Dynamic interfacial properties of human apolipoproteins A-IV and B-17 at the air/water and oil/water interface

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Abstract **Viscoelastic behavior of proteins at interfaces is a critical determinant of their ability to stabilize emulsions. We therefore used air bubble surfactometry and drop volume tensiometry to examine the dynamic interfacial properties of two plasma apolipoproteins involved in chylomicron assembly: apolipoprotein A-IV and apolipoprotein B-17, a recombinant, truncated apolipoprotein B. At the air/water interface apolipoproteins A-IV and B-17 displayed wide area-tension loops with positive phase angles indicative of viscoelastic behavior, and suggesting that they undergo rate-dependent changes in surface conformation in response to changes in interfacial area. At the triolein/water interface apolipoprotein A-IV displayed maximal surface activity only at long interface ages, with an adsorption rate constant of 1.0×10^{-3} sec^{-1}, whereas apolipoprotein B-17 lowered interfacial tension even at the shortest interface ages, with an adsorption rate constant of 9.3×10^{-3} sec^{-1}. Apolipoprotein A-IV displayed an expanded conformation at the air/water interface and a biphasic compression isotherm, suggesting that its hydrophilic amphipathic helices move in and out of the interface in response to changes in surface pressure. We conclude that apolipoproteins A-IV and B-17 display a combination of interfacial activity and elasticity particularly suited to stabilizing the surface of expanding triglyceride-rich particles.**—Weinberg, R. B., V. R. Cook, J. A. DeLozier, and G. S. Shelness. **Dynamic interfacial properties of human apolipoproteins A-IV and B-17 at the air/water and oil/water interface.** ***J. Lipid Res.*** **2000.** 41: **1419–1427.**

Supplementary key words surface tension • interfacial elasticity • drop volume tensiometry • chylomicron assembly • cholesterol • monolayers • triglyceride-rich particles

Apolipoprotein A-IV (apoA-IV) is a 46-kDa plasma glycoprotein (1), which makes it the largest member of a family of lipid-binding proteins that regulate plasma lipoprotein metabolism (2). ApoA-IV is synthesized by the intestinal enterocytes of mammalian species (3) during lipid absorption (4, 5), and enters the circulation on the surface of lymph chylomicrons (6, 7). ApoA-IV is a hydrophilic protein, and has the weakest lipid affinity of the human apolipoproteins (8–10). Consequently, its binding to lipoproteins is labile and sensitive to processes that alter the physical state of the lipoprotein surface (1, 11–15). We have proposed that this behavior allows apoA-IV to function as a barostat that maintains lipoprotein surface pressure and lipid packing within the critical limits required for maximal activity of enzymes and transfer proteins (16–18).

Although a broad spectrum of physiologic functions has been proposed for apoA-IV (19, 20), a preponderance of evidence suggests that its primary biological role is in intestinal lipid absorption (20). Furthermore, the observations that *1*) Pluronic L-81, a hydrophobic surfactant, selectively and simultaneously blocks intestinal synthesis and secretion of apoA-IV and chylomicrons (21–23); *2*) enterocyte apoA-IV synthesis does not increase during absorption of short-chain fatty acids (24), which are transported directly into portal blood rather than packaged into chylomicrons; and *3*) plasma apoA-IV levels are decreased in abetalipoproteinemia (6, 25) and hypobetalipoproteinemia (1), genetic disorders in which chylomicron synthesis is impaired, together suggest that apoA-IV plays a specific role in the process of chylomicron assembly.

Chylomicron assembly is the final and essential step in the absorption of dietary lipids. In the first stage of chylomicron assembly (26) apoB-48 is transcribed in the rough endoplasmic reticulum of intestinal enterocytes and is cotranslationally lipidated with a small amount of triglyceride by microsomal triglyceride transfer protein to form 100-Å-diameter nascent particles. Absence or truncation of apoB-48 (27), or absence or inhibition of microsomal triglyceride transfer protein (28), arrests this process. In the second stage, the nascent chylomicron particles, which already have apoA-IV on their surface (29), are transported to the Golgi apparatus, where they acquire additional triglyceride molecules and expand to diameters of 5,000–10,000 Å. Pluronic ethylene-propylene copolymer surfactants specifically block this stage (21–23). Finally, mature chylomicrons are exocytosed from the basolateral cell

Abbreviations: apo, apolipoprotein; DMPC, 1-α-dimyristoyl phosphatidylcholine; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

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membrane into the mesenteric lymphatics, from where they ultimately reach the circulation.

The thermodynamic requisites for the dispersion of hydrophobic dietary lipids within the intracellular aqueous milieu as large, stable particles are much the same as for the formation of an oil-in-water macroemulsion (30). In this regard, the elastic behavior of proteins at interfaces is a critical determinant of their ability to stabilize foams and emulsions (30). Although the interaction of plasma apolipoproteins with lipid monolayers has been extensively studied by surface balance techniques (16, 31–35), these approaches afford little insight into their dynamic behavior in the setting of rapidly changing interfacial geometry. Therefore, we used air bubble surfactometry and drop volume tensiometry to examine the dynamic interfacial properties of apoA-IV at the air/water and oil/water interface. As an important point of comparison we also studied the dynamic interfacial properties of apoB-17, a truncated form of human apolipoprotein B that includes the amino-terminal α_1 helical domain that is essential for the initiation of triglyceride-rich particle assembly (36) and is soluble in aqueous buffer.

EXPERIMENTAL PROCEDURES

Lipids and surfactants

l-α-Dimyristoyl phosphatidylcholine (DMPC) and triolein (Sigma, St. Louis, MO) were >99% pure by thin-layer chromatography on silica gel. DMPC was diluted to 0.1 mg/ml in high performance liquid chromatography-grade chloroform (Aldrich, Milwaukee, WI) and stored under nitrogen at −20°C. Phospholipid concentration was confirmed by phosphorus assay (37). Pluronic surfactants L-81, L-84, and 25R4 were obtained from BASF (Mount Olive, NJ).

Purification of human apoA-IV and apoA-I

ApoA-IV was isolated from lipoprotein-depleted plasma obtained from donors with the A-IV-1/1 genotype by adsorption to a phospholipid-triglyceride emulsion (38). ApoA-I was isolated from high density lipoproteins (39). Both apolipoproteins were purified by anion-exchange high pressure liquid chromatography, and were homogeneous by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Apolipoprotein concentration was determined by the bicinchoninic acid protein assay (40).

Cloning, expression, and purification of human apoB-17

The FLAG epitope (DYKDDDDK) was appended to the amino-terminal 17% of human apoB (amino acids 1–772 of apoB-100) by polymerase chain reaction. The modified apoB construct was subcloned into the vector pCMV5 and sequenced to confirm its identify. ApoB-17F was excised from the vector, using the flanking *Eco*RI (5′) and *Kpn*I (3′) restriction enzyme sites, and ligated to *Eco*RI- and *Kpn*I-digested pFastBac1 (Life Technologies, Rockville, MD). This plasmid was transposed into DH10Bac competent cells; recombinant baculovirus DNA was isolated and transfected into Sf9 cells at a density of 9×10^5 cells/35-mm dish. Virus was harvested after 72 h and titered by plaque assay. Viral stock was amplified by infecting a 1-liter culture of 3.2×10^6 Sf9 cells/ml in suspension in Sf00-II medium (Life Technologies) at a multiplicity of infection of 0.5.

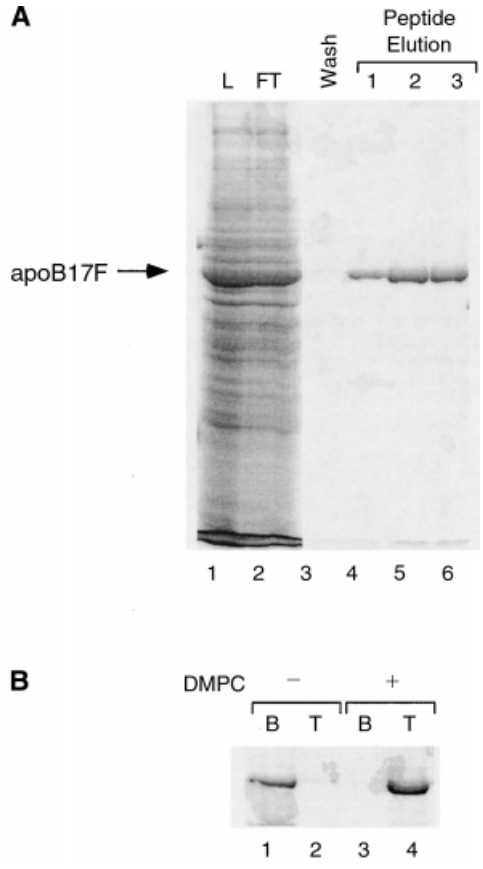

Fig. 1. Purification and vesicle binding of apoB-17F. (A) Supernatant from baculovirus-infected Sf9 cells was loaded onto an M2-agarose immunoaffinity column. The column was washed, and apoB-17F was eluted by FLAG peptide. Fractions were analyzed by 8% SDS-PAGE with Coomassie blue staining. Lane 1, Sf-9 cell supernatant loaded onto the column (L); lane 2, column flowthrough (FT); lane 3, final column wash; lanes 4–6, affinity-purified apoB-17 sequentially eluted by FLAG peptide. (B) Affinity-purified apoB-17F was incubated with (+) and without (−) DMPC vesicles for 20 h and analyzed by density gradient ultracentrifugation followed by 8% SDS-PAGE with Coomassie staining. Lanes 1 and 3, $d > 1.21$ g/ml bottom (B) fractions; lanes 2 and 4, $d < 1.21$ g/ml top (T) fractions.

After 48 h the cells were pelleted, and the supernatant (250 ml) was made 1 mM phenylmethylsulfonyl fluoride, 0.05% sodium azide, 0.5% Triton X-100, and applied at 3 ml/min to a 2-ml bed volume of anti-FLAG monoclonal antibody M2-conjugated agarose beads (Sigma). Most apoB-17 appeared in the effluent (**Fig. 1A**, lanes 1 and 2), presumably because the amount of protein present exceeded the column binding capacity; the flowthrough fraction was reapplied under the same conditions. The column was washed with phosphate-buffered saline (PBS), and apoB-17 was eluted with PBS containing FLAG peptide at 150 μg/ml (Fig. 1A, lanes 4–6). Eluate was concentrated with Centricon-10 centrifugal concentrators (Amicon-Millipore, Danvers, MA). ApoB-17 concentration was determined by densitometry of 8% SDS-polyacrylamide gels stained with Coomassie blue, using bovine serum albumin as a mass standard.

Binding of human apoB-17 to multilamellar vesicles

Multilamellar vesicles were made by adding DMPC in $CHCl_3$ to a glass flask, removing the solvent, suspending the lipid in PBS with 0.05% azide, and vortexing. ApoB-17 (200 μg/ml) was

incubated in the absence or presence of vesicles (lipid:protein weight ratio, 20:1) at 24°C with gentle inversion, and the optical density at 325 nm versus buffer was measured at 0, 0.25, and 20 h. Aliquots were adjusted to 1.25 g/ml with solid KBr, and subjected to density gradient centrifugation in a Beckman (Palo Alto, CA) TL-100 rotor (36). Protein was precipitated from the top d < 1.25 g/ml and bottom d > 1.25 g/ml fractions with 10% trichloroacetic acid and quantitated by 8% SDS-PAGE with Coomassie blue staining.

Dynamic behavior of apolipoproteins at the air/water interface

Dynamic interfacial behavior at the air/water interface was examined with a pulsating bubble surfactometer (Electronetics, Amherst, NY). This instrument sinusoidally oscillates a tiny air bubble in a plastic chamber filled with aqueous sample, records bubble pressure (P) as a function of bubble radius (r), and calculates interfacial tension (γ) from the Young-Laplace equation, $\Delta P = 2\gamma/r$ (41). Studies were conducted at 25°C with apolipoproteins at 0.4 mg/ml and Pluronic detergents at 0.02% (v/v) in 50 mM Tris, 100 mM NaCl, pH 7.5. Interfacial tension at minimum bubble radius reached stable values within 15 min of the start of bubble cycling. Thereafter, surface area (A)-tension (γ) loops were recorded at 20 cycles/min. Surface pressure at the air/water interface was taken as $\gamma_{buffer} - \gamma_{sample}$. Absolute elasticity ($\varepsilon$) was calculated as $\Delta\gamma/(\Delta A/A)$, and the viscoelastic component (ε_v) was calculated as $\varepsilon \sin(\phi)$, where ϕ is the phase angle of the γ-A loops (42, 43).

Dynamic behavior of apolipoproteins at the oil/water interface

Dynamic interfacial behavior at the oil/water interface was studied with a Kruss USA (Charlotte, NC) DVT-10 drop volume tensiometer. This instrument measures the interfacial tension between two immiscible liquids by pumping the lighter phase through a tungsten carbide capillary into a cuvette filled with the heavy phase and timing the drop interval with a photocell. At the instant of drop detachment the separation force equals the attachment force: $V_{drop}(\sigma_H - \sigma_L)g = \gamma\pi d$, where σ_H is the density of the heavy phase, σ_L is the density of the light phase, g is the gravitational constant, and d is the orifice diameter. Because the infusion rate is set, the drop interval gives V_{drop} and yields γ (44). Triolein (σ = 0.91 g/ml) served as the light phase and 41.3 mM Tris, pH 7.5 (σ = 0.9994 g/ml), served as the heavy phase. Apolipoproteins were studied at 10 μg/ml and Pluronic detergents at 0.02% (v/v) at light-phase flow rates of 5.0–0.02 ml/h. Rate constants were calculated by fitting the data to a Langmuir derived adsorption equation $\gamma(t) = \gamma_E \exp[A \exp(-kt)]$ (45). The effect of apoA-IV on the adsorption of cholesterol to the oil/water interface was studied by measuring the interfacial tension of increasing concentrations of free cholesterol ([CH]) dissolved in triolein, in the absence and presence of apoA-IV in the aqueous phase, at a light-phase flow rate of 0.2 ml/h. Maximal interfacial cholesterol concentration (Γ) was calculated from the Gibbs adsorption equation, $\Gamma = -(d\gamma/d\ln[CH])/RT$ (46).

Pressure-area isotherms of apoA-IV and apoA-I at the air/water interface

Mean molecular area (A)-surface pressure (Π) isotherms of apoA-IV and apoA-I at the air/water interface were determined with a KSV 5000 Langmuir film balance (KSV Instruments, Helsinki, Finland), equipped with a computer-controlled compression barrier and a Wilhelmy plate electrobalance, and enclosed in a cabinet maintained at 25°C and 70–75% relative humidity. A 150 × 250 mm Teflon trough was filled with degassed 50 mM Tris, 100 mM NaCl, pH 7.5, or 2 M KCl, 5 mM Tris, pH 7.5, and the buffer surface was cleaned by vacuum aspiration. Apolipoprotein solutions diluted to 0.10 mg/ml in buffer with 30% (v/v) ethanol were layered on the surface with a glass rod to initial pressures of <1 mN/m (47). Equal masses of apoA-IV and apoA-I were spread to enable comparison of an equivalent number of α helices. Protein films were left to equilibrate for 30 min, and were then isokinetically compressed at 5 mm/min. Before monolayer collapse the barrier was reversed back to its starting point. A-Π isotherms were calculated from the barrier displacement and the mass of protein spread on the surface; inflection points were identified from second derivatives of the curves. The interfacial molecular weight of apoA-IV was calculated by analyzing the low pressure region of it's a-Π isotherm with the ideal gas equation, $\Pi A = \Pi A_0 + nRT$, where A is total surface area and A_0 is the limiting area occupied by n moles of protein (48). Thus the intercept of a plot of $\Pi \cdot A$ versus Π yields the interfacial molecular weight, and the slope yields the limiting residue area.

RESULTS

Binding of human apoB-17 to DMPC multilamellar vesicles

When apoB-17 was incubated with DMPC multilamellar vesicles, the optical density at 325 nm compared with a buffer blank decreased from 1.4 to 0.9 at 15 min and to 0.3 at 20 h (data not shown). In the absence of DMPC vesicles most of the apoB-17 was recovered in the d > 1.25 g/ml (Fig. 1B, lanes 1 and 2); however, when apoB-17 was incubated with DMPC vesicles, most of the apoB-17 floated the d < 1.25 g/ml top fraction (Fig. 1B, lanes 3 and 4). These data establish that recombinant apoB-17 can bind to large multilamellar DMPC vesicles to form smaller vesicles and discoidal recombinant lipoproteins, as previously described (49).

Dynamic properties of apolipoproteins at the air/water interface

Apolipoproteins and Pluronic detergents bound to the air/water interface as evidenced by a decrease in bubble surface tension. Of the apolipoproteins, apoB-17 displayed the lowest interfacial tension and highest surface pressure at minimum (resting) bubble radius (**Table 1**). The FLAG peptide at equivalent concentrations demonstrated no surface activity; this suggests that FLAG epitope did not contribute to the interfacial behavior of recombi-

TABLE 1. Dynamic interfacial properties at the air/water interface

	γ	Π	ε	ϕ	ε_v
	mN/m	*mN/m*	*mN/m*	*deg*	*mN/m*
ApoB-17	34.0	37.8	19.0	76.3	18.4
ApoA-I	39.8	32.0	7.6	12.4	1.6
ApoA-IV	42.5	29.3	21.7	28.0	10.2
Pluronic L-81	33.3	38.5	2.8	ND	ND
Pluronic L-84	41.3	30.5	2.8	ND	ND
Pluronic 25R4	44.0	27.8	2.7	ND	ND

γ, Interfacial tension at minimum (resting) bubble surface area; Π, surface pressure at minimum (resting) bubble surface area; ε, elasticity; ϕ, γ-area loop phase angle; ε_v, viscous elastic component; ND, not determined.

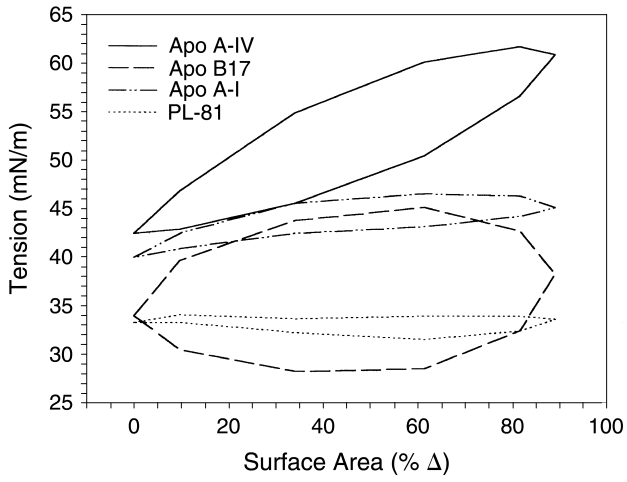

Fig. 2. Dynamic interfacial tension of apolipoproteins and Pluronic surfactants at the air/water interface. Surface area-tension loops of apolipoproteins (0.4 mg/ml) and Pluronic detergents (0.02%, v/v) in 50 mM Tris, 100 mM NaCl, pH 7.5, were measured with a pulsating bubble surfactometer at 20 cycles/min.

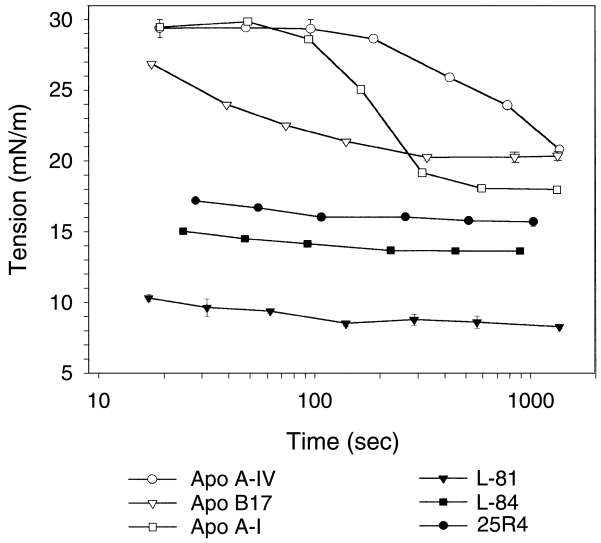

Fig. 3. Dynamic interfacial tension of apolipoproteins and Pluronic surfactants at the oil/water interface. Interfacial tension of apolipoproteins (10 μg/ml) and Pluronic detergents (0.02%, v/v) as a function of interface age was measured with a drop volume tensiometer at triolein flow rates of 5.0–0.02 ml/h. Data points represent means ± SD of 5 drops; where error bars are not visible, the standard deviation is less than the area of the data point.

nant apoB-17, although we cannot rule out the possibility that it might alter the three-dimensional structure of apoB-17 in a manner that affects its surface activity. Surface pressures at minimum (resting) bubble radius for apoA-IV and apoA-I were lower, and were similar to values determined by surface balance techniques (16, 32–35). Of the Pluronic detergents, L-81 displayed the highest surface activity, and reduced interfacial tension more effectively than any of the apolipoproteins; L-84 and 25R4, which are more hydrophilic, were less surface active. With bubble pulsation, the ΔA-γ loops for apoA-IV and apoB-17 displayed large changes in surface tension with changes in surface area, indicative of high elasticity at the air/water interface, and positive phase angles, indicative of viscous behavior (**Fig. 2**). These data suggest that apoA-IV and apoB-17 undergo rate-dependent changes in surface conformation in response to changes in interfacial area. These properties are characteristic of foaming and emulsifying agents that stabilize expanding interfaces (30). Conversely, the ΔA-γ loops for apoA-I and the Pluronic detergents were flat, consistent with little viscoelastic behavior. This property, particularly when combined with high surface activity, as with L-81, is characteristic of antifoaming agents that destroy interfaces and induce phase separation (30).

Dynamic properties of apolipoproteins at the oil/water interface

Apolipoproteins and Pluronic detergents bound to the oil/water interface as evidenced by a decrease in oil drop interfacial tension below 30 mN/m, the interfacial tension between pure triolein and buffer (**Fig. 3**). ApoB-17 lowered interfacial tension at the shortest interface ages, with maximal reduction at times >300 sec. Pure FLAG peptide at equivalent concentrations did not lower interfacial tension at any interface age, again suggesting that it did not contribute to the interfacial properties of recombinant apoB-17. ApoA-I displayed maximal reduction of surface tension between 100 and 500 sec; reduction of surface tension by apoA-IV did not reach a plateau even at >1,000 sec. Nonetheless, all apolipoproteins reduced interfacial tension to equivalent levels at the longest measured interface age. The derived rate constants were 9.3×10^{-3} sec^{-1} for apoB-17, 8.9×10^{-3} sec^{-1} for apoA-I, and 1.0×10^{-3} sec^{-1} for apoA-IV. In this regard, it is important to note that these kinetic parameters reflect a combination of diffusion, interfacial absorption, and conformational changes that maximize surface activity. Diffusion, which is fast, probably has the least impact on the observed differences among the apolipoproteins. Thus, the apoA-IV data suggest that either its interfacial absorption is slow, or that after absorption a slow conformational change must take place before it generates maximal surface activity. In contrast, Pluronic detergents were fast surfactants that lowered surface tension almost twice as effectively as apolipoproteins at all interface ages, in the same order as their hydrophobicity: L-81 > L-84 > 25R4 (50).

Pressure-area isotherms of apoA-IV and apoA-I at the air/water interface

We compared A-π isotherms for apoA-IV and apoA-I to examine the molecular basis for their different dynamic interfacial properties. ApoA-IV monolayers spread over buffer were more expanded at all pressures than apoA-I monolayers, and displayed a biphasic compression isotherm with a low pressure inflection at 15.9 mN/m, corresponding to a molecular area of 32.8 $Å^2$/residue, and a high pressure inflection at 24.4 mN/m, corresponding to 24.4 $Å^2$/residue (**Fig. 4**). Reexpansion of apoA-IV monolayers revealed the compression to be reversible with little

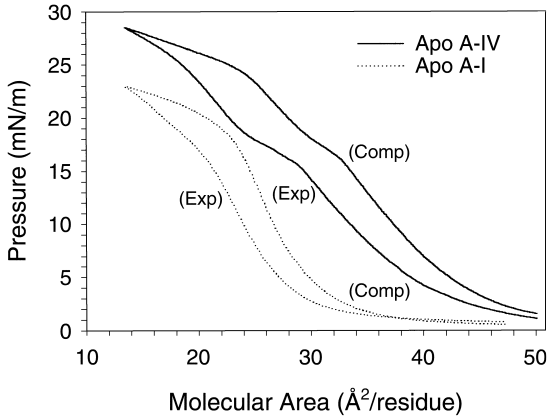

Fig. 4. Molecular area-surface pressure isotherms of apoA-IV and apoA-I at the air/water interface. Apolipoproteins were layered over 50 mM Tris, 100 mM NaCl, pH 7.5, and the protein films were compressed at 5 mm/min (Comp). Before monolayer collapse the monolayers were expanded (Exp) back to the starting points.

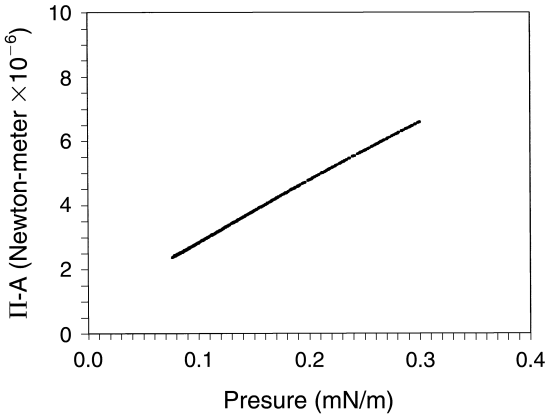

Fig. 6. Analysis of the low pressure portion (<0.5 mN/m) of apoA-IV compression isotherm on buffer using the ideal gas equation. The intercept of the plot of Π·A versus Π yields the interfacial molecular weight; the slope yields the limiting residue area (48).

hysteresis, and gave inflections at 18.5 and 15.3 mN/m, corresponding to 24.2 and 29.2 Å²/residue. In contrast, compression of apoA-I monolayers spread over buffer displayed a monotonic isotherm with a sharper increase in pressure between 30 and 24 Å²/residue and a single high pressure inflection at 17.1 mN/m, corresponding to 23.6 Å²/residue. ApoA-IV monolayers spread over 2 M KCl, an impermeable subphase (32, 47), were more condensed and displayed a monophasic compression isotherm with only a single high pressure inflection at 24.7 mN/m and 18.2 Å²/residue; the compression isotherm of apoA-I spread over 2 M KCl was nearly identical, with an inflection at 21.4 mN/m and 20.5 Å²/residue (**Fig. 5**). These data suggest that with changing surface pressure hydrophilic amphipathic helices in apoA-IV can reversibly move in and out of the interface. A plot of Π-A versus Π for $\Pi < 0.5$ was linear (**Fig. 6**) and yielded a calculated interfacial molecular weight of 45,316 with a limiting area of 24.4 Å/residue. These data indicate that despite its high affinity self-association in solution (51), like other apolipoproteins (48, 52), apoA-IV is monomeric at the interface.

Effect of apoA-IV on the adsorption of free cholesterol to the oil/water interface

With buffer as the aqueous phase, increasing concentrations of cholesterol dissolved in triolein caused a sharp decrease in surface tension between 20 and 70 mmol/liter, indicative of adsorption of cholesterol molecules to the oil/water interface (**Fig. 7**). The calculated maximal interfacial cholesterol concentration was 5.65×10^{-10} mol/cm², or 29.4 Å²/molecule (**Fig. 8**). This latter value is smaller than the cross-sectional area of condensed cholesterol monolayers, 37–38 Å (53); however, it is close to the calculated cross-sectional area of the cholesterol sterol ring (54), and may reflect the effective surface active cross-sectional area of cholesterol 3-hydroxyl groups embedded in the hydrophobic triolein matrix, as has been

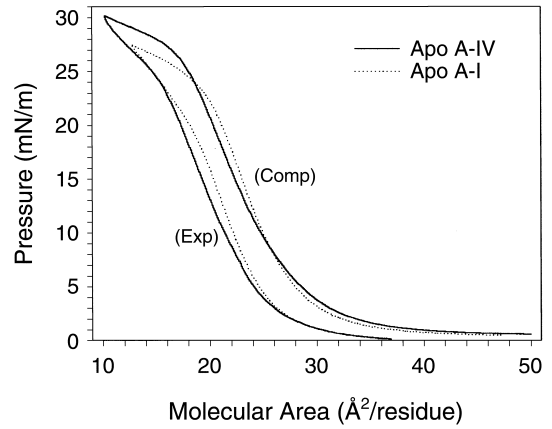

Fig. 5. Molecular area-surface pressure isotherms of apoA-IV and apoA-I layered over 2 M KCl, 5 mM Tris, pH 7.5, and compressed/expanded as in Fig. 3.

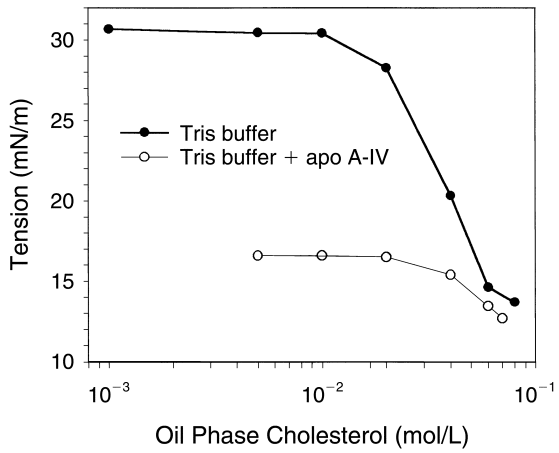

Fig. 7. Effect of apoA-IV on the adsorption of cholesterol to the oil/water interface. The interfacial tension at the oil/water interface was measured as a function of increasing oil-phase cholesterol concentration in the absence (solid circles) and presence (open circles) of apoA-IV at 50 μg/ml in the aqueous phase.

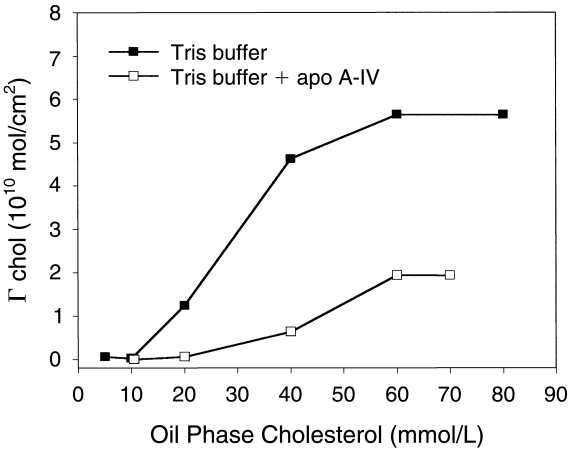

Fig. 8. Maximal interfacial cholesterol concentration (Γ) versus oil-phase cholesterol concentration, calculated using the Gibbs adsorption equation from the tension versus cholesterol concentration data in Fig. 7, in the absence (solid squares) and presence (open squares) of apoA-IV at 50 μg/ml in the aqueous phase.

observed for cholesterol-detergent monolayers (55). With apoA-IV at 50 μg/ml in the aqueous phase, the maximal interfacial cholesterol concentration decreased to 1.95×10^{-10} mol/cm^2. Assuming no change in the molecular area of cholesterol, this corresponds to occupancy of 65% of the oil drop surface area by apoA-IV molecules. In comparison, drop volume tensiometer studies by Handa et al. found that apoA-I at equivalent concentrations reduced cholesterol influx to the oil/water interface by only 36% (46). The higher effectiveness of apoA-IV may be due to its expanded surface conformation.

DISCUSSION

The physical events in chylomicron assembly, that is, the dispersion of dietary lipids within the intracellular milieu as large, stable particles, are much the same as for the formation of an oil-in-water macroemulsion. Hence, two critical conditions must be met for the process to be thermodynamically favorable (30): *1*) A mechanism is required to reduce the interfacial tension at the chylomicron surface; otherwise the free energy cost of expanding the hydrophobic interface would limit particle growth; and *2*) a mechanism is required to increase the resistance of the particles to the mechanical shock of Brownian-driven collisions; otherwise the particles would coalesce and an intracellular phase separation would ensue. Surfactants that simultaneously lower interfacial tension and increase elasticity serve these functions in the formation of an oil-in-water macroemulsion. Apolipoproteins may serve these functions in chylomicron assembly.

Our data establish that apoA-IV and apoB-17 exhibit a combination of moderate surface activity and high elasticity that is characteristic of effective emulsifying agents. Of the apolipoproteins, apoB-17 generated the lowest interfacial tension at the air/water interface, displayed high elasticity, and had the fastest adsorption to the oil/water interface. This behavior is in keeping with the intrinsic hydrophobicity of apoB (56, 57) and the cotranslational nature of apoB lipidation (26). The apoB-100 molecule contains two long β-sheet domains, separated by three α-helical domains (56) that can alter their surface conformation in response to changes in particle size (58–60). Studies with truncated apoB constructs suggest that the amino-terminal α_1 domain is critical for the initial lipidation of triglyceride-rich particles (57, 61). Our studies with apoB-17, which includes the entire α_1 domain, suggest that the dynamic interfacial properties of this domain may function to stabilize nascent particles. If the α_2 and α_3 domains have similar properties, then their role could be to facilitate particle growth. However, as the intestine produces only apoB-48, which lacks the α_2 and α_3 domains (62), the evolutionary appearance of intestinal apoB editing may have created a need for an auxiliary apolipoprotein to assume this function in chylomicron assembly.

The interfacial properties of apoA-IV may be optimally suited for this role. Although apoA-IV displayed higher surface tension at the air/water interface and slower adsorption to the oil/water interface, it was more elastic and less viscous than apoB-17, suggesting that it could better alter its surface conformation in response to collisional deformation, and thus better promote chylomicron stability. Moreover, because each chylomicron particle carries only one apoB-48 molecule (63), the stabilizing effects of apoB would rapidly diminish with particle growth. But continuous adsorption of apoA-IV molecules to the expanding chylomicron surface would maintain constant interfacial tension and elasticity at any particle size. This is analogous to the manner in which the insect protein apolipophorin III stabilizes hemolymph lipophorin particles as they acquire diacylglycerol from the fat storage organ (64, 65). ApoA-I, in contrast, was relatively inelastic, suggesting that it would not provide optimal particle stabilization. Furthermore, apoA-I may take a different intracellular path to the Golgi apparatus and hence may not be present in the early stages of chylomicron assembly (29).

The distinctive interfacial properties displayed by apoA-IV may be a consequence of its unique structural characteristics. Although apoA-IV has a high content of α-helical structure (66), its amphipathic α helices are hydrophilic (66) and have a radial charge distribution (67) that precludes deep penetration into condensed lipid monolayers (9, 10). Thus, a significant fraction of the α helices in apoA-IV may be excluded from the interface at high pressure (16). The monolayer compression data suggest that these features could enable apoA-IV to adopt an expanded, pressure-sensitive conformation at the chylomicron surface, such that with particle growth, its hydrophilic α-helices are recruited into the interface, thereby maintaining constant interfacial elasticity and molecular packing.

A further consequence of such behavior is that the presence of apoA-IV on the expanding nascent chylomicron surface could modulate interfacial free cholesterol concentration. In the oil drop experiments, adsorption of apoA-IV from the aqueous phase effectively reduced free

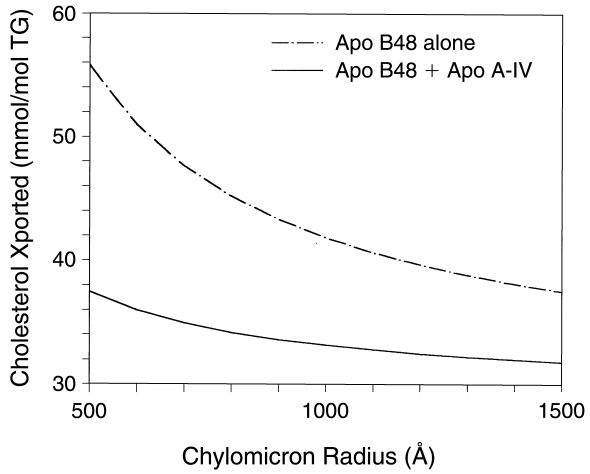

Fig. 9. Chylomicron cholesterol transport calculated as a function of particle radius and apolipoprotein composition. Calculations (Appendix A) are based on the molecular areas, volumes, and solubilities of the apoprotein and lipid components of the chylomicron surface and core (70, 71) for particles carrying one molecule of apoB-48 alone, or one molecule of apoB-48 and a sufficient number of apoA-IV molecules to maintain the fractional surface coverage by apolipoproteins at a constant initial value.

cholesterol influx to the expanding triglyceride/water interface and decreased maximal interfacial cholesterol concentration. Similarly, mathematical modeling (**Appendix**) predicts that, at any particle radius, chylomicron cholesterol transport decreases with increasing coverage of the particle surface by apoA-IV (**Fig. 9**). Disruption of chylomicron assembly abolishes cholesterol absorption (27), which suggests that cholesterol absorption may be sensitive to processes that alter the flux of cellular cholesterol into chylomicrons. Therefore, apoA-IV may be one of many factors that modulate the efficiency of intestinal cholesterol absorption.

Pluronic surfactants, such as L-81, selectively block intracellular chylomicron assembly and secretion (21) with a potency proportional to their hydrophobicity (22); the mechanism by which these agents act has not been determined. Electron micrographs of intestines from L-81-treated rats show accumulation of large triglyceride-filled droplets in the smooth endoplasmic reticulum (68). A remarkably similar histologic picture is seen in transgenic animals lacking intestinal apoB (27, 69). We observed that Pluronic surfactants display rapid binding, high activity, and low elasticity at hydrophobic interfaces, properties that are the hallmarks of antifoaming agents (30). This suggests that Pluronic surfactants may disrupt chylomicron assembly by destabilizing the surface of nascent chylomicrons so that they fuse into huge intracellular droplets, and further supports an important role of dynamic interfacial phenomena in the process of chylomicron assembly.

In summary, apoA-IV and apoB-17 display dynamic interfacial behavior particularly suited to the thermodynamic requisites of chylomicron assembly. Specifically, apoA-IV adopts an expanded, pressure-sensitive conformation with a high elasticity at hydrophobic interfaces, properties that are optimal for stabilizing expanding lipid/aqueous interfaces. Moreover, by maintaining a constant lipid packing density at the expanding chylomicron surface, apoA-IV may limit interfacial free cholesterol concentration, and thereby modulate the efficiency of intestinal cholesterol absorption. We conclude that the unique interfacial properties of apoA-IV may play an important auxiliary role in chylomicron assembly during intestinal lipid absorption.

APPENDIX

A model of the effect of apoA-IV on chylomicron cholesterol transport

A mathematical model of chylomicron cholesterol transport as a function of particle radius and apolipoprotein content was created, using the molecular areas and volumes of the protein and lipid components of the chylomicron surface and core (70) and the molar ratios of free cholesterol to phospholipid and cholesterol esters to triglyceride present in nascent chylomicrons (71).

If absorption of a volume of dietary triglyceride, V_{tg}, results in the secretion of a number of chylomicron particles, N_p, with core volume V_{core}, such that $V_{tg} = N_p \times V_{core}$, then for a homogeneous population of particles with outer radius r, covered with a phospholipid monolayer of thickness t, the number of chylomicron particles is given by

$$N_p = (3V_{tg})/4\pi(r - t)^3 \qquad \textit{Eq. 1)}$$

If the mass of cholesterol transported into circulation is solely a function of the total number of chylomicron particles formed and the number of free and esterified cholesterol molecules carried by each particle, then

$$CH_{xport} = \{N_p \times (n_{fc} + n_{ce})\}/N_A \qquad \textit{Eq. 2)}$$

where CH_{xport} is moles of cholesterol transported, n_{fc} is the number of free cholesterol molecules in each particle, n_{ce} is the number of cholesterol ester molecules in each particle, and N_A is Avogadro's number.

Assuming that only cholesterol ester and triglyceride molecules are located in the chylomicron core (70), then

$$4/3\pi(r - t)^3 = (n_{tg} \times v_{tg}) + (n_{ce} \times v_{ce}) \qquad \textit{Eq. 3)}$$

where n_{tg} is the number of core triglyceride molecules, n_{ce} is the number of core cholesterol ester molecules, v_{tg} is the partial specific volume of triglycerides (1,556 Å^3/molecule), and v_{ce} is the partial specific volume of cholesterol esters (1068 Å^3/molecule). If X_{cetg} is the molar ratio of cholesterol esters to triglyceride (n_{ce}/n_{tg}) in the chylomicron core (~0.03 mol/mol), then by substitution and rearrangement:

$$n_{ce} = \{(4/3)\pi(r - t)^3\}/\{1{,}556 / X_{cetg} + 1{,}068\} \qquad \textit{Eq. 4)}$$

Assuming that all the apolipoproteins, phospholipids, and free cholesterol molecules occupy the chylomicron outer shell, (70), then

$$4\pi r^2 = A_{APO} + (n_{pl} \times A_{pl}) + (n_{fc} \times A_{fc}) \qquad \textit{Eq. 5)}$$

where A_{APO} is the total surface area occupied by the apolipoprotein amino acid residues, n_{pl} is the number of phospholipid molecules, A_{pl} is the molecular area of phospholipid (71.0 Å^2/molecule), n_{fc} is the number of free cholesterol molecules, and A_{fc} is the molecular area of free cholesterol (39.1 Å^2/molecule). If X_{fcpl} is the molar ratio of free cholesterol to phospholipid (n_{fc}/n_{pl}) in the chylomicron surface (~0.2 mol/mol), then by substitution and rearrangement:

$$n_{fc} = \{4\pi r^2 - A_{APO}\}/\{71.0 / X_{fcpl} + 39.1\} \qquad Eq.\ 6)$$

In the hypothetical scenario in which only a single molecule of apoB-48 is present on the chylomicron surface, then $A_{APO} = n_{aa} \times A_{aa}$, where n_{aa} is the number of amino acid residues in apoB-48 (2,152) and A_{aa} is the molecular area of each residue (15.6 Å^2/molecule). In the scenario in which additional molecules of apoA-IV adsorb to the surface as the particle expands, thereby maintaining the fractional coverage by apolipoproteins fixed at an initial value, F_{APO}, then $A_{APO} = F_{APO}(4\pi r^2)$. Hep G2 cells transfected with an apoB-48 construct secrete HDL-like particles with a radius of 62 Å (72); using these parameters yields $F_{APO} = 0.695$.

Combining equations 1, 2, 4, and 6 yields

$$CH_{xport} = \{(3V_{tg}) / [N_A 4\pi(r - t)^3]\} \times \{(4\pi r^2 - 33,571) / (384) + [\pi(r - t)^3] / (29,976)\} \qquad Eq.\ 7)$$

for particles carrying only one molecule of apoB-48, and

$$CH_{xport} = \{(3V_{tg}) / [N_A 4\pi(r - t)^3]\} \times \{(4\pi r^2 \times 0.305) / (384) + [\pi(r - t)^3] / (29,976)\} \qquad Eq.\ 8)$$

for particles carrying one molecule of apoB-48 and enough additional apoA-IV molecules to maintain the fractional surface coverage by apolipoprotein fixed at the initial value of 0.695.

Assuming that as much as 2 g of biliary, dietary, and cellular cholesterol enters the intestinal lumen daily and that fractional cholesterol absorption is 50–60%, a subject ingesting an daily intake of 80 g of fat would absorb ~30 mmol of cholesterol/mole of dietary triglyceride. For an average chylomicron diameter of 1,200 Å (71), equation 8 predicts that ~35 mmol of cholesterol/mole of triglyceride is transported into plasma. The close agreement of these values supports the validity of this model.

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REFERENCES

1. Weinberg, R. B., and A. M. Scanu. 1983. The isolation and characterization of human apolipoprotein A-IV from lipoprotein depleted serum. *J. Lipid Res.* **24:** 52–59.
2. Luo, C. C., W. H. Li, M. N. Moore, and L. Chan. 1986. The isolation and characterization of human apolipoprotein A-IV from lipoprotein depleted serum. *J. Mol. Biol.* **187:** 325–340.
3. 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 the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
4. Hayashi, H., D. F. Nutting, K. Fujimoto, J. A. Cardelli, D. Black, and P. Tso. 1990. Transport of lipid and apolipoproteins apo A-I and apo A-IV in intestinal lymph of the rat. *J. Lipid Res.* **31:** 1613–1625.
5. Kalogeris, T. J., K. Fukagawa, and P. Tso. 1994. Synthesis and lymphatic transport of intestinal apolipoprotein A-IV in response to graded doses of triglyceride. *J. Lipid Res.* **35:** 1141–1151.
6. Green, P. H., 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. Green, P. H., 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.
8. Weinberg, R. B., and M. S. Spector. 1985. Structural properties and lipid binding of human apolipoprotein A-IV. *J. Biol. Chem.* **260:** 4914–4921.
9. Weinberg, R. B., and M. Jordan. 1990. Effects of phospholipid on the structure of human apolipoprotein A-IV. *J. Biol. Chem.* **265:** 8081–8086.
10. Weinberg, R. B., M. Jordan, and A. Steinmetz. 1990. Distinctive structure and function of human apolipoprotein variant, Apo A-IV-2. *J. Biol. Chem.* **265:** 18372–18378.
11. DeLamatre, J. G., C. A. Hoffmeier, A. G. Lacko, and P. S. Roheim. 1983. Distribution of apolipoprotein A-IV between the lipoprotein and lipoprotein-free fractions of rat plasma: possible role of lecithin-cholesterol acyltransferase. *J. Lipid Res.* **24:** 1578–1585.
12. Weinberg, R. B., and M. S. Spector. 1985. Human apolipoprotein A-IV: displacement from the surface of triglyceride-rich particles by HDL2-associated C-apoproteins. *J. Lipid Res.* **26:** 26–37.
13. Bisgaier, C. L., O. P. Sachdev, E. S. Lee, K. J. Williams, C. B. Blum, and R. M. Glickman. 1987. Distribution of apolipoprotein A-IV in human plasma. *J. Lipid Res.* **28:** 693–703.
14. Bisgaier, C. L., M. V. Siebenkas, C. B. Hesler, T. L. Swenson, C. B. Blum, Y. L. Marcel, R. W. Milne, R. M. Glickman, and A. R. Tall. 1989. Effect of a neutralizing monoclonal antibody to cholesterol ester transfer protein on the redistribution of apolipoproteins A-IV and E among human lipoproteins. *J. Lipid Res.* **30:** 1025–1031.
15. Lagrost, L., P. Gambert, V. Dangremont, A. Athias, and C. Lallemant. 1990. Role of cholesterol ester transfer protein (CETP) in the HDL conversion process as evidenced by using anti-CETP monoclonal antibodies. *J. Lipid Res.* **31:** 1569–1575.
16. Weinberg, R. B., J. A. Ibdah, and M. C. Phillips. 1992. Adsorption of apolipoprotein A-IV to phospholipid monolayers spread at the air/water interface. *J. Biol. Chem.* **267:** 8977–8983.
17. Weinberg, R. B., V. R. Cook, P. Kussie, and A. R. Tall. 1994. Interfacial properties of recombinant human cholesterol ester transfer protein. *J. Biol. Chem.* **269:** 29588–29591.
18. Weinberg, R. B., J. B. Jones, A. G. Lacko, and P. H. Pritchard. 1995. Effect of interfacial pressure on the binding and phospholipase A2 activity of recombinant human lecithin-cholesterol acyltransferase. *Biochem. Biophys. Res. Commun.* **211:** 840–846.
19. Lefevre, M., and P. S. Roheim. 1984. Metabolism of apolipoprotein A-IV. *J. Lipid Res.* **25:** 1603–1610.
20. Kalogeris, T. J., M. D. Rodriquez, and P. Tso. 1997. Control of synthesis and secretion of intestinal apolipoprotein A-IV. *J. Nutr.* **127:** 537S–543S.
21. Tso, P., J. A. Balint, M. B. Bishop, and J. B. Rodgers. 1981. Acute inhibition of intestinal lipid transport by Pluronic L-81 in the rat. *Am. J. Phys.* **241:** G487–497.
22. Tso, P., and S. R. Gollamundi. 1984. Pluronic L-81: a potent inhibitor of the transport of intestinal chylomicrons. *Am. J. Phys.* **247:** G32–G36.
23. Tso, P., and J. A. Balint. 1986. Formation and transport of chylomicrons by enterocytes to the lymphatics. *Am. J. Phys.* **250:** G715–726.
24. Kalogeris, T. J., F. Monroe, S. J. Demichele, and P. Tso. 1996. Intestinal synthesis and lymphatic secretion of apolipoprotein A-IV vary with chain length of intestinally infused fatty acids in rats. *J. Nutr.* **126:** 2720–2729.
25. Bisgaier, C. L., O. P. Sachdev, L. Megna, and R. M. Glickman. 1985. Distribution of apolipoprotein A-IV in human plasma. *J. Lipid Res.* **26:** 11–25.

26. Innerarity, T. L., J. Boren, S. Yamanaka, and S. O. Olofsson. 1996. Biosynthesis of apolipoprotein B-48-containing lipoproteins. *J. Biol. Chem.* **271:** 2353–2356.
27. Young, S. G., C. M. Cham, R. E. Pitas, B. J. Burri, A. Connoly, L. Flynn, A. S. Pappu, J. S. Wong, R. L. Hamilton, and R. V. Farese. 1995. A genetic model for absent chylomicron formation: mice producing apo B in the liver, but not in the intestine. *J. Clin. Invest.* **96:** 2932–2946.
28. Gordon, D. A. 1997. Recent advances in elucidating the role of microsomal triglyceride transfer protein in apolipoprotein B lipoprotein assembly. *Curr. Opin. Lipid.* **8:** 131–137.
29. Kumar, N. S., and C. M. Mansbach. 1999. Prechylomicron transport vesicle: isolation and partial characterization. *Am. J. Phys.* **276:** G378–386.
30. Rosen, M. J. 1989. Emulsification by surfactants. *In* Surfactants and Interfacial Phenomena. 2nd edition. John Wiley & Sons, New York. 304–322.
31. Krebs, K. E., M. C. Phillips, and C. E. Sparks. 1983. A comparison of the surface activities of rat plasma apolipoproteins C-II, C-III-0, and C-III-3. *Biochim. Biophys. Acta.* **751:** 470–473.
32. Krebs, K. E., J. A. Ibdah, and M. C. Phillips. 1988. A comparison of the surface activities of human apolipoprotein A-I and A-II at the air/water interface. *Biochim. Biophys. Acta.* **959:** 229–237.
33. Ibdah, J. A., and M. C. Phillips. 1988. Effects of lipid composition and packing on the adsorption of apolipoprotein A-I to lipid monolayers. *Biochemistry.* **27:** 7155–7162.
34. Ibdah, J. A., K. E. Krebs, and M. C. Phillips. 1989. The surface properties of apolipoproteins A-I and A-II at the air/water interface. *Biochim. Biophys. Acta.* **1004:** 300–308.
35. Ibdah, J. A., S. Lund-Katz, and M. C. Phillips. 1989. Molecular packing of high density and low density lipoprotein surface lipids and apolipoprotein A-I binding. *Biochemistry.* **28:** 1126–1133.
36. Ingram, M. F., and G. S. Shelness. 1997. Folding of the amino-terminal domain of apolipoprotein B initiates microsomal triglyceride transfer protein-dependent lipid transfer to nascent very low density lipoprotein. *J. Biol. Chem.* **272:** 10279–10286.
37. Bartlett, G. R. 1959. Phosphorus assay in column chromatography. *J. Biol. Chem.* **234:** 466–468.
38. Weinberg, R. B., R. A. Hopkins, and J. B. Jones. 1996. Purification, isoform characterization, and quantitation of human apolipoprotein A-IV. *Methods Enzymol.* **263:** 282–296.
39. Shore, B., and V. Shore. 1969. Isolation and characterization of polypeptides of human serum lipoproteins. *Biochemistry.* **8:** 4510–4516.
40. Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk. 1985. Protein assay using bicinchoninic acid. *Anal. Biochem.* **150:** 76–85.
41. Enhorning, G. 1977. Pulsating bubble technique for evaluating pulmonary surfactant. *Appl. Physiol.* **43:** 198–203.
42. Lucassen, J., and M. Van Den Tempel. 1972. Dynamic measurements of dilational properties of a liquid interface. *Chem. Eng. Sci.* **27:** 283–1291.
43. Benjamins, J., A. Cagna, and E. H. Lucassen-Reynders. 1996. Viscoelastic properties of triacylglycerol/water interfaces covered with proteins. *Colloids Surfaces.* **114:** 245–254.
44. Mollet, C., Y. Touhami, and V. Hornof. 1996. A comparitive study of the effects of ready-made and in-situ formed surfatants on interfacial tension measured by drop volume tensiometry. *J. Colloid Interface Sci.* **178:** 523–530.
45. Xu, Y. 1995. Dynamic interfacial tension between bitumen and aqueous sodium hydroxide solutions. *Energ. Fuel.* **9:** 148–154.
46. Handa, T., H. Saito, I. Tanaka, A. Kakee, K. Tanaka, and K. Miyajima. 1992. Lateral interactions of pig apolipoprotein A-I with egg yolk phosphatidylcholine and with cholesterol in mixed monolayers at the triolein-saline interface. *Biochemistry.* **31:** 1415–1420.
47. Phillips, M. C., and K. E. Krebs. 1986. Studies of apolipoproteins at the air-water interface. *Methods Enzymol.* **128:** 387–403.
48. Shen, B. W., and A. M. Scanu. 1980. Properties of human apolipoprotein A-I at the air-water interface. *Biochemistry.* **19:** 3643–3650.
49. Herscovitz, H., M. Hadzopoulou-Cladaras, M. T. Walsh, C. Cladaras, V. I. Zannis, and D. M. Small. 1991. Expression, secretion, and lipid-binding characterization of the N-terminal 17% of apolipoprotein B. *Proc. Natl. Acad. Sci. USA.* **88:** 7313–7317.
50. BASF website. 1999. www.basf.com/businesses/chemicals/performance/html/pluronic.html (7/18/2000).
51. Weinberg, R. B., and M. S. Spector. 1985. The self-association of human apolipoprotein A-IV: evidence for an in vivo circulating dimeric form. *J. Biol. Chem.* **260:** 14279–14286.
52. Yokoyama, S., Y. Kawai, S. Tajima, and A. Yamamoto. 1985. Behavior of human apolipoprotein E in aqueous solutions and at interfaces. *J. Biol. Chem.* **260:** 16375–16382.
53. Phillips, M. C., D. A. Cadenhead, R. J. Good, and H. F. King. 1971. Dipole interactions in monomolecular layers. *J. Coll. Interface Sci.* **37:** 437–441.
54. Slotte, J. P. 1992. Enzyme-catalyzed oxidation of cholesterol in pure monolayers at the air/water interface. *Biochim. Biophys. Acta.* **1123:** 326–333.
55. McGregor, M. A., and G. T. Barnes. 1978. Equilibrium penetration of monolayers. VI. Cholesterol-cetrimonium bromide systems. *J. Pharm. Sci.* **67:** 1054–1056.
56. Segrest, J. P., M. K. Jones, V. K. Mishra, G. M. Anatharamaiah, and D. W. Garber. 1994. 1992. Apolipoprotein B has a pentapartite structure composed of three amphipathic α helical domains alternating with two amphipathic β strand domains: detection by the computer program LOCATE. *Arterioscler. Thromb.* **14:** 1674–1685.
57. Mann, C. J., T. A. Anderson., J. Read, S. A. Chester, G. B. Harrison, S. Kochl, P. J. Ritchie, P. Bradbury, F. S. Hussain, J. Amey, B. Vanloo, M. Rosseneu, R. Infante, J. M. Hancock, D. G. Levitt, L. J. Banaszak, J. Scott, and C. C. Shoulders. 1999. The structure of vitellogenin provides a molecular model for the assembly and secretion of atherogenic lipoproteins. *J. Mol. Biol.* **285:** 391–408.
58. Walsh, M. T., and D. Atkinson. 1990. Calorimetric and spectroscopic investigation of the unfolding of apolipoprotein B. *J. Lipid Res.* **31:** 1021–1062.
59. McNamara, J. R., D. M. Small, Z. Li, and E. J. Schaefer. 1996. Differences in LDL subspecies involve alterations in lipid composition and conformational changes in apolipoprotein B. *J. Lipid Res.* **37:** 1924–1935.
60. Chauhan, V., X. Wang, T. Ramsamy, R. W. Milne, and D. L. Sparks. 1998. Evidence for lipid-dependent structural changes in specific domains of apolipoprotein B. *Biochemistry.* **37:** 3735–3742.
61. Shelness, G. S., M. F. Ingram, X. F. Huang, and J. A. DeLozier. 1999. Apolipoprotein B in the rough endoplasmic reticulum: translation, translocation and the initiation of lipoprotein assembly. *J. Nutr.* **129:** 456S–462S.
62. Chan, L., B. H. J. Chang, M. Nakamuta, W. H. Li, and L. C. Smith. 1997. Apobec-1 and apolipoprotein B mRNA editing. *Biochim. Biophys. Acta.* **1345:** 1–26.
63. Phillips, M. L., C. Pullinger, I. Kroes, D. A. Hardman, G. Chen, L. K. Curtiss, M. M. Gutierrez, J. P. Kane, and V. N. Schumaker. 1997. A single copy of apolipoprotein B-48 is present on the human chylomicron remnant. *J. Lipid Res.* **38:** 1170–1177.
64. Ryan, R. O. 1990. Dynamics of insect lipophorin metabolism. *J. Lipid Res.* **31:** 1725–1739.
65. Ryan, R. O., K. Oikawa, and C. M. Kay. 1993. Conformational, thermodynamic, and stability properties of *Manduca sexta* apolipophorin III. *J. Biol. Chem.* **268:** 1525–1530.
66. Weinberg, R. B. 1987. Differences in the hydrophobic properties of discrete alpha helical domains of rat and human apolipoprotein A-IV. *Biochim. Biophys. Acta.* **918:** 299–303.
67. Segrest, J. P., M. K. Jones, H. De Loof, C. G. Brouillette, Venkatachalapathi, Y. V, and G. M. Anatharamaiah. 1992. The amphipathic helix in exchangeable apolipoproteins: a review of secondary structure and function. *J. Lipid Res.* **33:** 141–166.
68. Reger, J. F., S. Frase, and P. Tso. 1989. Fine structure observations on rat jejunal epithelial cells during fat processing and resorption following L-81 exposure and reversal. *J. Submicrosc. Cytol. Pathol.* **21:** 399–408.
69. Hamilton, R. L., J. S. Wong, C. M. Cham, L. B. Nielsen, and S. G. Young. 1998. Chylomicron-sized lipid particles are formed in the setting of apolipoprotein B deficiency. *J. Lipid Res.* **39:** 1543–1557.
70. Shen, B. W., A. M. Scanu, and F. J. Kezdy. 1977. Structure of human serum lipoproteins inferred from compositional analysis. *Proc. Natl. Acad. Sci. USA.* **74:** 837–841.
71. Miller, K. M., and D. M. Small. 1987. Structure of triglyceride-rich lipoproteins: an analysis of core and surface phases. *In* Plasma Lipoproteins. A. M. Gotto, editor. Elsevier, Amsterdam. 1–75.
72. Spring, D. J., S-M. Lee, D. L. Puppione, M. Phillips, J. Elovson, and V. N. Schumaker. 1992. Identification of a neutral lipid core in a transiently expressed and secreted lipoprotein containing an apo B-48-like apolipoprotein. *J. Lipid Res.* **33:** 233–120.