Human apolipoprotein A-IV: displacement from the surface of triglyceride-rich particles by HDL_2-associated C-apoproteins

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Abstract Human apolipoprotein A-IV rapidly dissociates from the surface of lymph chylomicrons following their entry into circulation by an unknown mechanism. We have therefore investigated the binding of human apoA-IV to triglyceride-rich particles and the interaction of these apoA-IV/lipid complexes with human HDL_2. Human apoA-IV was purified from lipoprotein depleted serum (*J. Lipid Res.* 1983. **24**:52–59). Triglyceride-rich particles of well-defined properties were isolated from Intralipid®, a commercially available phospholipid-triglyceride emulsion. Various concentrations of radiolabeled human apoA-IV were incubated at 24 °C with a fixed quantity of lipid particles; the particles were reisolated by centrifugation, and bound and free apoA-IV were quantitated. In 50 mM Tris, pH 7.4, apoA-IV bound to the triglyceride-rich particles in a non-cooperative manner, with a K_d of 2.0 μM. The calculated maximal binding was 4.96×10^{-4} mol of apoA-IV bound per mol of phospholipid. The addition of increasing amounts of human HDL_2 to the incubations caused the progressive dissociation of apoA-IV from the triglyceride-rich particles. Analysis of the reisolated particles by isoelectric focusing demonstrated the presence of C-apoproteins, suggesting their transfer from HDL_2. Addition of purified apoC-III-1 to the incubations at concentrations equivalent to those present in HDL_2 caused a similar dissociation of apoA-IV. HDL_2 was modified to selectively remove C-apoproteins, without alteration of other physical characteristics. This modified HDL_2 was four times less effective in causing apoA-IV dissociation. These results demonstrate that the lipid binding properties of human apoA-IV may be quantitatively examined using triglyceride-rich particles as model chylomicrons. This approach reproduces in vitro the dissociation of apoA-IV that occurs in vivo when mesenteric lymph chylomicrons enter the circulation, and suggests that the primary mechanism for this phenomenon is the transfer of C-apoproteins from high density lipoproteins to the triglyceride-rich particle surface. We hypothesize that this mechanism may play an important role in the modulation of chylomicron apoA-IV content in man.—**Weinberg, R. B., and M. S. Spector.** Human apolipoprotein A-IV: displacement from the surface of triglyceride-rich particles by HDL_2-associated C-apoproteins. *J. Lipid Res.* 1985. **26**: 26–37.

Supplementary key words Intralipid® • high density lipoprotein • lipid binding • lipoprotein modification • apoprotein exchange

Apolipoprotein A-IV is an acidic plasma protein of molecular weight 46,000 which is synthesized in the intestine and liver of several mammalian species (1–4) and man (4–10). The synthesis and secretion of apoA-IV by the enterocytes of the small intestine are significantly increased during lipid absorption (8, 11, 12). The nascent protein combines with lipid in the Golgi apparatus of these cells (13), and is thereafter secreted into mesenteric lymph incorporated onto the surface of triglyceride-rich lipoproteins (2–4, 6). Studies of the intravascular behavior of apoA-IV in the rat and man indicate that it rapidly dissociates from the surface of lymph chylomicrons following their entry into circulation (14, 15); chylomicrons isolated from plasma thus contain little apoA-IV. In the rat, a large fraction of apoA-IV reassociates with high density lipoproteins following dissociation from the surface of chylomicrons (14). The intravascular behavior of human apoA-IV is distinctive in that it does not reassociate with HDL to the same extent as in other species; apoA-IV circulates primarily unassociated with lipoproteins (5–7, 16) with a half-life of less than 24 hr (15).

Recent reports have documented the persistence of apoA-IV on the surface of triglyceride-rich particles in dyslipoproteinemic states characterized by the accumulation of chylomicron remnants (17–19). These observations suggest that apolipoprotein A-IV may affect the hepatic clearance of these particles, and therefore factors influencing the chylomicron affinity of apoA-IV may modulate the intravascular metabolism of chylomicron remnants. It is well established

Abbreviations: HDL, high density lipoprotein; VLDL, very low density lipoprotein; TRP, phospholipid-triglyceride emulsion particles.

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that lymph chylomicrons undergo a complex series of interactions with high density lipoproteins following entry into the circulation (20–22). Although in vitro observations have suggested that the presence of HDL accelerates the dissociation of apoA-IV from chylomicrons (6, 23), the systematic investigation of this phenomenon has been hampered by the difficulty in obtaining human mesenteric lymph from which nascent chylomicrons and purified apoA-IV could be isolated. We have recently described a method for the isolation of human apoA-IV from plasma (9), which yields sufficient quantities of protein to allow the in vitro study of its interaction with model chylomicrons. We report here studies of the binding of human apoA-IV to triglyceride-rich particles isolated from a phospholipid-triglyceride emulsion, and the interaction of these apoA-IV/lipid complexes with human HDL_2.

METHODS

Preparation of apolipoprotein A-IV

Apolipoprotein A-IV used in these studies was isolated from human serum by a modification of the technique of Weinberg and Scanu (9). Blood was obtained from normolipemic male donors following a 12-hr fast. The blood was collected in plastic centrifuge tubes on ice in the presence of 1 mM Na_2EDTA, and allowed to clot for 1 hr. Serum was separated by low speed centrifugation, and its density was raised to 1.25 g/ml by the addition of solid NaCl and NaBr. Centrifugation was performed in a Beckman L8-70 ultracentrifuge in a Ti-60 rotor at 55,000 rpm for 48 hr. The floating lipoproteins were removed by aspiration, and the lipoprotein-free infranatants were dialyzed exhaustively against 150 mM NaCl, 50 mM K-phosphate buffer, pH 7.4, 0.05% Na_2EDTA (henceforth referred to as PBS) at 4°C. To 250 ml of lipoprotein-depleted serum, 250 ml of PBS and 140 g of solid NaCl were added with constant stirring, and the pH was readjusted to 7.4 with solid KOH. Triglyceride-rich particles isolated from 100 ml of Intralipid® (Cutter Corporation) were added, and the mixture was incubated under nitrogen for 1 hr at 37°C. The triglyceride-rich particles were reisolated by centrifugation at 10^6 g-min in an SW-28 rotor, and delipidated with 40 volumes of diethyl ether–absolute ethanol 3:1 at 4°C overnight.

The protein precipitate was collected by centrifugation and dissolved in 50 mM Tris, pH 8.2, and dialyzed against this same buffer for 12 hr at 4°C. Twenty-five mg of protein was applied to a 1.6 × 40 cm column of DEAE cellulose (DE-52, Whatman) equilibrated at 4°C with 50 mM Tris, pH 8.2, 7.2 M deionized urea. The column was washed with 200 ml of equilibration buffer at 15 ml/hr, and was then eluted with a linear gradient from 50 mM to 90 mM Tris, pH 8.2, 7.2 M urea in a total volume of 800 ml. Column effluent was continuously monitored at 280 nm and 4-ml fractions were collected. Fractions containing apoA-IV were exhaustively dialyzed against 5 mM NH_4HCO_3, pH 8.6, at 4°C and lyophylized. Prior to binding studies apoA-IV was solubilized in buffer.

Preparation of HDL_2 and C-apoproteins

VLDL and HDL_2 were prepared by sequential flotation of human serum (24). The VLDL fraction was isolated and recentrifuged at d 1.006 g/ml. Apolipoproteins C-III-1 and C-III-2 were isolated from delipidated human VLDL by anion exchange chromatography (25). The proteins thus prepared gave single bands on alkaline urea polyacrylamide gel electrophoresis and on isoelectric focusing. The HDL_2 fraction was isolated at d 1.125 g/ml, and was recentrifuged at the same density. Prior to displacement studies, the HDL_2 was dialyzed against 50 mM Tris, pH 7.4. The lipoprotein thus isolated gave a band of α_1 mobility on agarose electrophoresis, and a band with an apparent molecular weight of 330,000 on gradient pore electrophoresis.

Preparation of phospholipid-triglyceride emulsion particles (TRP)

Intralipid® 10% was obtained from Cutter Laboratories and stored at 4°C. A 2-ml aliquot was mixed with solid sucrose to yield a final sucrose concentration of 5%. This solution was layered underneath 10 ml of 150 mM NaCl, 0.05% Na_2EDTA, pH 7.0, in a polyallomer tube for the SW-40 rotor, and centrifuged for 30 min at 28,000 rpm at 4°C. The floating lipid cake was recovered by tube slicing and resuspended to its original volume in 50 mM Tris, pH 7.4. The preparation was used within 2 hr.

Radioiodination of apoA-IV

ApoA-IV was iodinated with Na ^{125}I using solid state lactoperoxidase (26). Fifty μg of human apoA-IV in 100 mM phosphate buffer, pH 7.2, was incubated with 1 mCi of Na ^{125}I in the presence of activated Enzymobeads® (Bio-Rad Laboratories) for 20 min. The reaction mixture was then quenched with carrier NaI and was rapidly fractionated on a 1 × 15 cm column of Bio-Gel P-6 (Bio-Rad Laboratories) equilibrated with PBS containing 0.1% bovine serum albumin. The protein fractions of highest iodine incorporation were pooled and dialyzed against 50 mM Tris, pH 7.4. Iodine incorporation was 35% and the specific activity of the radiolabeled protein was 7 μCi/μg. Labeled protein radioactivity comigrated exclusively with unlabeled apoA-IV on SDS and alkaline urea polyacrylamide gel electrophoresis.

Protocol for binding studies

Radiolabeled apoA-IV was added to 500 μl of apoA-IV stock solution to yield solutions giving 171 to 525 cpm/μg of apoA-IV. All binding studies were performed in 800-μl capacity ultracentrifuge tubes in 50 mM Tris, pH 7.4. Each incubation contained 50 μl of TRP solution, varying amounts of apoA-IV stock solution, and sufficient 50 mM Tris, pH 7.4, to bring the volume to 250 μl. The mixtures were then incubated for 30 min at 24°C. ApoA-IV bound to TRP was separated from unbound protein by ultracentrifugation; 25 μl of 60% sucrose in 50 mM Tris, pH 7.4, was added to each tube. Using meticulous care, 350 μl of buffer was layered over the reaction mixtures without mixing of phases. The tubes were then centrifuged in an SW-50.1 rotor, fitted with tube adapters, for 15 min at 30,000 rpm at 4°C. The floating TRP cake containing bound apoA-IV was cleanly separated from the infranate by tube slicing, and was resuspended in 500 μl of buffer. Both the floating TRP and the infranate fractions were assayed for radioactivity to quantitate bound and free apoA-IV. Gamma counting was performed in 10 × 75 mm glass tubes using a Packard 5360 Gamma spectrometer with a sample elevator setting of 1.5 cm. In this configuration, count rate was independent of sample volume. The TRP were further analyzed for recovered phospholipid by the method of Bartlett (27).

In all experiments, the total amount of protein added to each incubation was accounted for by the sum of the bound and free fractions. Chemical assay of free protein in the subnatants yielded values that agreed with those determined by gamma counting. The ratio of bound to free protein determined at each concentration was independent of stock solution specific activity over a 100-fold range. Phospholipid recovery averaged 94.6 ± 3.3%. In control incubations containing no TRP, less than 0.4% of the total counts appeared in the top 50 μl of the tube. Bound apoA-IV was normalized for phospholipid recovery. Binding constants were derived from double reciprocal transformation of the data (28).

Procedure for displacement experiments

The general procedures and conditions of the binding experiments were followed. TRP were precoated with apoA-IV at a concentration of 5 μM. Lipoproteins or apoproteins in 50 mM Tris, pH 7.4, were added at various concentrations. These reaction mixtures were incubated for 30 min at 24°C, following which the TRP were recovered and analyzed as described above. Control incubations containing no added lipoproteins or apoproteins were performed simultaneously, and bound apoA-IV, corrected for phospholipid recovery, was expressed as percent of control. Displacement curves were linearized by log-logit transformation (29). In some experiments the recovered TRP were partially delipidated by washing with several milliliters of diethyl ether, and were analyzed for bound apolipoprotein by analytic isoelectric focusing. To determine the fate of displaced apoA-IV, aliquots of each subnatant were applied to non-denaturing polyacrylamide gradient pore gels. Following electrophoresis the gels were stained, dried, and exposed to Kodak XAR-5 film for 72 hr. The radioautograms were then scanned with a laser densitometer to determine the percent distribution of apoA-IV.

Modification of HDL_2

Human HDL_2 was modified to selectively reduce its C-apoprotein content by incubation with an excess of triglyceride-rich particles. Five mg of HDL_2 in PBS was incubated with 1 ml of TRP solution for 1 hr at 37°C. The TRP were removed from the mixture by centrifugation for 10^6g-min in an SW-40 rotor at 4°C. An additional ml of TRP solution was added to the subnatant and the procedure was repeated. Following the final removal of all TRP, the density of the HDL_2 solution was raised to 1.063 g/ml by the addition of solid NaCl and the solution was centrifuged in a 40.3 rotor at 40,000 rpm for 24 hr at 10°C. The top 2 ml of each tube was discarded and the infranate density was raised to 1.125 g/ml by the addition of solid NaBr. This solution was centrifuged in the 40.3 rotor at 40,000 rpm for 48 hr. The floating HDL_2 was collected at the top of each tube and dialyzed against 50 mM Tris, pH 7.4. Recovery of HDL_2 averaged 54%

Analytic procedures

The protein content of apolipoprotein and lipoprotein solutions was determined by the method of Lowry et al. (30), using crystalline bovine serum albumin as a standard. Protein in the subnatants from the binding studies was determined by the method of Bradford (31). Phospholipid was determined by the method of Bartlett (27), using a conversion factor of 25. Triglyceride was determined by the method of Biggs, Erickson, and Moorehead (32), using triolein as a standard. Free and esterified cholesterol were determined enzymatically (33). Polyacrylamide gel electrophoresis was performed in the presence of SDS (34), or in alkaline 8 M urea (35). Gels were stained with Coomassie blue. The molecular weight of protein bands was established by comparison to the mobility of purified protein standards of known molecular weight. Isoelectric focusing inthe presence of 8 M urea and 2% pH 4–6 Ampholine® (LKB) was performed by the method of Gidez, Swaney, and Murnane (36). Gels were stained as described by Blakesley and Boezi (37). Lipoprotein electrophoresis in 0.7% agarose gel was performed as described by Breckenridge (38). Gradient pore gel electrophoresis was performed in 0.08 M Tris-

borate buffer, pH 8.4, with a linear gradient of 3–30% acrylamide. The molecular weight of HDL_2 was calculated by comparison of its mobility with that of purified protein standards. Isopycnic density gradient centrifugation was performed as described by the method of Nilsson et al. (39). Dialysis was performed at 4°C using Spectrapor-2 tubing (Spectrum Medical Industries). Scanning densitometry of isoelectric focusing gels and radioautograms was performed on a Zeineh SL-504-XL soft laser densitometer (Biomed Instruments). Amino acid analyses of apoproteins were performed on a Dionex 502D amino acid analyzer following 24 hr invacuo hydrolysis in 6 N HCl. Quasi-elastic light scattering of triglyceride-rich particle solutions was performed in the laboratory of Dr. Kenneth Schmitz, University of Missouri, Kansas City, as described (40).

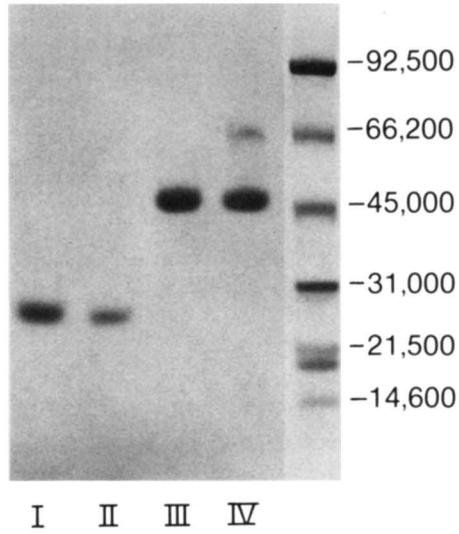

Fig. 2 SDS polyacrylamide gel electrophoresis of fractions from DEAE-cellulose chromatography. Twenty-five micrograms of protein was solubilized in 50 mM Tris, pH 8.6, 1% SDS and applied to each gel. Molecular weights of protein standards are indicated at the right. Roman numerals refer to fractions identified in Fig. 1.

RESULTS

Anion exchange chromatography on DEAE-cellulose in the presence of 7.2 M urea rapidly effected the separation of apoA-IV from the crude mixture of apoproteins absorbed onto Intralipid. Four peaks were characteristically obtained under the conditions described (**Fig. 1**). Molecular weight determination (**Fig. 2**) and amino acid analysis of peaks I and II established them to be apoA-I, although isoelectric focusing showed that peak II was relatively enriched with the more acidic isoforms of this apoprotein (**Fig. 3**). ApoA-IV eluted in peak III, with an apparent molecular weight of 45,900 (Fig. 2) and a major isoform focusing at a pI of 5.48 (Fig. 3). Amino acid analysis of this peak agreed with published values for apoA-IV (9). ApoA-IV thus prepared did not crossreact to monospecific antisera against human serum albumin, LDL, Lp(a), apoA-I, apoA-II, apoE, or apoC-III. This protein was utilized in subsequent binding studies. In some preparations a fourth peak was observed, with an apparent molecular weight of 45,900. Amino acid analysis of this peak was also identical to published values for apoA-IV, yet its isoform pattern was charge shifted to the anode, with the major isoform focusing at a pI of 5.43 (Fig. 3). The molecular properties of this subfraction are currently under investigation.

Triglyceride-rich particles of $S_f > 400$ isolated from Intralipid® constituted 82% of the triglyceride and 35% of the phospholipid present in the emulsion. The lipid composition of the recovered particles was 91.0% triglyceride, 8.6% phospholipid, and 0.4% free cholesterol by weight. No cholesteryl esters or protein were detected. The particles were stable in Tris buffer for at

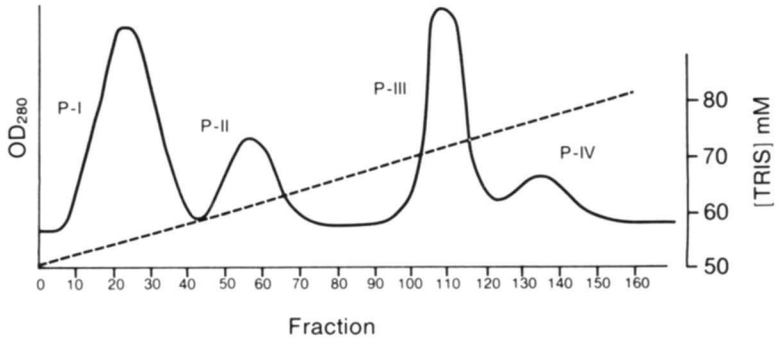

Fig. 1 Anion exchange chromatography of apoproteins absorbed onto Intralipid. Twenty-five milligrams of protein was applied to a 1.6 × 40 cm column of DEAE cellulose equilibrated with 50 mM Tris, pH 8.2, 7.2 M urea. Elution was performed with a Tris gradient from 50 mM to 90 mM in a total volume of 800 ml. Four milliliter fractions were collected.

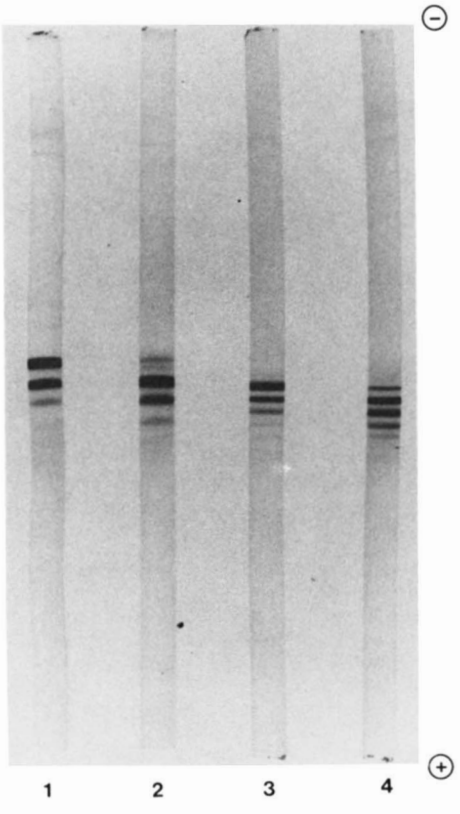

Fig. 3 Analytical isoelectric focusing of fractions from DEAE-cellulose chromatography in the presence of 8 M urea. The gels were 7.5% acrylamide and 2% pH 4–6 Ampholine®. Gel 1, fraction I; gel 2, fraction II; gel 3, fraction III; gel 4, fraction IV.

least 24 hr, as evidenced by near quantitative recovery of phospholipid upon ultracentrifugation. Multiple centrifugations caused a loss of less than 5% of the particles with each flotation; moreover, the triglyceride/phospholipid ratio of the recovered particles remained constant, indicating that the lipid composition of the particles was unchanged by ultracentrifugation. Quasi-elastic light scattering demonstrated that the particles constituted a homogenous population with respect to size, with a mean radius of 1,421 ± 50 Å (n = 30). These particles have been shown to have the same pseudomicellar structure characteristic of plasma lipoproteins (41); thus the lipid composition and particle radius were utilized to calculate the number of phospholipid molecules per particle by the method of Shen, Scanu, and Kezdy (42). By such analysis it was determined that each particle contained 3.444×10^5 molecules of phospholipid, and that the TRP solution prepared as described contained 9.5×10^{12} particles per ml. The density of these particles, as calculated from the known particle volume and lipid composition, was 0.907 g/ml. The physical properties of these particles are summarized in **Table 1.**

Human apolipoprotein A-IV reproducibly bound to the surface of triglyceride-rich particles in 50 mM Tris buffer at pH 7.4. As the concentration of apoA-IV in the incubations was increased, increasing amounts of apoprotein bound to the surface of the particles (**Fig. 4**). The addition of apoA-IV did not appear to alter the stability of the particles, as phospholipid recovery was not altered. The shape of the curve was consistent with a non-cooperative Michealian mode of binding, with saturation occurring at an apoA-IV concentration greater than 10 μM. A double reciprocal transformation of this data was performed to allow calculation of binding constants (**Fig. 5**). ApoA-IV bound to TRP with an apparent dissociation constant K_d of 2.0 μM. At saturation, B_{max} was 4.96×10^{-4} moles of apoA-IV per mole of phospholipid.

HDL_2 significantly influenced the binding of apoA-IV to the particles. The addition of increasing concentrations of human HDL_2 to TRP precoated with apoA-IV caused progressive displacement of the apoA-IV. The addition of HDL_2 did not alter the stability of the particles, as evidenced by near quantitative recovery of TRP phospholipid. Analysis of the particles for free cholesterol revealed that at 24°C there was no significant transfer of free cholesterol from HDL_2 to the surface of the TRP. A log-logit transformation of percent apoA-IV displacement versus HDL_2 concentration yielded a straight line (**Fig. 6**). The intercept of this line with an ordinate value of zero occurred at 342 μg/ml HDL_2 protein, corresponding to that concentration of HDL_2 which displaces 50% of the apoA-IV on the TRP surface. The presence of 1 mM DTNB did not alter the displacement reaction. Isoelectric focusing of delipidated TRP recovered from this experiment demonstrated that, following incubation with the highest concentration of HDL_2, little detectable apoA-IV remained on the surface of the particles; instead, four bands of lower pI were present, which corresponded to apoC-II and three isoforms of apoC-III (**Fig. 7**). Examination of the subnatants by non-denaturing gradient pore electrophoresis and radioautography revealed that very little apoA-IV bound to HDL_2. At an

TABLE 1. Physical properties of triglyceride-rich particles isolated from Intralipid®

Structure[a]	Phospholipid surface monolayer; triglyceride core
Flotation	$S_f > 400$
Density	0.907 gm/ml
Particle radius	1421 ± 50 Å
Lipid composition[b]	
Triglyceride	91.0%
Phospholipid	8.6%
Cholesterol	0.4%

[a]Schoefl (41)
[b]Expressed as percent total weight.

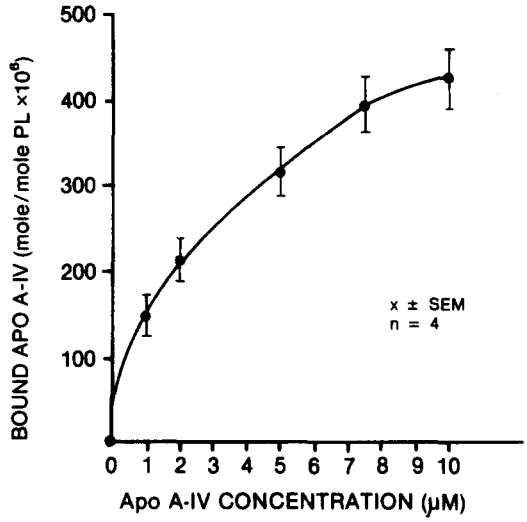

Fig. 4 Binding of human apolipoprotein A-IV to triglyceride-rich particles. Total concentration of apoA-IV in the incubations is indicated in μM along the X-axis.

HDL_2 concentration of 750 μg/ml, only 1.3% of the subnatant total radioactivity was associated with the HDL_2 fraction. The absolute amount of apoprotein bound, as calculated from the known molecular weights and subnatant concentrations of apoA-IV and HDL_2, was 2.9×10^{-2} mol of apoA-IV bound per mol of HDL_2. At lower HDL_2 concentrations, even smaller ratios were observed.

As these findings suggested that HDL_2 apoproteins caused the dissociation of apoA-IV, we next added purified apoC-III-1 to the incubations at concentrations equivalent to those present in HDL_2. We assumed that apoproteins of the C family constitute 10% of HDL_2 protein by weight. ApoC-III-1 was much more potent than HDL_2 on a weight basis in displacing apoA-IV (Fig. 6). However, when expressed as 10% of HDL_2 protein mass, the displacement curve for apoC-III-1 closely approximated that of HDL_2. Identical results were obtained with apoC-III-2. These data provided further evidence that the transfer of C-apoproteins from HDL_2 to the surface of the TRP effected the displacement of apoA-IV. As a correlate of this hypothesis, it would follow that HDL_2 depleted of C-apoproteins would be less effective in displacing apoA-IV from the triglyceride-rich particles. We had previously observed that co-incubation with triglyceride-rich particles reduces the C-apoprotein content of HDL_2 (43). In the present study, HDL_2 recovered following treatment with an excess of triglyceride-rich particles was found to be selectively depleted of C-apoproteins, but otherwise structurally intact (**Table 2**). Analytic isoelectric focusing demonstrated a 90% reduction in the amount of C-apoproteins present (**Fig. 8**). However, no significant change in hydrated density, apparent molecular weight, or agarose electrophoretic mobility was noted. Moreover, no significant change in lipid content occurred with the exception of a reduction in free cholesterol. This modified HDL_2, when added to the incubations, was much less effective in

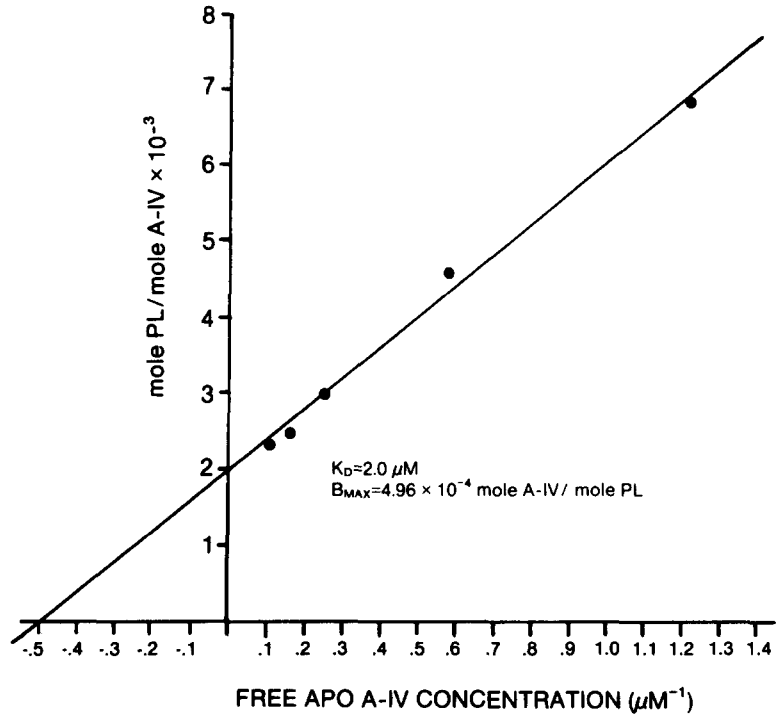

Fig. 5 Double reciprocal plot of the data from Fig. 1. Y-intercept is B_{max}^{-1}. X-intercept is $-K_d^{-1}$.

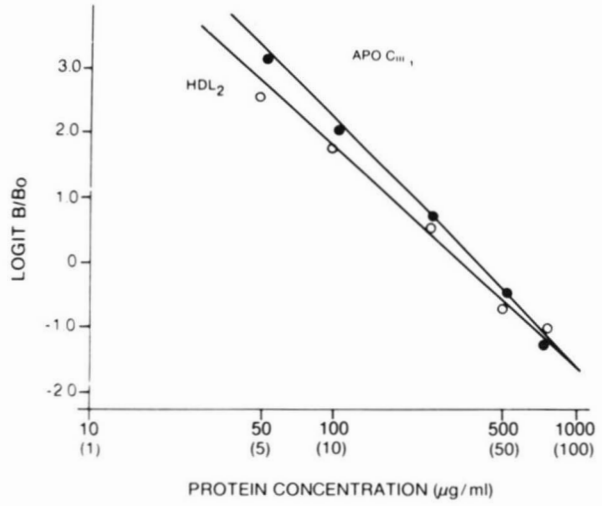

Fig. 6 Displacement of human apolipoprotein A-IV from the surface of triglyceride-rich particles. HDL_2 (○), with protein concentration along X-axis in boldface. ApoC-III-1 (●), with protein concentration along X-axis in parentheses.

displacing apoA-IV (**Fig. 9**). The slope of the displacement curve was considerably shallower and intersected an ordinate value of zero at 1,208 μg/ml, indicating that the concentration of modified lipoprotein required to displace 50% of the apoA-IV, is almost four times greater than native HDL_2. Analytic isoelectric focusing of delipidated TRP showed residual apoA-IV, but did not demonstrate the presence of C-apoproteins. Examination of the subnatants revealed that no detectable radioactivity was associated with the modified HDL_2 fraction.

DISCUSSION

The study of plasma apolipoproteins by in vitro recombination with lipid has yielded important knowledge of their structure-function relationships, and has contributed towards the elucidation of their biologic actions (44). Although several lines of evidence suggest that apolipoprotein A-IV plays as important role in the intravascular metabolism of plasma lipoproteins, relatively little is known of its biophysical properties and biological behavior, undoubtedly because of previous difficulty in its isolation and purification. Our results demonstrate that the lipid binding properties of human plasma apolipoprotein A-IV may be quantitatively examined using particles isolated from a phospholipid-triglyceride emulsion as model chylomicrons. Moreover, this approach reproduces, in vitro, the prompt dissociation of apoA-IV that occurs in vivo when nascent chylomicrons enter circulation (14, 15), and suggests that the primary mechanism for this phenomenon is the transfer of C-apoproteins from HDL to the triglyceride-rich particle surface.

Unilamellar vesicles of pure phospholipid, or mixtures of phospholipid and cholesterol, have been used extensively in the investigation of apolipoprotein/lipid interactions (44). However, because recent evidence indicates that particle size may significantly influence the affinity of purified apoproteins for model lipoproteins (45) and because apoA-IV displays highest in vivo affinity for large triglyceride-rich particles (5–8), we used particles isolated from Intralipid®, a phospholipid-stabilized triglyceride emulsion, to examine the lipid binding properties of apoA-IV. Like plasma lipoproteins, particles isolated from this emulsion have a pseudo-micellar structure, with a phospholipid surface monolayer surrounding a triglyceride core (41). The physical characteristics of these particles demonstrate close similarity to the physical properties of human chylomicrons (46). Moreover, recent investigations have demonstrated that these particles reproducibly interact in vitro with human apolipoproteins (47–50), lipoproteins (43, 49, 51), and lipolytic enzymes (51, 52) in a manner similar to lymph chylomicrons.

The binding isotherm generated by addition of increasing amounts of apoA-IV to a fixed quantity of TRP indicates that this apoprotein binds to TRP by a non-cooperative, saturable mechanism, and implies a reversible interaction between individual protein molecules and equivalent binding sites on the TRP surface. Current theories suggest that the ability of lipoproteins to bind to lipid is dependent upon their

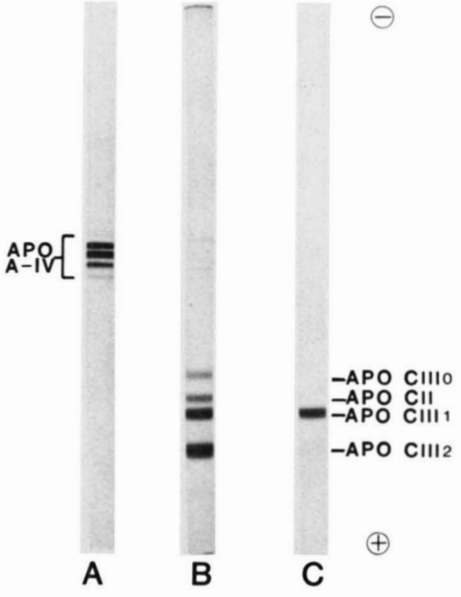

Fig. 7 Analytic isoelectric focusing of apolipoproteins associated with triglyceride-rich particles following displacement studies. Gels are 7.5% acrylamide, 8 M urea, 2% pH 4–6 Ampholine®. A, Control incubations containing only apoA-IV-coated TRP; B, following incubation with 750 μg/ml human HDL_2; C, following incubation with 75 μg/ml apoC-III-1.

TABLE 2. Physical properties of native and modified human HDL_2

	C-Apoprotein[a] Content	Lipid Composition[b] PRT	PL	EC	FC	TG	Apparent Molecular Weight	Average[c] Hydrated Density	Electrophobic Mobility
Native HDL_2	15.8	37.6	30.4	21.3	4.9	5.8	330,000	1.078	α_1
Modified HDL_2	1.7	37.8	34.1	21.8	1.5	4.8	325,000	1.080	α_1[d]

[a]Percent of total protein as determined by densitometry of IEF gel (no correction for relative apoprotein chromogenicity).
[b]Expressed as percent total weight; protein (PRT), phospholipid (PL), cholesteryl ester (EC), free cholesterol (FC), triglyceride (TG).
[c]Expressed in gm/ml.
[d]Slightly delayed.

ability to form amphipathic α-helixes, either spontaneously or in the presence of lipid (44). We have recently reported preliminary data which show that apoA-IV spontaneously assumes α-helical structure at physiologic pH, and that its lipid affinity under various conditions is related to its α-helical content (53). We therefore propose that apoA-IV binds to the surface of TRP by hydrophobic interaction with phospholipid alkyl chains, and that the apolipoprotein molecules intercalate between the phospholipid head groups of the surface monolayer. If this model is correct, then the maximal binding calculated from the known physical properties of TRP and apoA-IV should correspond closely to the experimentally derived B_{max}. The surface area of the TRP accessible to apoA-IV will be the difference of the total surface area, 2.555×10^7 Å^2, and the area occupied by the phospholipid head groups. Using the technique of Shen et al. (42), we calculated from the TRP radius and lipid composition that each particle contains 3.444×10^5 molecules of phospholipid. Assuming that each head group occupies an area of 71 Å^2 (42), the total area occupied by the head groups is 2.445×10^7 Å^2, and thus the surface area available for apoprotein binding is 1.101×10^6 Å^2. Finally, assuming that the area occupied by a single amino acid residue is 15.6 Å^2 (42), and that apoA-IV contains 400 amino acid residues (7), the theoretical maximal number of molecules of apoA-IV per triglyceride-rich particle is 176. This value agrees closely with the observed B_{max} of 4.96×10^{-4} mol of A-IV/mol of phospholipid, or 171 molecules of apoA-IV per triglyceride-rich particle.

The use of physical separation methods in ligand binding studies can result in binding constants which are perturbed from their equilibrium value if the time needed to effect the separation is longer than the rate constant for ligand dissociation, or if the separation itself introduces conditions that directly influence the interaction of the ligands (28). In the case of the association of apolipoproteins and lipids, the rate constants are thought to be of an extremely small order in comparison to the time required for separation by techniques such as ultracentrifugation or column chromatography (54, 55). In the specific case of apoA-IV, it has been demonstrated that repeated ultracentrifugation or agarose gel chromatography of lymph chylomicrons can cause a significant reduction of their apoA-IV content (5, 6, 7). We must, therefore, assume that the K_d for apoA-IV derived by our experimental approach is not a true equilibrium value. We believe, however, that the value has not been significantly perturbed, for it is of the same order of magnitude as dissociation constants calculated for the other apolipoproteins using equilibrium techniques (56, 57). Moreover, the binding phenomena demonstrated by this model are functioning in the same concentration range as that of total plasma apoA-IV in man (7, 8, 9, 15).

Our results suggest that the primary mechanism by which HDL effects the concentration-dependent dissociation of apoA-IV from model chylomicrons is by the

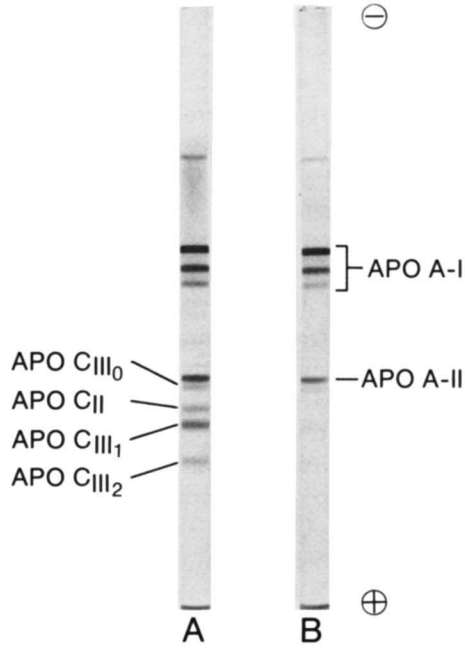

Fig. 8 Analytic isoelectric focusing of native and apoC-depleted human HDL_2. Gels are 7.5% acrylamide, 8 M urea, 2% pH 4–6 Ampholine®. Fifty μg of protein was partially delipidated with diethyl ether and applied directly to each gel. A, Native HDL_2; B, HDL_2 modified by exposure to an excess of triglyceride-rich particles.

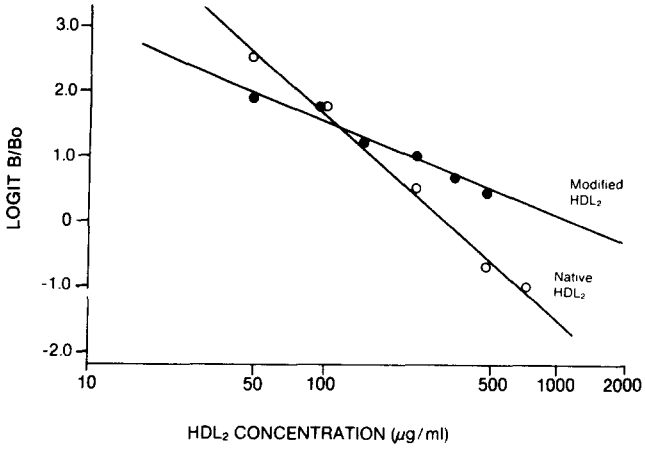

Fig. 9 Displacement of human apolipoprotein A-IV from the surface of triglyceride-rich particles by native HDL_2 (○) and apoC-depleted HDL_2 (●).

transfer of C-apoproteins to the surface of these lipid particles. The non-cooperative nature of the displacement suggests that the biophysical basis for this phenomenon is a differential affinity at equilibrium of the C-apoproteins for the TRP surface. Tajima, Yokoyama, and Yamamoto (45), using large triolein particles similar to those in this study, recently reported that the affinity of several C-apoproteins is almost fourfold greater than our observed value for apoA-IV. Thus, at equilibrium, C-apoproteins partition between the TRP and HDL, displacing apoA-IV from the TRP because of their higher surface activity. In such a scheme HDL serves primarily as a passive reservoir of C-apoproteins, a function which is further suggested by the finding that apoC-III-1 or apoC-III-2, when added in concentrations equivalent to those present in HDL_2, cause a similar dissociation of apoA-IV. HDL does not appear to compete directly with the TRP for apoA-IV, as following the displacement experiments we found very little apoA-IV associated with HDL_2.

The failure of the two major apoproteins of HDL, apoA-I and apoA-II, to participate in this displacement reaction may similarly be explained on the basis of differential surface affinity. Although the K_d of apoA-I for large triolein particles has been determined to be close to our observed value for apoA-IV (45, 49), apoA-I appears to have higher affinity for smaller particles with greater surface curvature (45), and hence in a system containing HDL and large triglyceride-rich particles, the A-I will remain on the smaller HDL surface. Free apoA-II is a potent displacer of other apoproteins in several lipoprotein model systems (55, 58). Nonetheless, its surface activity and mean helical hydrophobic moment are so great (59), that once bound to the surface of HDL, its dissociation becomes highly energetically unfavorable.

An increase in the cholesterol content of phospholipid vesicles or large triolein particles can decrease the binding of apolipoproteins (49, 54), probably by an alteration of the steric properties of the phospholipid surface monolayer. However, in the present study, this mechanism did not contribute to the dissociation of apoA-IV because the incubation conditions precluded the transfer of significant amounts of free cholesterol to the TRP. Although previous studies have shown that the free cholesterol content of TRP can increase up to fourfold following a 2-hr incubation with HDL_2 at 37°C (43, 49), no significant change was noted after 30 min at 24°C. This difference is explained by the observation of McLean and Phillips (60) that the rate constant of cholesterol transfer between egg-yolk phospholipid vesicles is temperature dependent, and is more than tenfold slower at 24°C than 37°C.

Human HDL_2 were easily depleted of C-apoproteins by repeated exposure to TRP. The mechanism of this depletion is, again, likely to be the redistribution of C-apoproteins onto the surface of TRP present in excess. The preservation of the physical-chemical properties of HDL by this maneuver is a feature of other surface displacement techniques which have been used to modify the apoprotein content of this lipoprotein (55). The observation that apoC-depleted HDL_2 is considerably less effective in causing the dissociation of apoA-IV is further evidence that the role of HDL_2 in this reaction is that of a passive reservoir of C-apoproteins. Yet, it is not clear why apoC-depleted HDL_2 remains capable, albeit much less effectively, of causing apoA-IV dissociation. If the small amount of residual C-apoproteins was responsible, then the displacement curve should have been shifted rightward and parallel to the curve for native HDL_2. However, C-apoproteins were not electrophoretically detectable on the recovered TRP, and the curve for the modified HDL_2 had a shallower slope, suggesting the existence of a different and less efficient dissociation mechanism. Although an increased affinity of apoA-IV for the modified HDL_2 would be an attractive alternative mechanism, this was not observed. It is possible that small amounts of other apoproteins, such as apoA-I, were transferred to the TRP in quantities below the limit of detection. Since the K_d of apoA-I for large triolein particles (45) is near that observed for apoA-IV, and since, as noted, it displays higher affinity for HDL, it is expected that the displacement efficiency of this mechanism would be appropriately low.

TRP isolated from Intralipid® demonstrate physical-chemical and physiologic (61, 62) similarity to chylomicrons. Extending the present observations to the behavior of mesenteric lymph chylomicrons entering circulation, we hypothesize that the prompt dissociation of apoA-IV from the surface of these triglyceride-rich lipoproteins is primarily a consequence

of displacement by HDL-associated C-apoproteins, which transfer to their surface. This hypothesis predicts that incomplete dissociation of apoA-IV from intestinal triglyceride-rich lipoproteins will be observed in dyslipoproteinemic states where the C-apoprotein pool available for transfer to these particles is relatively reduced, either because of decreased levels of HDL, or an expanded pool of triglyceride-rich particles, or both. And indeed, several investigators have observed abnormalities of apoA-IV metabolism under such conditions. Beisegel and Utermann (5) found apoA-IV present in the d < 1.006 g/ml lipoproteins in 5 of 30 patients with fasting hypertriglyceridemia, although they did not comment upon any distinguishing characteristics of this subgroup. Nestel and Fidge (19) have documented that, in the hyperlipidemia of chronic renal failure, VLDL and IDL are enriched with apoA-IV, in association with a fourfold elevation of plasma apoA-IV concentration; they attributed these abnormalities to a defect in hepatic clearance of remnant particles of intestinal origin. Arbeny et al. (63) documented the persistence of apoA-IV in VLDL and IDL in association with severe hypertriglyceridemia and profound depression of HDL induced by cholesterol feeding in diabetic rats. Ghisellei et al. (18) found that apoA-IV is present in the beta-VLDL circulating in human apolipoprotein E deficiency.

These observations notwithstanding, abnormalities of apoA-IV metabolism have not been observed in the more common hyperlipidemias, and it is likely that other factors besides surface displacement phenomena influence its lipoprotein affinity and distribution. We have recently observed that apoA-IV present in lymph may undergo an intravascular charge modification that alters its lipid binding ability (64). Furthermore, DeLamatre et al. (65) have demonstrated in the rat that the action of LCAT, and possibly other plasma enzymes, can cause the movement of apoA-IV from the lipoprotein-free fraction onto plasma lipoproteins. Further investigation will be required to elucidate the specific function of human apolipoprotein A-IV in plasma lipoprotein metabolism. Nonetheless, our present studies predict that certain features of its biologic behavior in vivo may be determined by its relatively low lipid affinity, and by the ability of HDL-associated C-apoproteins to displace it from the surface of triglyceride-rich lipoproteins.

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REFERENCES

1. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoprotein: characterization by SDS gel electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
2. Imaizumi, I., R. J. Havel, M. Fainaru, and J-L. Vigne. 1978. Origin and transport of the A-I and arginine-rich apolipoproteins in mesenteric lymph of rats. *J. Lipid Res.* **19:** 1038–1046.
3. Wu, A. L., and H. G. Windmueller. 1978. Identification of circulating apolipoproteins synthesized by rat small intestine in vivo. *J. Biol. Chem.* **253:** 2525–2528.
4. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the d < 1.006 lipoproteins of human and canine lymph, homologous with rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
5. Beisiegel, U., and G. Utermann. 1979. An apolipoprotein homolog of rat apolipoprotein A-IV in human plasma: isolation and partial characterization. *Eur. J. Biochem.* **93:** 601–608.
6. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins: studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
7. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma: isolation and quantitation. *Eur. J. Biochem.* **99:** 333–343.
8. Green, P. H. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV: intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
9. Weinberg, R. B., and A. M. Scanu. 1983. Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. *J. Lipid Res.* **24:** 52–59.
10. Gordon, J. I., C. L. Bisgaier, H. F. Sims, O. P. Sachdev, R. M. Glickman, and A. W. Strauss. 1984. Biosynthesis of human preapolipoprotein A-IV. *J. Biol. Chem.* **359:** 468–474.
11. Krause, B. R., C. H. Sloop, C. K. Castle, and P. S. Roheim. 1981. Mesenteric lymph apolipoproteins in control and ethinyl estradiol-treated rats: a model for studying apolipoproteins of intestinal origin. *J. Lipid Res.* **22:** 610–619.
12. Green, P. H. R., J. H. Lefkowitch, R. M. Glickman, J. W. Riley, E. Quinet, and C. B. Blum. 1982. Apolipoprotein localization and quantitation in the human intestine. *Gastroenterology.* **83:** 1223–1230.
13. Swift, L. L., P. D. Soulé, M. E. Gray, and V. S. LeQuire. 1984. Intestinal lipoprotein synthesis. Comparison of nascent Golgi lipoproteins from chow-fed and hypercholesterolemic rats. *J. Lipid Res.* **25:** 1–13.
14. Fidge, N. H. 1980. The redistribution and metabolism of iodinated apolipoprotein A-IV in rats. *Biochim. Biophys. Acta.* **619:** 129–141.
15. Fidge, N. H., and P. Nestel. 1981. Studies on the in vivo metabolism of A-IV apoprotein in human subjects. *Circulation.* **64:** Supp IV: 159A.

16. Bisgaier, C., O. Sachdev, L. Megna, and R. M. Glickman. 1983. Plasma distribution of human apoA-IV. *Circulation*. **68: Supp III:** 215A.
17. Mahley, R. W. 1978. Alterations in plasma lipoproteins induced by cholesterol feeding in animals including man. *In* Disturbances in Lipid and Lipoprotein Metabolism. J. M. Dietschy, A. M. Gotto, and J. A. Ontko, editors. American Physiological Society, Bethesda, MD. 181–198.
18. Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science*. **214:** 1239–1241.
19. Nestel, P. J., N. H. Fidge, and M. H. Tan. 1982. Increased lipoprotein remnant formation in chronic renal failure. *N. Engl. J. Med.* **307:** 329–333.
20. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins in man. *J. Clin. Invest.* **52:** 32–38.
21. Robinson, S. F., and S. H. Quarfordt. 1978. Chylomicron apoprotein alteration after plasma exposure. *Biochim. Biophys. Acta.* **541:** 492–503.
22. Schaefer, E. J., L. L. Jenkins, and H. B. Brewer. 1978. Human chylomicron apolipoprotein metabolism. *Biochem. Biophys. Res. Commun.* **80:** 405–412.
23. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712–722.
24. Havel, R. J., H. A. Eder, and J. H. Bradgon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
25. Herbert, P. N., R. S. Shulman, R. J. Levy, and D. S. Fredrickson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins. *J. Biol. Chem.* **248:** 4941–4946.
26. David, G. S., and R. A. Reisfeld. 1974. Protein iodination with solid state lactoperoxidase. *Biochemistry.* **13:** 1014–1021.
27. Bartlett, G. R. 1959. Phosphorous assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
28. Levitzki, A. 1980. Quantitative aspects of ligand binding to receptors. *In* Cellular Receptors for Hormones and Neurotransmitters. D. Schulster and A. Levitzki, editors. John Wiley & Sons, New York. 9–28.
29. Zettner, A. 1973. Principles of competitive binding assays (saturation analyses). I. Equilibrium techniques. *Clin. Chem.* **19:** 699–705.
30. Lowry, O. H., N. J. Rosebrough, A. C. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
31. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
32. Biggs, H. G., J. M. Erikson, and W. R. Moorehead. 1975. A manual colorimetric assay of triglycerides in serum. *Clin. Chem.* **21:** 437–441.
33. Gallo, L. L., R. Atasoy, G. V. Vahouny, and C. R. Treadwell. 1978. Enzymatic assay for cholesterol ester hydrolase activity. *J. Lipid Res.* **19:** 913–916.
34. Weber, K., and H. Osborn. 1969. The reliability of molecular weight determination by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
35. Kane, J. P. 1973. A rapid electrophoretic technique for identification of a subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.
36. Gidez, L. I., J. B. Swaney, and S. Murnane. 1977. Analysis of rat serum apolipoproteins by isoelectric focusing. I. Studies on the middle molecular weight subunits. *J. Lipid Res.* **18:** 59–68.
37. Blakesley, R. W., and J. A. Boezi. 1977. A new staining technique for proteins in polyacrylamide gels using Coomassie Brilliant Blue G-250. *Anal. Biochem.* **82:** 580–582.
38. Breckenridge. W. C., and G. F. Maquire. 1981. Quantification of sinking pre-beta lipoprotein in human plasma. *Clin. Biochem.* **14:** 82–86.
39. Nilsson, J., V. Mannickarottu, C. Edelstein, and A. M. Scanu. 1981. An improved detection system applied to the study of serum lipoprotein after single-step gradient ultracentrifugation. *Anal. Biochem.* **110:** 342–348.
40. Parthasarathy, N., and K. S. Schmitz. 1980. Quasi-elastic light scattering by biopolymers. IV. Tertiary collapse of calf thymus DNA in 5.5 M LiCl. *Biopolymers.* **19:** 1137–1151.
41. Schoefl, G. I. 1966. The ultrastructure of chylomicra and of the particles in an artificial fat emulsion. *Proc. Roy. Soc.* **169:** 147–152.
42. Shen, B. W., A. M. Scanu, and F. J. Kezdy. 1976. The structure of human serum lipoproteins inferred from compositional analysis. *Proc. Natl. Acad. Sci. USA.* **74:** 837–841.
43. Weinberg, R. B., and A. M. Scanu. 1982. In vitro reciprocal exchange of apoproteins and nonpolar lipids between human high-density lipoproteins and an artificial triglyceride-phospholipid emulsion (Intralipid). *Atherosclerosis.* **44:** 141–152.
44. Morrisett, J. D., R. L. Jackson, and A. M. Gotto. 1977. Lipid-protein interactions in the plasma lipoproteins. *Biochim. Biophys. Acta.* **472:** 93–133.
45. Tajima, S., S. Yokoyama, and A. Yamamoto. 1983. Effect of lipid particle size on association of apolipoproteins with lipid. *J. Biol. Chem.* **258:** 10073–10082.
46. Zilversmit, D. B. 1965. The composition and structure of lymph chylomicrons in dog, rat, and man. *J. Clin. Invest.* **44:** 1610–1622.
47. Sata, T., R. J. Havel, L. Kotite, and J. P. Kane. 1976. New protein in human blood plasma, rich in proline, with lipid binding properties. *Proc. Natl. Acad. Sci. USA* **73:** 1063–1067.
48. Polz, E., and G. M. Kostner. 1979. Binding of B_2-glycoprotein I to Intralipid: determination of the dissociation constant. *Biochem. Biophys. Res. Commun.* **90:** 1305–1312.
49. Erkelens, D. W., C. Chen, C. D. Mitchell, and J. A. Glomset. 1981. Studies of the interaction between apolipoproteins A and C and triacylglycerol-rich particles. *Biochim. Biophys. Acta.* **665:** 221–233.
50. Connelly, P. W., and A. Kuksis. 1983. Influence of divalent cations on rat apolipoprotein transfers to synthetic lipoprotein-like lipid emulsions in vitro. *Can. J. Biochem. Cell Biol.* **61:** 63–71.
51. Glomset, J. A., C. D. Mitchell, W. C. King, K. A. Applegate, K. R. Norum, and E. Gjone. 1980. In vitro effects of lecithin:cholesterol acyltransferase on apoprotein distribution in familial lecithin: cholesterol acyltransferase

deficiency. *Ann. N.Y. Acad. Sci.* **348:** 224–243.
52. Huttunen, J. K., and E. A. Nikkilä. 1973. Postheparin plasma lipase in human subjects. Studies with chylomicrons, very low density lipoproteins and a fat emulsion. *Eur. J. Clin. Invest.* **3:** 483–490.
53. Weinberg, R. B., and M. S. Spector. 1984. Apolipoprotein A-IV: structural determinants of lipid binding. *Clin. Res.* **32:** 215A.
54. Pownall, H. J., J. B. Massey, S. K. Kusserow, and A. M. Gotto. 1979. Kinetics of lipid-protein interactions: effects of cholesterol on the association of human plasma high-density apolipoprotein A-I with L-α-dimyristoylphosphatidylcholine. *Biochemistry.* **18:** 574–579.
55. Lagocki, P., and A. M. Scanu. 1980. In vitro modulation of the apolipoprotein composition of high density lipoprotein. *J. Biol. Chem.* **255:** 3701–3706.
56. Trauble, H., G. Middlehoff, and V. W. Brown. 1974. Interaction of a serum apolipoprotein with ordered and fluid lipid bilayers. Correlation between lipid and protein structure. *FEBS Lett.* **49:** 269–275.
57. Gwynne, J., G. Palumbo, H. B. Brewer, and H. Edelhoch. 1975. The interaction of apoA-I from human high density lipoprotein with lysolecithin. *J. Biol. Chem.* **250:** 7300–7306.
58. Mao, S. J. T., K. Kluge, and S. J. Squillace. 1981. Apolipoprotein A-II can incorporate into plasma very low density lipoproteins. *FEBS Lett.* **132:** 289–292.
59. Krebs, K. E., and M. C. Phillips. 1983. The helical hydrophobic moments and surface activities of serum apolipoproteins. *Biochim. Biophys. Acta.* **754:** 227–230.
60. McLean, L. R., and M. C. Phillips. 1982. Cholesterol desorption from clusters of phosphotidylcholine and cholesterol in unilamellar vesicle bilayers during lipid transfer or exchange. *Biochemistry.* **21:** 4053–4059.
61. Hallberg, D. 1965. Studies on the elimination of exogenous lipids from the bloodstream. The kinetics of the elimination of a fat emulsion studied by single injection technique in man. *Acta Physiol. Scand.* **64:** 306–313.
62. Erkelens, D. W., J. D. Brunzell, and E. L. Bierman. 1979. Availability of apolipoprotein C-II in relation to the maximal removal capacity for an infused triglyceride emulsion in man. *Metabolism.* **28:** 495–501.
63. Arbeeny, C. M., D. Edelstein, S. R. Freedman, and H. A. Eder. 1980. Serum lipoproteins of diabetic rats fed a high cholesterol diet. *Diabetes.* **29:** 774–777.
64. Weinberg, R. B., and M. S. Spector. 1984. Intravascular charge modification influences the chylomicron affinity of human apolipoprotein A-IV. *Gastroenterology.* **86:** 129A.
65. DeLamatre, J. G., C. A. Hoffmeier, A. G. Lacko, and P. S. Roheim. 1983. Distribution of apolipoprotein A-IV between the lipoprotein and the lipoprotein-free fractions of rat plasma: possible role of lecithin: cholesterol acyltransferase. *J. Lipid Res.* **24:** 1578–1585.