE–Intralipid complexes are somewhat less effective than the initial apoE–Intralipid complex in binding to the LDL receptor, as is seen with reisolated apoE–Intralipid, HTG-VLDL, or apoE-trypsinized HTG-VLDL (Fig. 1). It is important to note that the free apoE, at concentrations equivalent to the TGRP plus apoE, failed to compete with LDL binding. Thus the competition observed with TGRP plus apoE is due to the complex and not to free apoE.

The use of model triglyceride-rich lipoproteins allowed us to ask the question whether apoE, in the absence of apoB, on the surface of large particles could attain the appropriate conformation for binding to the LDL receptor. In Fig. 2 we compared the ability of apoE–Intralipid complexes to compete with LDL for the LDL receptor. The complexes are as competent as LDL in binding to the LDL receptor. This provides direct evidence that apoE on large TG-rich lipoproteins can interact with the LDL receptor in the absence of apoB. Furthermore, the interaction of the apoE with the Intralipid phospholipid matrix is essential, since neither Intralipid alone nor apoE alone (Fig. 4 and Table 4) effectively competed with LDL for the receptor. ApoA-I alone or apoA-I–Intralipid complexes were also incapable of effectively competing for binding, uptake, and degradation with ^{125}I-labeled LDL for the LDL receptor (Table 4). Thus competition by apoE–Intralipid is specific to apoprotein E.

These model studies have allowed us to conclude that not only is apoE necessary for interaction of large triglyceride-rich particles with the LDL receptor, it is sufficient. Conversely, not only is apoB insufficient for this interaction, it is unnecessary.

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