Apolipoprotein C-III displacement of apolipoprotein E from VLDL: effect of particle size

Emelita D. Breyer,* Ngoc-Anh Le,† Xianzhou Li,† Deborah Martinson,† and W. Virgil Brown[1,†]

Department of Chemistry,* Georgia State University, Atlanta, GA 30302, and Division of Atherosclerosis and Lipid Metabolism,† Emory University, Atlanta, GA 30322

Abstract **ApoC-III and apoE are important determinants of intravascular lipolysis and clearance of triglyceride-rich chylomicrons and VLDL from the blood plasma. Interactions of these two apolipoproteins were studied by adding purified human apoC-III to human plasma at levels observed in hypertriglyceridemic subjects and incubating under specific conditions (2 h, 37°C). As plasma concentrations of apoC-III protein were increased, the contents in both VLDL and HDL were also increased. Addition of apoC-III at concentrations up to four times the intrinsic concentration resulted in the decreasing incremental binding of apoC-III to VLDL while HDL bound increasing amounts without evidence of saturation. No changes were found in lipid content or in particle size of any lipoprotein in these experiments. However, distribution of the intrinsic apoE in different lipoprotein particles changed markedly with displacement of apoE from VLDL to HDL. The fraction of VLDL apoE that was displaced from VLDL to HDL at these high apoC-III concentrations varied among individuals from 20% to 100% its intrinsic level. The proportion of VLDL apoE that was tightly bound (0% to 80%) was found to be reproducible and to correlate with several indices of VLDL particle size. In the group of subjects studied, strongly adherent apoE was essentially absent from VLDL particles having an average content of less than 50,000 molecules of triglyceride. Addition of apoC-III to plasma almost completely displaces apoE from small VLDL particles. Larger VLDL contain tightly bound apoE which are not displaced by increasing concentration of apoC-III.**—Breyer, E. D., N-A. Le, X. Li, D. Martinson, and W. V. Brown. **Apolipoprotein C-III displacement of apolipoprotein E from VLDL: effect of particle size.** ***J. Lipid Res.*** **1999.** 40: **1875–1882.**

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Apolipoprotein C-III (apoC-III) and apolipoprotein E (apoE) are found in human plasma as components of chylomicrons, very low density lipoproteins (VLDL), and high density lipoproteins (HDL). Both belong to a group of apolipoproteins which are bound to the surface of these lipoproteins but also participate in a dynamic exchange among various lipoproteins during their transport in the blood stream. The interaction of these two proteins has been the subject of recent interest because of their effect on the clearance of triglyceride-rich lipoproteins (TG-rich Lp) has been demonstrated to be counter balancing and complex, with implications for various manifestations of hypertriglyceridemia.

ApoC-III provides a strong negative charge on the surface of lipoproteins preventing nonspecific interactions with cell surfaces (1) and perhaps with other lipoproteins. This may serve the function of reducing futile cycles in triglyceride transport by preserving the particles for high affinity interactions such as with lipoprotein lipase or specific cell surface receptors e.g., such as those binding to apoE or apoB. Plasma concentrations of apoC-III in human populations correlate well with triglyceride levels (2, 3). The genetically determined deficiency of apoC-III in humans has been shown to increase the rate of triglyceride clearance from plasma by 6- to 7-fold (4). A similar enhancement of triglyceride clearance was observed in mice made apoC-III deficient by gene knockout experiments (5). Overexpression of apoC-III produces hypertriglyceridemia in transgenic mouse models via inhibition of clearance of TG-rich particles (6). It is now clear that normal physiological systems responsible for triglyceride transport are partially determined by the plasma content of apoC-III. Although it is not clear how this protein contributes to the familial hypertriglyceridemic syndromes, recent studies have found that two classes of drugs that are effective in lowering plasma triglycerides in these patients, act through suppression of apoC-III gene transcription in rodents (7, 8).

In contrast to apoC-III, apoE facilitates the removal of plasma lipoproteins. The initial phase of triglyceride clearance in peripheral tissues is dependent on the endothelial-

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; ISL, intermediate size lipoprotein; HDL, high density lipoprotein; LRP, LDL receptor-related protein; HuCIIITg, human apoC-III transgenic mice; HTGL, hepatic triglyceride lipase; LpL, lipoprotein lipase; apoC-III-0, CIII-1, and CIII-2, isoforms of apoC-III that differs in the number of sialic acid residues; ELISA, enzyme-linked immunosorbent assay.

[1] To whom correspondence should be addressed.

bound lipoprotein lipase. The products of this enzyme action are chylomicron and VLDL remnants. Virtually all of the chylomicron remnants and a large fraction of the VLDL remnants are taken up by the liver and degraded. This uptake of remnants is a high affinity process that is greatly facilitated by apoE (1, 9–11). ApoE may also have important enhancing effects on the action of lipoprotein and hepatic triglyceride lipases (12–16), the enzymes important in the conversion of VLDL remnants into LDL. When apoE is deficient or defective, remnants accumulate and this can lead to dysbetalipoproteinemia (17). However, when apoE is made completely deficient in gene knockout experiments, hypercholesterolemia is the result with only modest increases in plasma triglycerides (18).

The mechanism by which apoC-III reduces triglyceride clearance has been the subject of debate. There is considerable evidence that this is due in great part to competition with apoE, either interfering with its interaction with cell surface receptors or actual displacement of apoE from the surface of TG-rich lipoproteins. In hepatic perfusion studies clearance of VLDL is markedly reduced when apoC-III is added to the lipoproteins in vitro (1, 19). When present in excess, apoC-III has been shown to inhibit lipoprotein lipase and hepatic triglyceride lipase (20–23). Thus, apoE appears to increase hepatic uptake and lipolysis of TG-rich particles, while apoC-III inhibits both processes (13, 14, 24–26).

Both apoC-III and apoE are known to exchange between VLDL and HDL particles (26–31). Blum (31) has shown that short term perturbations can change the distribution of apoE among the particles. Assessing this phenomenon by in vitro experiments is complicated by the presence of exchangeable (loose binding) and non-exchangeable (tight binding) pools of apoE and apoC-III on human VLDL and HDL (32, 33). In some individuals, one-half or more of the mass of these apolipoproteins may be tightly bound to the lipoprotein and essentially non-exchangeable. The determinants of the tight and loose binding among different individuals have not been systematically studied. This paper reports a series of experiments that examine the alterations of apoE distribution among the various lipoproteins produced by adding apoC-III to human plasma. One major determinant of the fraction of apoE which is tightly bound to VLDL is shown to be the size of the lipoprotein particle.

MATERIALS AND METHODS

Isolation and characterization of purified apolipoprotein C-III

Isolation of apoC-III from VLDL was performed after lipid extraction using the procedure reported by Jackson and Holdsworth (34). The apolipoproteins were solubilized from the final residue in aqueous buffer using 6 M urea and 0.1% sodium decyl sulfate. Stock urea solutions used in the experiment were deionized and stored with a mixed cation–anion exchange resin (Rexyn, Fisher, Atlanta, GA) at 4°C. Apolipoproteins C-I, C-II, and C-III were separated from the other urea-soluble proteins by gel-filtration chromatography on a 2.5 × 100 cm Sephadex G-100 (Sigma, St. Louis, MO) column, eluent 50 mM Tris, pH 8.6, in 6 M urea. The fractions were dialyzed against 10 mM Tris, pH 8.2, in 6 M urea and apoC-I, C-II, C-III-0, C-III-1, C-III-2 were separated on a 2.5 × 30 cm anion-exchange column, DEAE-Sephacel (Whatman, Maidstone, England), using a linear gradient of added NaCl from 0 to 0.125 M NaCl (total volume of 1 liter). The apolipoprotein fractions were dialyzed against 10 mM NH_4HCO_3, lyophilized, and stored at −80°C. Purity of each fraction was determined using the PhastGel IEF (Pharmacia, Piscataway, NJ) with pH gradient of 4.0 to 6.5. The mass of apolipoproteins C-II and C-III was determined by specific ELISA (see below).

Incubation of apoC-III

Venous blood was collected in 0.01% EDTA from volunteer subjects (fasting >12 h) with plasma triglyceride concentrations ranging from 125 mg/dl to 510 mg/dl. Fresh plasma from each subject was incubated with or without added apoC-III at 37°C for 2 h, then stored in an ice bath for 2–6 h prior to FPLC separation.

FPLC of plasma incubated with apolipoprotein C-III

VLDL, LDL, and HDL particles from 300 μl of incubated plasma were isolated using the Fast Protein Liquid Chromatography system with a 1.5 × 35 cm Superose 6 column and an eluting buffer of 0.05 M phosphate, 0.10 M NaCl, 0.01% EDTA and 0.02% NaN_3 (35). Lipoprotein profiles for each subject were obtained in 22 0.6-ml fractions (see Fig. 1). Fractions 4–8, 9–16, and 17–22 were pooled to give the VLDL, IDL plus LDL, and HDL, respectively. Lipid and apolipoprotein analyses were performed on these pooled fractions and on the original plasma. Lipid assays demonstrated more than 90% recovery after FPLC. Experiments with total recovery of apolipoproteins below 80% were excluded and repeated. Lipid and apolipoprotein values were normalized for recovery and intra-assay error.

Lipid analysis

All lipid analyses were performed using the Beckman CX5 chemistry analyzer instrument from Beckman (Fullerton, CA). Cholesterol and triglyceride values were determined using Beckman reagents while phospholipids were analyzed using the Wako (Richmond, VA) Phospholipid B reagent.

Apolipoprotein analysis

All apolipoproteins A-I, B, C-II, C-III, and E were analyzed using ELISA as developed in our laboratory. Apolipoproteins B, A-I, and E were quantitated by a double antibody sandwich ELISA technique using purified specific IgG prepared from immunized goats by immunoaffinity chromatography in this laboratory. Both apolipoprotein C-II and C-III mass values were determined by competitive ELISA technique using human VLDL coated plates, goat anti-human IgG, specific for either apolipoprotein C-II or C-III, for first antibody and rabbit anti-goat IgG alkaline phosphatase conjugate as a second antibody. Pooled normal human plasma was used for standards and calibrators. The analyses of incubations performed with plasma from a single subject were done in one assay. All samples were performed in triplicate with a coefficient of variation below 10%.

ApoE genotype analyses

Genomic DNA from potassium EDTA anti-coagulated whole blood was isolated using the procedure outlined by Miller, Dykes, and Polesky (36). ApoE genotypes were determined using the method described by Bolla et al. (37).

Data analysis

Curve-fitting and analysis of the ELISA-derived data were performed using the Immunofit program from Beckman (Fullerton, CA). Statistical calculations, including nonlinear regression anal-

ysis, were performed with Statistica from STATSOFT (Tulsa, OK) programs and graphic presentations were developed with software from Quattro Pro (Corel, Orem, UT).

RESULTS

Apolipoprotein and lipid distribution

Fast protein liquid chromatographic separation of the lipoproteins was performed with plasma samples from different individuals after incubations with varying amounts of added apoC-III. Lipid values (i.e., cholesterol and triglyceride) analyzed on each fraction documented the integrity of the particle size during the incubation and separation procedure (**Fig. 1A** and **B**). Plasma incubated with up to 4 times the intrinsic apoC-III level did not show a measurable effect on the size or lipid content of the different lipoprotein particles. The reproducibility of the elution pattern of apolipoproteins such as apoB in VLDL (Fig. 1C) and ISL/LDL fractions (**Fig. 2**) and of apoA-I in HDL fractions (data not shown) further support this observation. Recovery after FPLC for specific apolipoproteins was greater than 80% while that for lipids was consistently greater than 90%.

Figure 3 shows the loss of apoE from VLDL and ISL particles and its accumulation in the fractions with HDL, as the amount of exogenous apoC-III is increased in the plasma sample. Note that in subject S6, approximately 20% of the apoE in VLDL remains bound to this lipoprotein even with amounts of added apoC-III which were at or near saturation of the binding capacity as indicated by the unbound peaks in Figs. 3A and C. This residual apoE bound to VLDL is referred to as non-displaceable E. Replicate analyses on subject S6 using plasma drawn on a later date confirmed the 80% displacement of apoE upon addition of 4× the baseline value of apoC-III (Fig. 3). The initial addition of apoC-III resulted in binding primarily to VLDL but this reached an apparent maximum with further addition. The content of apoC-III in HDL continued to increase over the entire range tested in these experiments regardless of the basal apoC-III content of HDL (Figs 3A, 3C, and 2B). The plot of the VLDL bound apoE per VLDL particle (VLDL E/VLDL B) suggests that above the molar ratio of 28 to 30 of apoC-III per particle (VLDL apoC-III/VLDL B) no further apoE is displaced. Repeat experiments done with subject S1 were also consistent in demonstrating almost complete displacement of VLDL apoE to HDL at saturating levels of apoC-III (Figs. 2 and 3D) indicating that these results are characteristic of the individual's lipoproteins.

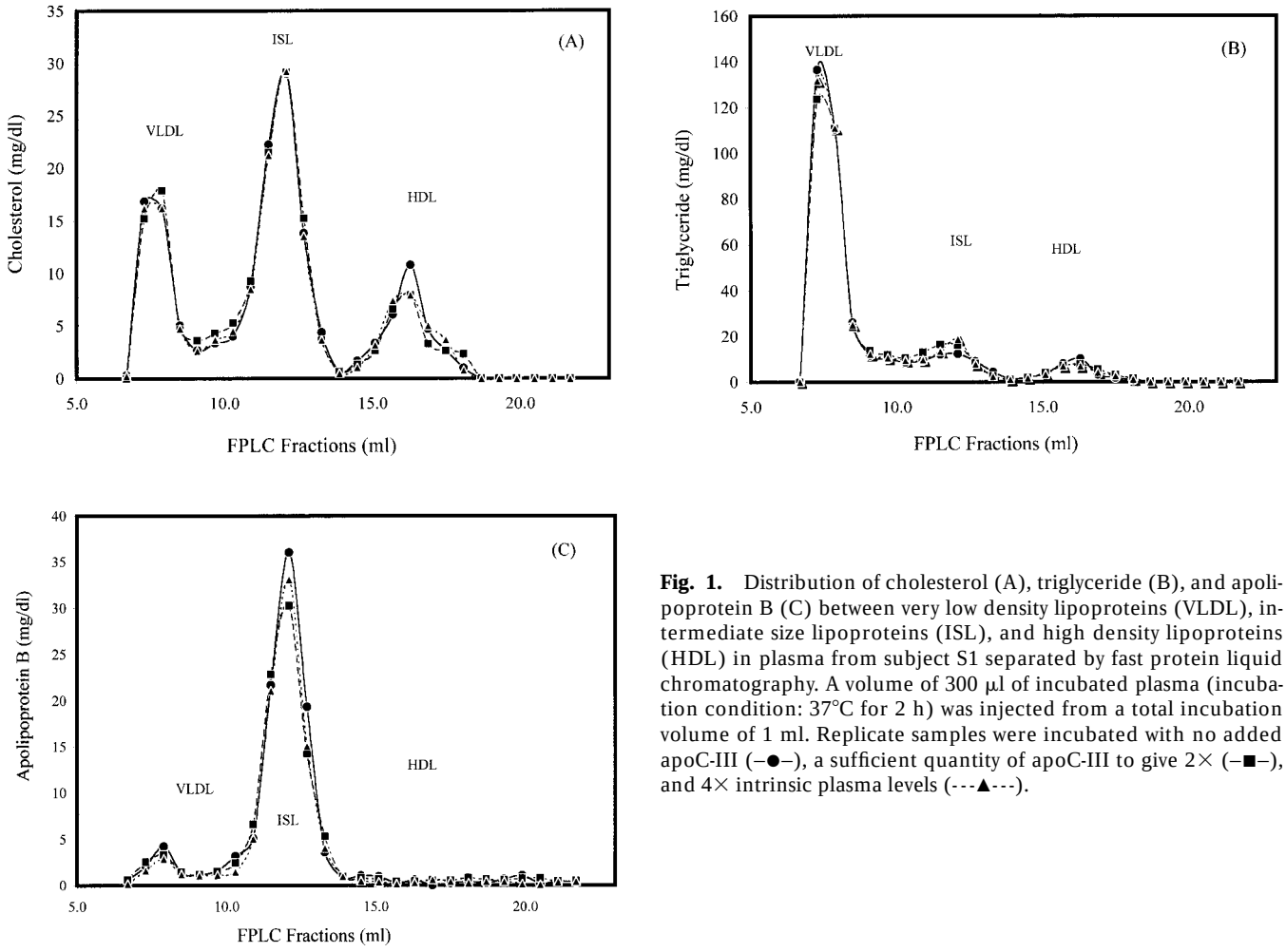

Fig. 1. Distribution of cholesterol (A), triglyceride (B), and apolipoprotein B (C) between very low density lipoproteins (VLDL), intermediate size lipoproteins (ISL), and high density lipoproteins (HDL) in plasma from subject S1 separated by fast protein liquid chromatography. A volume of 300 μl of incubated plasma (incubation condition: 37°C for 2 h) was injected from a total incubation volume of 1 ml. Replicate samples were incubated with no added apoC-III (–●–), a sufficient quantity of apoC-III to give 2× (–■–), and 4× intrinsic plasma levels (---▲---).

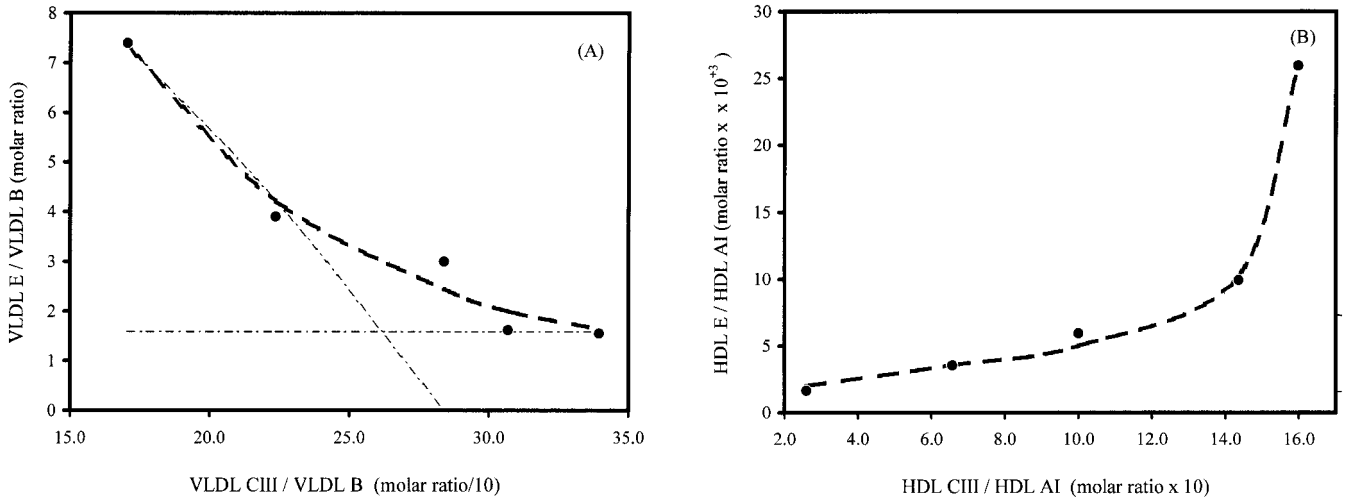

Fig. 2. In subject S6, increasing the concentration of plasma apoC-III by 2.5 times the baseline plasma concentration results in a 4.5-fold decrease in VLDL E per apoB particle and a corresponding increase in HDL E per A-I particle. This graphic display of the bound apoE per particle of VLDL (VLDL E/VLDL B) suggests that above molar ratio of 28 to 30 of apoC-III per particle (VLDL CIII/VLDL B) no further apoE is displaced (plasma apoB = 83.9 mg/dl, VLDL B = 0.375×10^{-2} mm).

Evaluation of non-displaceable pool of apoE

Plasma samples from 10 individuals were studied (**Table 1**) with triglyceride concentrations ranging from 125 to 510 mg/dl (1.41 to 5.77 mmol/l) and cholesterol concentrations from 157 to 332 mg/dl (4.07 mmol/l to 8.60 mmol/l). With the addition of apoC-III over the range discussed above, all samples showed at least partial displacement of apoE from VLDL and ISL to HDL. However, the characteristics of the displacement curves varied widely among individuals studied. Examples of these curves are given in **Fig. 4**. The estimated non-displaceable apoE fraction gave values ranging from 0% to 78% in the ten subjects (**Table 2**).

The binding curves of apoE to VLDL (Fig. 4) can be described by equation 1, which has three adjustable regression parameters, β_0, β_1, β_2.

$$\text{apoE}_{(\text{bound})} = \beta_0 + \beta_1 * e^{-\beta_2 * [\text{apoC-III}]} \qquad \textit{Eq. 1})$$

where $\text{apoE}_{(\text{bound})}$ is the amount of apoE per VLDL particle and [apoC-III] is the total plasma concentration in mmol per liter of apoC-III normalized to the total plasma apoB in mmoles per liter. Normalization of plasma apoC-III with respect to plasma apoB corrects for the variability in the number of apoB-containing particles in the different subjects. The equation $[E] = \beta_0 + \beta_1 \exp(-\beta_2[\text{C-III}])$ is an empirical function that is characteristic of the data and that is known to adequately describe other systems involving ligands with surfaces or receptors. The first parameter, β_0, is the asymptotic amount of apoE bound at very high levels of apoC-III. β_0 represents the non-displaceable E that is characteristic of the specific lipoprotein particles. β_2 represents the effectiveness of apoC-III to displace apoE and is somewhat related to the equilibrium constant, and again can be a characteristic of the binding lipoproteins. β_1 represents the amount of displaceable apoE. The equation allows the assessment of each of these constants in an individual by varying the apoC-III content of plasma over a sufficient range and does not require the addition of apoC-III to saturating levels. Saturation of the VLDL particle with apoC-III is not essential in order to reach near maximum displacement as shown for subject S1 in Figs. 3C and D. In this plasma, almost 99% of apoE was displaced from VLDL before reaching a plateau in the VLDL apoC-III concentration.. No mechanistic interpretation of equation 1 is proposed at this time.

The value of β_0 represents the average number of apoE molecules per particle of VLDL at infinite concentrations of apoC-III. Essentially all or nearly all of the apoE in the VLDL was displaced to HDL in three of these subjects when the maximum amounts of apoC-III were added to the plasma, thus β_0 approaches zero in these subjects. However, the β_1 values varied considerably as measured in moles of apoE displaced per VLDL particle. This constant reflects the sensitivity of the TG-rich particle to the amount of apoC-III added. In these three subjects the average molar ratio of VLDL triglyceride to VLDL apoB ranged from 20,000 to 37,000. A significant increase in the non-displaceable pool of apoE was further observed for VLDL containing more than 50,000 triglyceride molecules per particle.

β_0 is correlated with the average size of the VLDL particles ($r = 0.7560$, $P < 0.001$), as estimated by the number of moles of triglyceride per mole of apoB (**Fig. 5**, Table 2). The ratios of the initial content (per particle) of VLDL apoE, apoC-III, and apoC-II were also found to correlate with β_0 with coefficients $r = 0.88$ ($P < 0.01$), $r = 0.73$ ($P < 0.01$), and $r = 0.71$ ($P < 0.01$), respectively (data not shown). The amount of these exchangeable apolipoproteins increases with the triglyceride content and consequently the size of the particle, thus corroborating the significance of particle size in the affinity of apoE for this

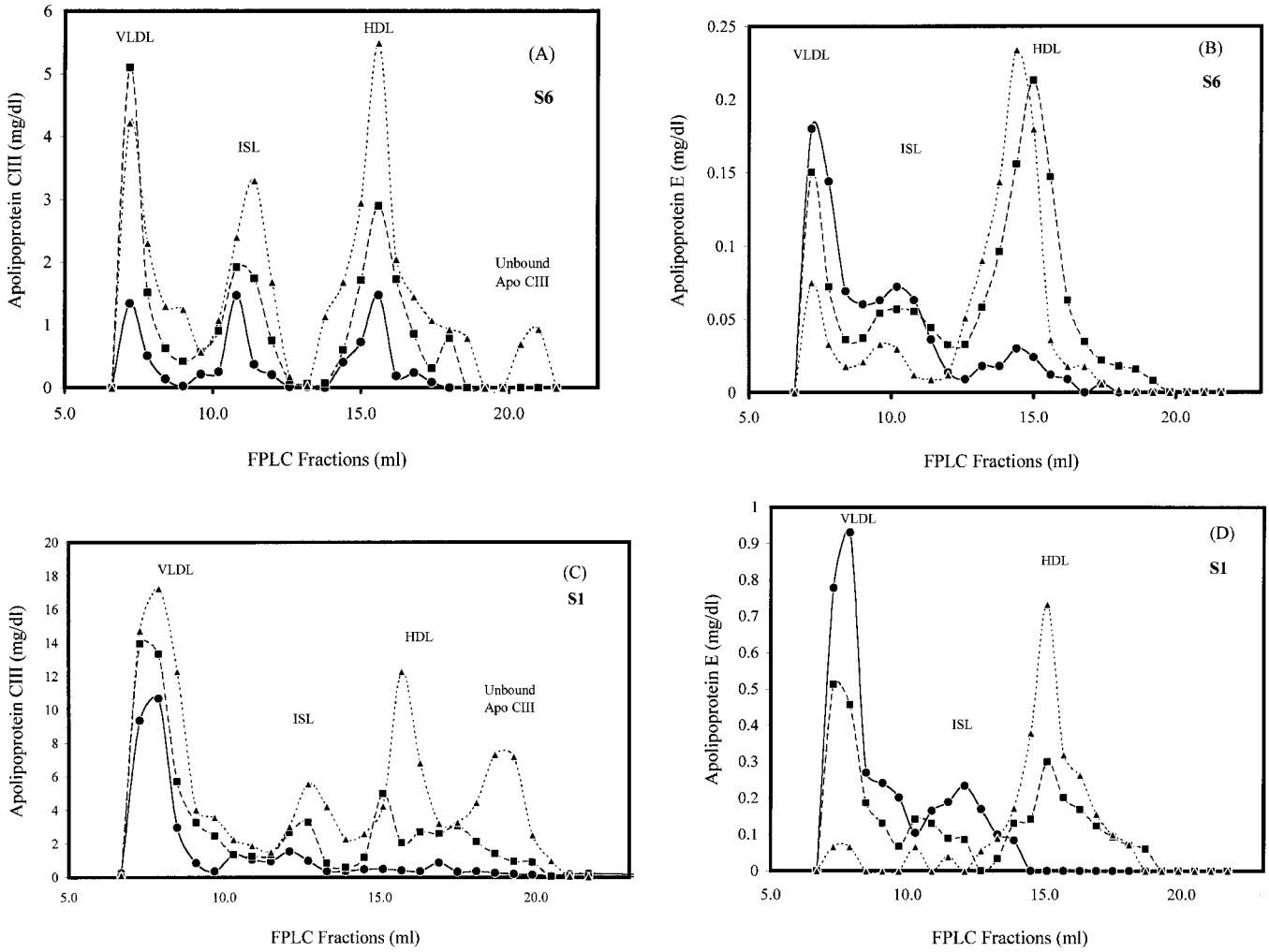

Fig. 3. Displacement of apoE from VLDL to HDL with increasing plasma concentrations of apoC-III. Replicate samples were incubated with no added apoC-III (–●–), a sufficient quantity of apoC-III to give 2× (–■–), and 4× intrinsic levels (---▲---). FPLC profile of apoE in the subject S6 has a β_0 = 1.23 and β_1 = 67.29, while S1 has a β_0 = 0.0 and β_1 = 13.14 (equation 1). Addition of saturating concentration of apoC-III (A) for subject S6 resulted in 80% transfer of apoE (B) to VLDL. A 4-fold increase in apoC-III (C) plasma concentration for subject S1 results in 99% transfer of apoE (D) from VLDL to HDL.

particle. There are no significant correlations of β_0 with HDL cholesterol, total plasma apoB, apoE, and apoC-III. The effect of apoE genotype was also considered in this study of ten subjects but there was no correlation between the different isoforms and parameters in equation 1. The possibility of apoB-48 contributing to the binding affinity of apoE was considered. Electrophoresis of the apolipoproteins from VLDL isolated by ultracentrifugation found no apoB-48 except a trace amount in one out of five subjects.

A second interesting finding from this work was the relatively small capacity of VLDL to bind additional apoC-III. However, HDL appears to have a very large available surface for accepting added apolipoproteins, both apoC-III and apoE (Fig. 2A, B). A small fraction of the unbound apoC-III observed at the highest level of added protein represents variable saturation levels among individuals (Fig. 3A, C).

TABLE 1. Baseline lipid and apolipoprotein levels of ten selected subjects for the study of apoE displacement by apoC-III

Subject	Sex	TRIG	CHOL	ApoC-III	ApoC-II	ApoE	ApoB	ApoA-I
				mg/dl				
S1	M	383	157	13.32	2.90	5.80	107.7	90.0
S2	F	125	197	9.73	1.80	1.10	73.1	145.1
S3	M	437	172	18.08	6.70	4.30	72.2	98.5
S4	M	174	180	11.79	2.80	1.00	71.7	137.0
S5	F	220	329	16.92	5.00	1.70	161.2	120.9
S6	M	261	200	21.10	4.40	1.80	83.9	90.0
S7	M	333	254	28.80	4.30	1.30	110.2	124.4
S8	M	510	331	33.83	5.20	4.50	147.1	162.1
S9	M	174	261	11.37	4.00	4.40	119.5	158.7
S10	M	261	247	15.73	4.40	2.70	91.4	147.4

All subjects have HDL cholesterol within 30–56 mg/dl and LDL cholesterol within 66–250 mg/dl.

DISCUSSION

The existence of equilibrating and non-equilibrating pools of apoE in human VLDL was initially demonstrated by experiments using radiolabeled lipoproteins (33). After adding ^{125}I-labeled VLDL to whole plasma, radioactivity of

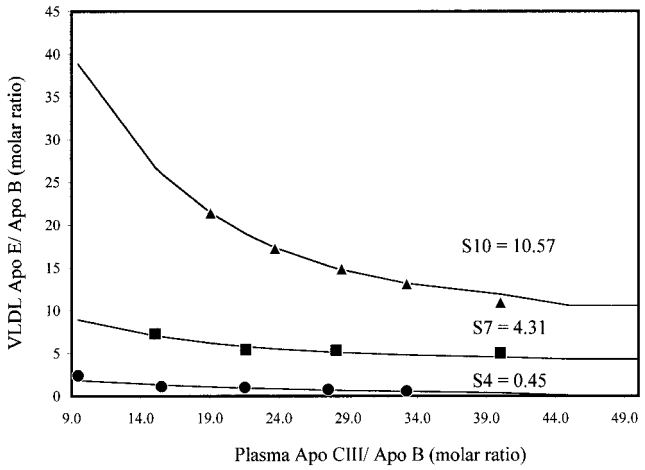

Fig. 4. Application of equation 1 on the mechanism of VLDL E transfer to HDL as a function of increasing concentration of apoC-III in plasma. Extrapolation of the apoC-III concentration allows for an estimation of the parameter β_0 which represents the non-displaceable pool of apoE in VLDL. β_0 varies with different individuals as illustrated with the data from three subjects S4 ([E] = 0.45 + 3.08exp(−0.09[CIII])); S7 ([E] = 4.31 + 11.18exp(−0.093[CIII])); and S10 ([E] = 10.57 + 73.11exp(−0.1001[CIII])).

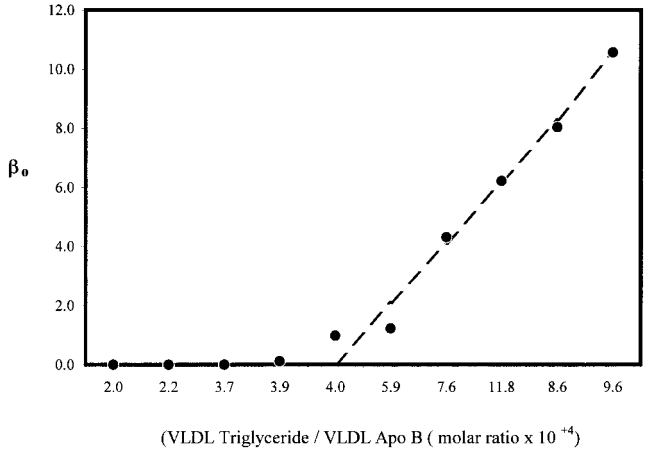

Fig. 5. Plot of β_0 versus the molar ratio of VLDL triglyceride to VLDL apoB ($\beta_0 = (1.023 \times 10^{-4}) \times$ VTRIG/VB − 2.02, $r = 0.756$, $P < 0.001$) for ten subjects as presented in Table 1. The VLDL triglyceride content provides an index of particle size when divided by VLDL particle number, a value equal to VLDL apoB as one molecule of apoB is present in each particle. β_0 increases with VLDL particle size, reaching a value significantly larger than zero when the particle size is greater than 50,000 triglyceride molecules per particle.

apoE in other plasma lipoproteins was increased to a stable but significantly lower value than that of the residual apoE in the VLDL fractions. In those experiments, non-exchangeable pools of apoE were demonstrated in HDL as well. This existence of a tightly bound component is not unique to apoE having been identified in both in vitro and in vivo studies using radioactive tracers of apoC-III (28), apoC-II (32), and apoA-IV (38).

Increased levels of other exchangeable apolipoproteins may also displace apoE from VLDL. ApoC-I, C-II, and C-III were found to prevent binding of added apoE to rabbit β-VLDL, thereby preventing its receptor mediated uptake by cells expressing the LDL receptor-like protein (LRP) (26). ApoC-I was the most effective competitor in those experiments. However, these studies did not examine the displacement of endogenous apolipoproteins on the native lipoproteins and focused on the special case of β-VLDL from hypercholesterolemic rabbits (26).

The findings that larger TG-rich particles contain a greater amount of non-exchangeable apoE conform with

TABLE 2. Relationship between the parameter β_0 with the size of the VLDL particles as measured by the triglyceride to VLDL apoB ratio

Subject	ApoE Genotype	Original Particle Composition[a] (molar ratio) VLDL E/B	VLDL CIII/B (×10)	TRIG/VLDL B (×10⁺⁴)	Percent of Actual VLDL E Displaced[b]	Percent of Theoretical VLDL E Displaced[c]	Calculated Parameters[d] ApoE Molecules/Particle β_0[e]	β_1	β_2
S1	E3/E4	7.70	6.00	2.02	96.4	100	0.00	13.14	0.084
S2	E3/E2	3.46	7.18	3.73	78.0	100	0.00	4.69	0.042
S3	E3/E2	6.28	28.00	2.23	80.0	100	0.00	9.21	0.027
S4	E3/E3	2.05	6.16	3.91	64.0	78	0.45	3.80	0.090
S5	E3/E3	4.95	26.20	4.00	44.0	80	0.98	5.99	0.092
S6	E3/E4	7.39	36.90	5.88	79.0	83	1.23	67.29	0.171
S7	E3/E4	7.27	11.30	7.57	27.0	41	4.31	11.18	0.093
S8	E3/E4	15.73	36.00	11.80	52.0	60	6.21	59.16	0.131‘
S9	—	11.34	18.00	8.61	22.0	29	8.03	10.47	0.256
S10	E3/E3	22.99	67.80	9.57	31.0	54	10.57	73.11	0.100

β_0 is defined as the amount of apoE retained after addition of saturating level of apoC-III. β_0 decreases in value with decreasing VLDL triglyceride per particle.

[a] The assays were performed in triplicate with a coefficient of variation below 10% for apolipoproteins and within the CDC standards for the lipids.

[b] Percent apoE displaced at the highest apoC-III added.

[c] The percent apoE displaced at infinite amount of apoC-III added.

[d] Parameters estimated by using the equation $[\text{VLDL apoE/B}] = \beta_0 + \beta_1 e^{(-\beta 2 * [\text{Plasma apoC-III/B}])}$.

[e] Minimum amount of non-displaceable apoE in the particle.

the demonstration that the full release of apoE for binding to HDL occurs when the VLDL are hydrolyzed in vitro by lipoprotein lipase (31). The observation that the content of apoE is higher in larger TRL (58.2%) compared to smaller remnant-like particles (ISL, 26.4%) in patients with hypertriglyceridemia (Type IV) is also consistent with the result of our experiments (39). However, the tight binding implied by the existence of non-exchangeable apoE does not appear to be a straightforward result of particle size. Experiments using triglyceride phospholipid emulsions (mean sizes 50, 80, 150 nm) with apoE isolated from rat serum found a higher concentration of the apolipoproteins on the smaller particle (40). Conformational studies of apoE using circular dichroism suggested a larger component of α-helical structure when bound to the smaller particles. On incubation with whole plasma, the smaller size emulsions contained higher ratios of apoE to apoC-III. These data suggest that the lower curvature of the surface of larger lipoproteins or the less tight packing of phospholipid molecules on such surfaces are not the necessary elements for binding to the non-exchangeable apoE molecules.

Perhaps a very specific conformation of these apoE proteins predominates during synthesis, providing initial strong binding followed by subsequent conversion to the exchangeable form during metabolism of the VLDL. Another possibility is that other apolipoproteins complex with a limited number of apoE molecules producing a tightly adherent complex. ApoB is known to change its conformation as particles are reduced in size making the region serving as ligand for the LDL receptor more available for interaction (41). Perhaps there is a concomitant reduction in the binding to apoE molecules by the apoB with VLDL hydrolysis. However, if protein–protein interactions are responsible for this phenomenon, the several copies of apoE present on each particle require multiple binding sites on the same or different apolipoproteins.

The common apoE isoforms (E2, E3, and E4) are known to differ in their conformation with resulting effects on receptor affinity (42). However, we found no relationship between the fraction of non-exchangeable apoE and the isoforms present in the plasma specimens from the group of individuals in this study. It should be noted that dysbetalipoproteinemic VLDL with homozygous E2 was not studied in these experiments.

Recent evidence using synthetic peptides containing the LDL receptor binding sequence of amino acids 141–155 from apoE demonstrates that the affinity of apoE for the lipoprotein particle can have a profound effect on the uptake of lipoproteins in vivo (43). When injected intravenously into apoE-deficient mice, weakly binding peptide had little effect on plasma lipoprotein levels. The high affinity of acetylated forms caused a rapid fall in VLDL and IDL levels. The physiological relevance of the tightly bound apoE pool in large VLDL is unclear. In transgenic mice with a marked increased in apoC-III concentration (8- to 15-fold), the residual apoE on VLDL is approximately one-fourth that of the native mouse VLDL. No data regarding the ability of the residual apoE to exchange are currently available. These particles are taken up by liver cells, suggesting that the residual apoE may continue to function as a ligand for cell surface receptors although at a slower rate.

ApoE tightly bound to VLDL may provide for clearance of TG-rich particles under circumstances of low hydrolytic rates and/or when apoC-II and apoC-III content may be excessive. This concept is compatible with the extremely high plasma triglyceride concentrations (4,521 ± 6,394 mg/dl) in apoC-III transgenic mice that have been bred from an apoE knockout background (44). With the native apoE gene present, the triglyceride levels were much lower (957 ± 158 mg/dl) in mice transgenic for human apoC-III (6). If the mouse VLDL particles behave as observed with the human lipoproteins in these studies, very little exchangeable apoE should be present on these particles and thus the functioning apoE would be of the tightly adherent variety. The hypertriglyceridemia must be due to both the inhibitory properties of excessive apoC-III (44) and the displacement of apoE from these triglyceride-rich particles (45).

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