Effect of acylglyceride content on the structure and function of reconstituted high density lipoprotein particles

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Abstract The effects of different acylglycerides on the conformation and charge of apolipoprotein A-I (apoA-I) have been investigated in reconstituted high density lipoproteins (LpA-I). Various amounts of diacylglycerol (DG) and triacylglycerol (TG) were incorporated into sonicated spherical LpA-I particles containing 2 molecules of apoA-I and 80 molecules of phospholipid. Inclusion of 30 molecules of TG into the LpA-I particle increases the net negative charge of apoA-I (−8.5 to −9.3 mV), but has little effect on the amount and thermodynamic stability of the α helices in apoA-I. Incorporation of 30 molecules of DG into the lipoprotein complex promotes a small increase in the α-helix content and stability, but greatly increases the net negative charge of apoA-I (−8.5 to −11.2 mV). Inclusion of DG increases the immunoreactivity of two epitopes in the N terminus of apoA-I, but decreases the exposure of a domain closer to the C terminus (residues 148–186) of the apoprotein. In contrast, TG increases the exposure of epitopes over the entire apoA-I molecule; TG increases the immunoreactivity of epitopes for 13 different monoclonal antibodies to apoA-I. Incubations with purified lecithin:cholesterol acyltransferase show that cholesterol esterification is stimulated by DG, but inhibited by TG. The data show that TG and DG have different effects on apoA-I structure and function and this suggests that the TG-to-DG ratio in HDL may directly affect the metabolism of this lipoprotein class.—Braschi, S., C. R. Coffill, T. A-M. Neville, D. M. Hutt, and D. L. Sparks. **Effect of acylglyceride content on the structure and function of reconstituted high density lipoprotein particles. *J. Lipid Res.* 2001.** 42: **79–87.**

Supplementary key words HDL • apolipoprotein A-I • diglyceride • triglyceride • charge • stability • conformation • immunochemistry • LCAT

Numerous studies have shown that plasma high density lipoprotein (HDL) cholesterol levels are inversely related to the risk of atherosclerotic cardiovascular disease (1, 2). Many investigations have attempted to elucidate the metabolic function of this lipoprotein class and the mechanism by which HDL affects cholesterol metabolism and decrease atherosclerotic risk. Studies have shown that changes in HDL composition dictate unique structural characteristic properties to this molecule that alter the functional properties of HDL particles (3–10). Changes in HDL structure and charge also directly affect HDL metabolism in vivo and appear to regulate HDL clearance from the plasma (11, 12). Therefore, the composition-dependent biophysical properties of HDL appear able to govern the metabolism of this lipoprotein class and affect cholesterol homeostasis.

Human plasma HDL has been shown to be approximately 50% protein and 50% lipid by weight. Phospholipid (PL) and cholesteryl esters (CE) are the major lipid constituents in this lipoprotein, while triacylglycerol (TG) is thought to constitute only 10–20% of the lipid mass (13). More recent studies suggest that TG may actually not be the predominant acylglyceride in HDL particles. Several years ago, Vieu et al. (14) showed that the methods commonly used for measuring TG have overestimated TG levels by not accounting for the contributions of diacylglycerol (DG). Their work showed that DG is the most abundant acylglyceride in HDL and may exist at DG-to-TG ratios ranging from 2 to 7, for normolipidemic human HDL. DG was not shown to be confined to the lipoprotein core like TG, but was predominantly localized on the lipoprotein surface. While this is still considered somewhat controversial, other groups have made similar observations (9, 15). In addition, DG has been shown to be the primary substrate for human hepatic lipase (HL) and the amount

Abbreviations: apoA-I, apolipoprotein A-I; CD, circular dichroism; CE, cholesteryl ester; DG, diacylglycerol; ED_{50}, particle concentration required for 50% inhibition of maximal antibody binding; FC, free (unesterified) cholesterol; GGE, gradient gel electrophoresis; HDL, high density lipoprotein; HL, hepatic lipase; LCAT, lecithin:cholesteryl acyltransferase; LpA-I, reconstituted HDL; MAb, monoclonal antibodies; NMR, nuclear magnetic resonance; PBS, phosphate-buffered saline; PL, phospholipid; POPC, palmitoyl-oleoyl phosphatidylcholine; TG, triacylglycerol.

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of DG in HDL appears to regulate the rate of hydrolysis of both PL and TG by HL (9). These observations confirm that DG is a major constituent of plasma HDL particles, but the role that lipids may have in the remodeling and the metabolic fate of HDL particles has never been investigated.

To determine how DG may affect the structure and function of HDL, we characterized the effect of DG on the charge and structure of apolipoprotein A-I (apoA-I) in homogeneous reconstituted HDL particles (LpA-I). Of all the HDL lipid constituents that have been characterized in this laboratory, DG appears to have the most profound effects on the charge and structural properties of apoA-I. These properties confer unique metabolic characteristics on the lipoprotein particles, which appear to affect significantly the remodeling and metabolism of HDL.

TABLE 1. Effect of the acylglyceride content of LpA-I complexes on their size and charge

Particle Composition (POPC:DG:TG:ApoA-I)[a]		Hydrodynamic Diameter[b]	Surface Potential[c]	Valence (per ApoA-I)[d]
Initial	Final			
mol:mol:mol:mol		*nm*	*−mV*	*−e*
120:0:0:2	82:0:0:2	7.8	8.5	3.0
120:0:40:2	74:0:26:2	7.7	9.3	2.9
120:10:40:2	72:6:26:2	7.8	9.7	3.3
120:40:10:2	81:22:8:2	7.6	10.5	3.4
120:40:0:2	74:32:0:2	7.7	11.2	3.6

[a] LpA-I complexes were prepared by sonication as indicated in text. POPC, DG, TG, and apoA-I molar ratios are indicated before (initial) and after (final) reisolation on a Superose 6 column. Values are representative of three different preparations of LpA-I.

[b] Particle diameters from nondenaturing gradient gel electrophoresis ± 0.5 nm (SD).

[c] Charge potential at the particle surface ± 0.2 (SD).

[d] Number of excess negative charges in electronic units per molecule of apoA-I ± 0.1 (SD).

EXPERIMENTAL PROCEDURES

Materials

Triolein was purchased from Sigma (St. Louis, MO) and 1,3-diolein was acquired from NuChek Prep (Elysian, MN). 1-Palmitoyl-2-oleoyl phosphatidylcholine (POPC) was obtained from Avanti Polar Lipids (Birmingham, AL). [^{3}H]cholesterol was purchased from DuPont-NEN Research Products (Mississauga, Ontario, Canada). Antibodies 2G11 and 4A12 were purchased from Sanofi (Marnes-la-Coquette, France). A03, A05, A07, A11, A17, A16, and A44 were obtained from the Institute Pasteur (Lille, France). 3D4, 2F1, 4H1, and 5F6 were produced in our laboratory. All other reagents were analytical grade.

Purification of human apoA-I

Human HDL (1.063 g/ml < d < 1.210 g/ml) was isolated from fresh plasma by sequential density gradient ultracentrifugation. HDL was delipidated in chloroform and methanol and purified apoA-I was isolated by size-exclusion chromatography on a Sephacryl S-200 HR column (16). ApoA-I was stored in lyophilized form at −80°C. Prior to use, apoA-I was resolubilized in 6 M guanidine hydrochloride, 10 mM Tris, pH 7.2, and dialyzed extensively against 50 mM phosphate buffer, 150 mM NaCl, pH 7.2 (PBS).

Preparation of spherical LpA-I complexes

Reconstituted LpA-I complexes were prepared by cosonication of POPC, TG, DG, and apoA-I (16). Specific amounts of POPC, TG, and DG in chloroform (see **Table 1** for initial concentrations) were dried under nitrogen into a 12 × 75 mm glass test tube and 800 μl of PBS was added. The lipid-buffer mixture was sonicated for 1 min in a 15°C water bath, incubated for 30 min at 37°C, and sonicated again for 5 min. ApoA-I (2 mg of a 1.4-mg/ml mixture in PBS) was subsequently added to the lipid suspension and the protein-lipid mixture was sonicated four times (1 min each time), punctuated by 1-min cooling periods. All LpA-I complexes were filtered through a 0.22-μm syringe tip filter and reisolated by size-exclusion chromatography on a Superose 6 column.

Determination of LpA-I physical characteristics

The size and homogeneity of apoA-I complexes were estimated by nondenaturing gradient gel electrophoresis (GGE) on precast 8–25% acrylamide gels (Phastgel; Pharmacia, Uppsala, Sweden) (17). Total glycerides and POPC concentrations were determined enzymatically with kits from Roche Diagnostics (Indianapolis, IN). In some experiments, radioactive diolein was included in the lipids to allow for the determination of particle DG content. [^{14}C]diolein was produced and purified as previously described (9) from a 4-h incubation at 37°C of 5 μCi of 1,2-[^{14}C]oleoyl phosphatidylcholine, 1 ml of phospholipase C (0.2 units/ml in phosphate buffer, pH 7.3), 1 ml of 1% $CaCl_2$, and 1 ml of diethyl ether. The relative proportion of di- and triacylglycerols was calculated subtractively from the total glyceride concentration and the radioactive DG in the LpA-I particle.

Circular dichroism

The average secondary structures of LpA-I apoA-I were monitored by circular dichroism (CD) spectroscopy on a JASCO (Tokyo, Japan) J40A spectropolarimeter calibrated with a 0.1% (w/v) *d*-10-camphorsulfonic acid solution (18). CD spectra were measured at 24°C in a 0.1-cm path length quartz cell with a sample protein concentration of 66.7 μg/ml in PBS. The percentage of α helices in apoA-I was calculated from the molar ellipticity at 222 nm, using a mean residue weight of 115.3. The effect of guanidine hydrochloride concentration on the secondary structure of apoA-I in various LpA-I complexes was monitored by the changes in molar ellipticity at 222 nm. Aliquots of each complex (33 μg of protein per ml of buffer) were incubated with 0–6 M guanidine hydrochloride in PBS for 72 h at 4°C. The midpoint of denaturation and free energy of unfolding of apoA-I on the surface of LpA-I complexes was calculated as described previously (17). Experiments were performed on three different LpA-I preparations.

Immunoreactivity of LpA-I

Immunoreactivity measurements were determined from a competitive solid-phase radioimmunometric assay similar to that previously described (10) and a panel of 13 different antibodies that recognize defined epitopes (residues) in apoA-I: 4H1 (2–8), 2F1 (8–82), A16 (14–29, 60–82), A05 (25–82), 2G11 (25–96), 3D4 (98–121), A17 (98–121), A11 (98–132), 5F6 (118–141), A03 (135–148), A07 (148–186), A44 (148–186), and 4A12 (173–205). Briefly, Removawells (Immulon 2; Dynatech Laboratories, Chantilly, VA) were coated with 100 μl of apoHDL (0.2 μg in 15 mM Na_2CO_3, 35 mM $NaHCO_3$, 0.02% NaN_3. pH 6.9), washed with PBS, and saturated with 0.5% gelatin (Bio-Rad, Hercules, CA) in PBS. Anti-apoA-I monoclonal antibodies (MAb, at a predetermined dilution) were mixed with serial dilutions of LpA-I particles (with 0.1% gelatin in PBS) and transferred to the previously

coated and saturated Removawells for a 1-h incubation at room temperature. After three washes with 0.05% Tween 20 in PBS, the Removawells were incubated for 1 h with an ^{125}I-labeled anti-mouse IgG antibody in PBS with 0.1% gelatin (200,000 cpm/well). Wells were washed three times with 0.05% Tween 20 in PBS, and their radioactivity was measured. The immunoreactivity of the antibodies for the various LpA-I particles was estimated from the ED_{50}, which represents the LpA-I concentration required to inhibit 50% of the maximal binding of the antibodies to the apoHDL-coated plate. Results are expressed in micrograms per milliliter and represent means ± SD of triplicate determinations. Experiments were conducted on two or three different LpA-I preparations.

Purification and activity assay of lecithin:cholesterol acyltransferase

Lecithin:cholesterol acyltransferase (LCAT) was purified from normolipidemic plasmas as previously described (19). LCAT activity was measured by incubating reconstituted LpA-I (containing 10 mol of cholesterol and 0.01 μCi of [^{3}H]cholesterol per mol of apoA-I) with 0.3 units of purified LCAT, 2.5 mg of bovine serum albumin, 2.5 mM 2-mercaptoethanol, and Tris buffer, pH 8.0, in a final volume of 500 μl at 37°C for 10 min. Reaction products were extracted in ethanol and hexane and the amount of ^{3}H associated with CE was determined by thin-layer chromatography as described (19). Under these conditions, initial rates were estimated with minimal substrate conversion; less than 5% of LpA-I cholesterol was esterified at enzyme saturation. LCAT activity was expressed as units of enzyme activity, where 1 unit represents 1 nmol of cholesterol esterified per ng of purified protein per hour of incubation. The maximal velocity (V_{max}) and the apparent K_m of cholesterol esterification by LCAT were determined from double-reciprocal plots of the CE formed as a function of the substrate concentration.

RESULTS

Effect of DG on size and charge of LpA-I complexes

Sonicated LpA-I particles were prepared to contain various amounts of DG and TG, while maintaining a relatively constant amount of POPC (see Table 1 for starting and final compositions). All recombinant complexes contained two molecules of apoA-I per particle and exhibited only one band on nondenaturating gradient gels (9). Inclusion of 10 to 40 molecules of DG and/or TG in the LpA-I complexes had no detectable effect on the particle size; all complexes were about 7.7 nm in diameter (Table 1).

As indicated in Table 1, addition of about 40 molecules of TG to the LpA-I complexes only slightly increased the negative surface potential of the particle. In contrast, the DG content of the LpA-I complex had a profound effect on the particle surface potential. Progressive increases in DG were associated with increases in net negative charge; inclusion of 40 molecules of DG gave rise to an increase in the magnitude of surface potential of approximately 2.7 mV. This increase of the LpA-I negative charge was associated with parallel changes in the net charge (valence) on apoA-I, and therefore appears to be primarily due to alterations in the conformation of the apoA-I molecule (Table 1). This may indicate that the presence of DG on an LpA-I complex increases its negative surface charge by promoting the exposure of negatively charged residues in apoA-I.

Effect of LpA-I DG content on secondary structure and stability of apoA-I

The apoA-I organization on the LpA-I particles was evaluated by quantifying the amount of the amphipatic α-helical structures in apoA-I on the various LpA-I complexes. As indicated in **Table 2**, inclusion of 10 to 40 molecules of TG and/or DG in an LpA-I complex did not significantly affect the α-helical content of the apoA-I molecule. In contrast, variations in the glyceride content of LpA-I particles had distinct effects on the unfolding of apoA-I secondary structure by guanidine hydrochloride. **Figure 1** shows that the addition of TG and/or DG molecules to an LpA-I complex directly affected the ability of guanidine hydrochloride to unfold the α-helical structure of apoA-I. Table 2 illustrates this point and shows that the midpoint of denaturation ($D_{1/2}$) for apoA-I on LpA-I complexes increased when TG content was increased, and then fell slightly when a few molecules of DG were further added. The inclusion of DG molecules only into the LpA-I complex had little effect on the $D_{1/2}$ for apoA-I. While the in-

TABLE 2. Effect of the acylglyceride content of LpA-I complexes on their α-helix content and denaturation characteristics

LpA-I Complex (POPC:DG:TG:ApoA-I)[a]	α-Helix Content[b]	$D_{1/2}$[c]	ΔG_D^0[d]	Δn[e]
mol:mol:mol:mol	*%*	*M GdnHCl*	*kcal/mol of ApoA-I*	*mol of GdnHCl/ mol of apoA-I*
ApoA-I	49	1.0	2.4*	15.4
120:0:0:2	58	1.5	1.4	5.5
120:0:40:2	61	2.5*	1.3	3.8*
120:10:40:2	62	2.2*	1.4	5.4
120:40:10:2	58	1.6	1.7*	6.5
120:40:0:2	61	1.6	2.0*	7.6*

Significance of difference from the control LpA-I (120:2, POPC:apoA-I): * $P < 0.01$.

[a] POPC, DG, TG, and apoA-I molar ratio of the LpA-I complexes. Values are representative of three different preparations of LpA-I.

[b] ApoA-I α-helix content determined from molar ellipticities at 222 nm ± 4% (SD).

[c] Midpoint of guanidine hydrochloride (GdnHCl) denaturation curve (Fig. 1) ± 0.03 M (SD).

[d] Free energy of denaturation at zero GdnHCl concentration ± 0.2 kcal (SD).

[e] GdnHCl bound during denaturation ± 0.1 mol/mol (SD).

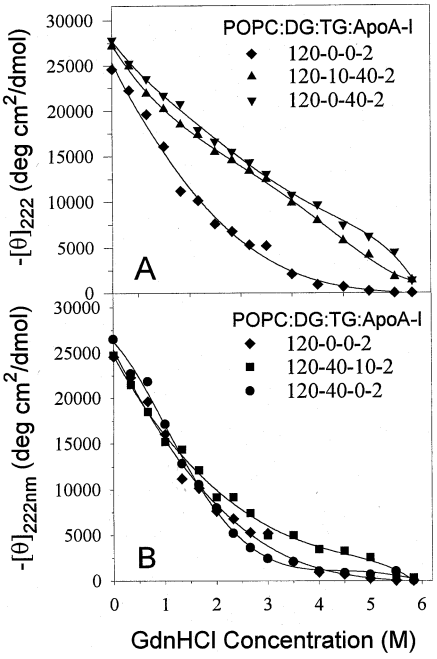

Fig. 1. Effect of guanidine hydrochloride concentration on the molar ellipticity of apoA-I on LpA-I particles. Aliquots of LpA-I particles (POPC:DG:TG:FC:apoA-I molar ratios: circles, 120:40:0:2; squares, 120:40:10:2; triangles, 120:10:40:2; inverted triangles, 120:0:40:2; diamonds, 120:0:0:2) were incubated with from 0 to 6 M guanidine hydrochloride in 0.05 M PBS for 72 h at 4°C. Circular dichroism spectra were measured at 24°C in a 0.1-cm path length quartz cell with sample protein concentrations of 33.3 μg/ml in PBS and four scans from 230 to 200 nm were collected and averaged at each guanidine hydrochloride concentration. Results represent the means of triplicate determinations and the values are representative of those observed for three different LpA-I preparations.

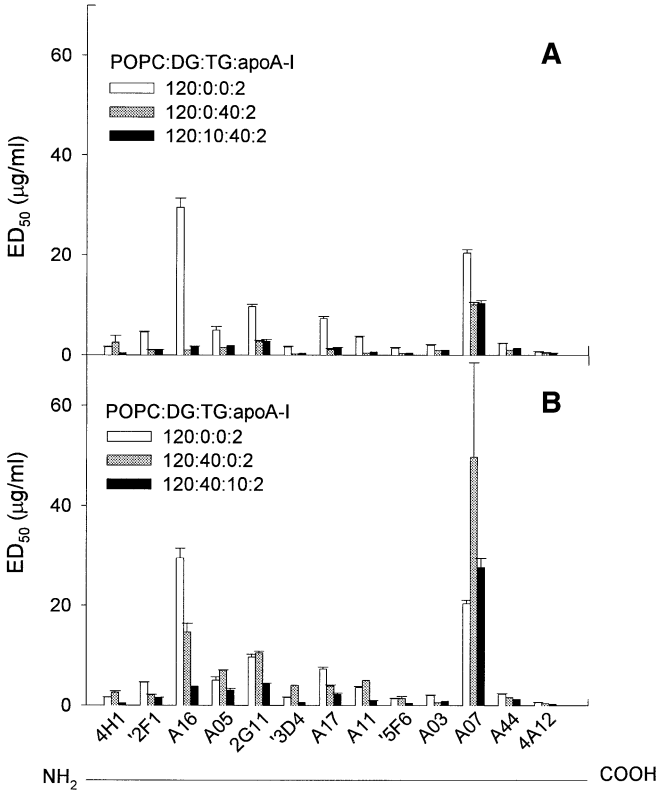

Fig. 2. Effect of the glyceride content of LpA-I complexes on apoA-I immunoreactivity. The apoA-I immunoreactivity of the various LpA-I complexes to each MAb was evaluated by a radioimmunometric assay determining the ED_{50}, which represents the LpA-I concentration inhibiting half the maximum binding of the MAb on the plate. The MAb are positioned on the x axis as their epitope appear in the apoA-I sequence. They can be grouped as representative of the N-terminal (4H1 to 2G11), the central (3D4 to A03), and the C-terminal regions (A07 to 4A12) of the apoA-I molecule. Results represent means ± SD of triplicate determinations and are representative of experiments conducted with two or three different LpA-I preparations.

creased $D_{1/2}$ suggests that the apoA-I molecule is more stable in the presence of TG, analysis of the denaturation curves with the binding model of Aune and Tanford suggests that this is actually not the case (17). Analysis of LpA-I denaturation curves shows that the free energy of stability (ΔG°_{D}) of apoA-I is actually slightly decreased in the presence of TG (Table 2). The addition of 40 molecules of TG to an LpA-I complex decreased the ΔG°_{D} for apoA-I by 12%. In contrast, inclusion of 40 DG molecules in an LpA-I complex was associated with an increase in the ΔG°_{D} (30%). Overall, the data show that the stability of apoA-I secondary structure is uniquely affected by different acylglycerides in HDL.

Effect of LpA-I glyceride content on epitope expression of apoA-I

The conformation of the apoA-I molecule on the different LpA-I complexes was evaluated by competitive solid-phase radioimmunometric assay, using a panel of 13 anti-apoA-I MAbs specific for epitopes that range from the amino terminus to the carboxyl terminus of the apoA-I (20). Competitive binding curves were parallel for all MAbs and therefore ED_{50} values were determined and are presented in **Fig. 2**. ED_{50} values show clear differences in apoA-I immunoreactivity when different acylglycerides are incorporated into the LpA-I complexes. As illustrated in Fig. 2A, the inclusion of TG, with or without small amounts of DG, into an LpA-I complex was associated with a decrease in the ED_{50} for most of the antibodies studied. Indeed, all epitopes but one, 4H1 corresponding to residues 2 to 8 (21) in the N terminus of the apoA-I molecule, were more exposed in the presence of TG. This suggests that TG has a major effect on the conformation of apoA-I on LpA-I particles.

Inclusion of DG in an LpA-I complex had a different effect on apoA-I conformation, as illustrated in Fig. 2B. While the exposure of some epitopes located in both the N terminus and the central domain of the apoA-I molecule was enhanced (epitope of MAb 2F1, A16, A17, and A03) in the presence of DG, most of the other epitopes located throughout the apoA-I molecule were unaffected by this lipid. In contrast, the immunoreactivity of MAb A07 epitope in the C-terminal domain of apoA-I was affected by DG and differently from that observed with TG. The

exposure of the MAb A07 epitope in apoA-I, residues 149 to 186 (21), was significantly reduced in the presence of DG. Inclusion of 40 molecules of DG in an LpA-I complex was associated with an increase in ED_{50} between 20 and 50 μg/ml. In contrast, incorporation of similar amounts of TG increased the exposure of the A07 epitope and inclusion of small amounts of TG with DG also increased the exposure of this epitope.

Effect of LpA-I glyceride content on cholesterol esterification by LCAT

Increasing the acylglyceride content in LpA-I particles has a significant effect on the lipoprotein structural characteristics and therefore would be expected to also affect LpA-I functional properties. The effect of the glyceride content on the rate of cholesterol esterification by LCAT was investigated with particles similar to that presented in Table 1, except that the particles also contained 20 molecules of free cholesterol (FC) per particle. Because the amount of apoA-I is constant in the LpA-I characterized in this study (two molecules per particle), substrate curves are presented as a function of apoA-I concentration and therefore represent the ability of the different lipoproteins to act as a substrate for LCAT. As illustrated in **Fig. 3**, addition of TG or DG to the LpA-I complexes had opposite effects on cholesterol esterification by LCAT. The addition of TG into an LpA-I complex was associated with a significant decrease in initial velocity at maximum substrate concentration. In contrast, inclusion of DG counteracted the inhibitory effect of TG in particles containing both lipids. When added to the LpA-I particle alone, DG caused a major stimulation in the rate of cholesterol esterification by LCAT.

Because the initial velocities shown in Fig. 3 were estimated with minimal substrate conversion, variations in the saturation points for the different curves correspond

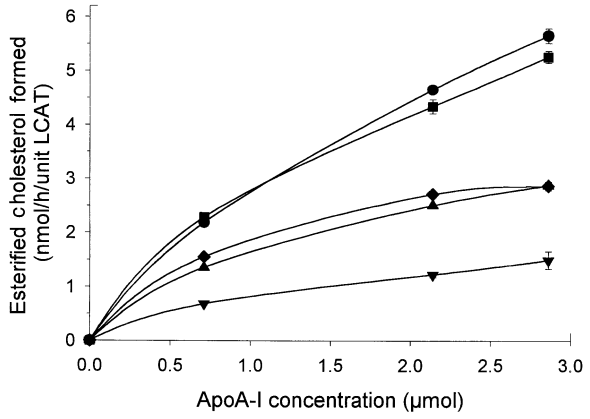

Fig. 3. Effect of the glyceride content of LpA-I on LCAT activation. The esterification by LCAT of [^{3}H]cholesterol in different LpA-I particles (POPC:DG:TG:FC:apoA-I molar ratios: circles, 120:40:0:20:2; squares, 120:40:10:20:2; triangles, 120:10:40:20:2; inverted triangles, 120:0:40:20:2; diamonds, 120:0:0:20:2) is shown. LpA-I were prepared by sonication as described in text and were incubated with purified LCAT. Values represent means ± SD of triplicate determinations and are representative of three different preparations of LpA-I complexes.

TABLE 3. Effect of LpA-I acylglyceride composition on LCAT kinetic parameters

LpA-I Complex (POPC:DG:TG:FC:ApoA-I)[a]	V_{max}[b]	K_m[c]
mol:mol:mol:mol:mol	*nmol CE/h*	*μM apoA-I*
120:0:0:20:2	4.1 ± 0.3	1.2 ± 0.2
120:0:40:20:2	2.3 ± 0.1	1.6 ± 0.1
120:10:40:20:2	4.5 ± 0.9	1.6 ± 0.1
120:40:10:20:2	8.6 ± 1.5	2.0 ± 0.6
120:40:0:20:2	11.3 ± 0.4	3.0 ± 0.3

[a] POPC, DG, TG, and apoA-I molar ratio of the LpA-I complexes. Values are representative of three different preparations of LpA-I.
[b] Apparent V_{max} and [c] K_m values (± SD) were estimated from double-reciprocal plots of the data shown in Fig. 3.

to changes in the V_{max} of cholesterol esterification by LCAT. This is evident in **Table 3**, which illustrates the apparent Michaelis-Menten kinetic constants estimated from a double-reciprocal plot analysis of the data shown in Fig. 3. Addition of 40 molecules of TG to an LpA-I complex was associated with a ~50% decrease in the V_{max} of cholesterol esterification by LCAT and an increase in the concentration of apoA-I required for half-maximal velocity (K_m). As such, it would appear that increases in the TG content of an LpA-I complex may act in a mixed noncompetitive inhibitory manner. In contrast, addition of 40 molecules of DG to an LpA-I complex caused an almost 3-fold increase in the LCAT V_{max} with a parallel increase in the K_m. This relationship between K_m and V_{max} values is similar to that observed in previous studies and shows that optimal rates of esterification by LCAT may require lower affinity binding, which facilitates a rapid interlipoprotein movement by this enzyme (22).

A comparison of the effect of DG on apoA-I structure and LCAT reactivity suggests that the rate of cholesterol esterification by LCAT may be directly affected by DG-mediated alterations in apoA-I conformation, charge, and stability. A direct relationship between LCAT activity and apoA-I surface charge is evident and indicated on **Fig. 4**; inclusion of DG in an LpA-I complex is associated with an increased LpA-I net negative charge and higher LCAT V_{max} values. This is in good agreement with previous studies, which have suggested that the rate of cholesterol esterification by LCAT is directly affected by the lipoprotein, protein-dependent, electrostatic properties (19, 22). The presence of DG in an LpA-I complex has a direct effect on apoA-I conformation and decreases the exposure of the A07 MAb epitope (residues 148 to 186). Reduced exposure of residues 148 to 186 at the surface of the apoA-I molecule is associated with an increased LpA-I net negative charge ($r = 0.7$) and a higher LCAT activity (Fig. 4). This supports our observation that LCAT is sensitive to apoA-I conformation-dependent surface charge (22). This unique LCAT-reactive conformation of apoA-I appears to promote an increased thermodynamic stability on the complex and a strong relationship also exists between an increased apoA-I stability and increased LCAT V_{max} values (Fig. 4). Overall, the data show that DG promotes the formation of more negatively charged and sta-

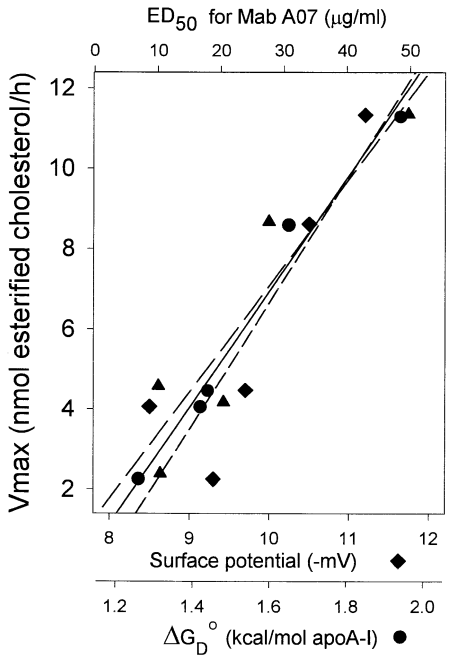

Fig. 4. Relationship between apparent LCAT V_{max} values and LpA-I biophysical characteristics. LCAT reaction V_{max} values were estimated as described in text and are plotted against LpA-I surface potential values (diamonds), the free energy of denaturation of apoA-I in the various LpA-I complexes (ΔG_D°) (circles) and the ED_{50} value for MAb A07 (triangles). Calculated regression lines are shown for the different series of data.

ble LpA-I particles, which exhibit a unique apoA-I conformation that appears to activate LCAT. Progressive increases in HDL TG result in a less negatively charged lipoprotein, which poorly activates LCAT.

DISCUSSION

Effect of DG on charge of apoA-I in LpA-I particles

Diacylglycerol has traditionally been thought to be a minor constituent of plasma lipoproteins, but has been shown to be the predominant acylglyceride of lipophorins, the insect hemolymph lipoproteins (23). More recent studies, however, show that DG is a major component of human HDL as well (9, 14, 15). This investigation shows that DG has major effects on the biophysical characteristics of apoA-I. ApoA-I electrostatic properties are extremely sensitive to the DG content of LpA-I complexes. Addition of a few molecules of DG has a significant effect on the LpA-I surface charge (Table 1). This result is similar to our earlier reports, which showed that the presence of neutral lipids in an LpA-I complex is indirectly responsible for much of its net negative charge (16). Previous work has shown that while about 10% of the charge on HDL comes from anionic lipids (i.e., phosphatidylinositol and fatty acids), the bulk of its net negative charge comes directly from the surface bound apolipoproteins, and is conformation sensitive (10, 16, 24). The present data show that this protein conformation-dependent charge is strongly dependent on the type of neutral lipid present in the lipoprotein; DG has a much greater effect on decreasing HDL surface charge than TG or CE [Table 1 and (16)]. Because neither CE nor TG are able to confer a surface charge to an LpA-I complex equivalent to that of a native HDL_3 particle (24), it appears that DG may be a major determinant of the HDL charge in vivo. Changes in lipoprotein charge, associated with different neutral lipids, appear as a consequence of unique conformational changes of the resident apoA-I molecules. As expected, a calculation of the molecular valence of apoA-I (which represents the number of excess negative charges) showed that the increased negative surface potential of the LpA-I complexes containing DG was due to an increase in the number of negative charges exposed at the surface of the apoA-I molecule (Table 1).

Effect of DG on apoA-I secondary structure and stability

Inclusion of DG or TG into an LpA-I complex had little effect on the amount of α-helical structure in apoA-I (Table 2). This result is similar to our previously reported findings and confirms the view that while CE increases the α-helix content of apoA-I, acylglyceride molecules have little effect on the secondary structure of this protein (16). However, different acylglyceride molecules can have unique effects on the stability of the α helices in apoA-I. Incorporation of DG into the LpA-I complex is associated with a major increase in the stability of apoA-I α helices to denaturation by guanidine hydrochloride (Table 2). Consistent with this finding, DG enrichment also appeared to prevent the dissolution of lipoprotein complexes after long-term storage at 4°C or nondenaturing electrophoresis (9). Because the inclusion of DG does not directly affect the α-helix content of apoA-I, the data suggest that DG molecules may interact closely with the apoA-I α helices and induce conformational changes that stabilize them. Indeed, DG significantly decreased the exposure of the A07 epitope, and a relationship was evident between the MAb ED_{50} values and the thermodynamic stability of the α helices of apoA-I ($r = 0.9$). Similar observations have been made with cholesterol (18). The study showed that cholesterol can also stabilize apoA-I structure, without increasing the amount of α helices in the protein. The lipid appeared to do so through direct lipid-protein interactions (18) and it appears that DG may interact with a similar domain in apoA-I.

Inclusion of large amounts of TG into an LpA-I particle profoundly changed the guanidine hydrochloride denaturation profiles and midpoints of denaturation. However, while the midpoint of denaturation increased significantly, isothermal denaturation analyses suggested that the thermodynamic stability of apoA-I was actually reduced in the presence of TG. This discrepancy between $D_{1/2}$ and ΔG_D° values is consistent with previous observations and highlights the importance of differentiating between guanidine accessibility and protein stability (10, 16, 17). This analysis actually appeared to corroborate our finding that the TG-enriched LpA-I showed an increased propensity to allow for apoA-I dissociation after storage at

4°C or nondenaturing electrophoresis. As such, it appears that TG may compete with apoA-I for binding to the phospholipid acyl chain interface. In this manner, TG may be able to partially displace apoA-I from the phospholipid interface and promote the reorganization of the apoA-I molecule into a less stable conformation. If apoA-I is less stable, the molecule may tend to dissociate from the LpA-I complex, as previously described (16). This may explain the "loosely bound apoA-I" phenomenon observed in the plasma of hypertriglyceridemic subjects, where apoA-I has been suggested to spontaneously dissociate from TG-enriched HDL (25). Variations in the TG-to-DG ratio in HDL particles may therefore affect the stability of HDL particles in vivo and influence their clearance from plasma (12, 25).

Effect of DG on apoA-I tertiary structure in LpA-I particles

Previous studies have shown that the binding of apoA-I to lipids can promote major changes in both the secondary and tertiary structures of apoA-I (26–28). Similar observations have been made with other exchangeable apoproteins, that is, apolipoprotein E and apolipophorin III, and studies suggest that the globular structure of the proteins can expand or open up in the presence of lipid (29, 30). Conformational changes in apoA-I were the most apparent with inclusion of TG. Almost every epitope in the molecule appeared to be more exposed and immunoreactive with the panel of MAbs studied. An increased immunoreactivity in the presence of TG was most apparent for the discontinuous epitope associated with the MAb, A16 (residues 14–29, 60–82). This may highlight major conformational changes in the N terminus of apoA-I and a decreased contact of this region with the lipoprotein surface lipids. Similar changes, albeit slightly less in magnitude, are apparent throughout the apoA-I molecule. The data suggest that TG may reduce the depth of penetration of apoA-I into the phospholipid surface and promote a more spread-out organization on the lipoprotein surface. This appears to promote a reduced stability of the molecule, as some of the protein-lipid hydrophobic interactions are lost (16).

DG has a more complex effect on apoA-I conformation and affects mostly epitopes located in the N-terminal and C-terminal regions of apoA-I (Fig. 2). Similar to TG, DG increases the immunoreactivity of two epitopes (2F1, A16) in the N terminus of apoA-I. However, in contrast to that observed with TG, DG decreases the expression of the A07 epitope, which corresponds to an α-helical region located between residues 148 and 186 in the C-terminal domain of the apoA-I molecule (21). This reduced immunoreactivity of the C-terminal region may indicate a more tight packing of these helices or a submersion into the surface lipid milieu. It is this unique DG-dependent conformational change in apoA-I that appears most closely associated with the observed changes in the charge and function of the molecule. We observed a direct relationship between the ED_{50} for A07 and the LpA-I surface potential ($r = 0.7$) and LCAT V_{max} values (Fig. 4). These data suggest that the exposure of the A07 epitope is associated with an increase in LpA-I charge and that these changes in conformation-dependent charge directly affect the action of LCAT.

The unique effects of DG on apoA-I conformation could be due to an effect of this lipid on the lipoprotein surface lipid structure or could relate to specific interactions between DG and apoA-I due to the unique distribution of DG both at the surface and in the core of the lipoprotein particle. Unlike TG and CE molecules, where hydrophobicity dictates their localization in the core of the HDL, DG appears to have a quite different distribution in the lipoprotein particle. ^{13}C nuclear magnetic resonance (NMR) studies using ^{13}C-enriched DG have shown that DG molecules are able to lie parallel to the phospholipid molecules in phospholipid bilayers, with both carbonyls exposed to the aqueous layer (31). Vieu et al. (14) observed that about 60% of the DG in human HDL was accessible to pancreatic lipase and therefore suggested that DG may partition between the core and the surface of HDL particles. This is consistent with the ^{13}C NMR work by Wang et al. (23), which showed a similar core/surface distribution of DG in lipophorin particles. The data are also strikingly similar to the distribution observed for FC in both LDL and HDL particles (32). While both DG and FC appear to be predominantly localized at the surface of lipoprotein particles, the lipids are also able to shuttle rapidly between the core and surface lipid domains. In addition, both lipids have little effect on the secondary structure of apoA-I, but significantly increase the negative charge and thermodynamic stability of the protein (18). This increase in stability of apoA-I, in the presence of DG and FC, appears to be associated with a decreased exposure of a central to C-terminal domain and a concomitant enhanced ability to stimulate FC esterification by LCAT.

Effect of LpA-I DG content on activation of LCAT

This investigation shows that DG and TG have different effects on the activation of LCAT. Incorporation of 40 molecules of TG into an LpA-I complex is associated with an almost 50% reduction in the V_{max} for LCAT and a parallel increase in apparent K_m values. While the catalytic mechanism involved is complex and does not fit most classic kinetic modeling assumptions, the data suggest that TG may inhibit LCAT in a mixed inhibitory fashion. This is unique and different from the inhibition observed with variations in the surface lipid constituents of HDL. In general, increases in PL have little effect on apparent K_m values, for apoA-I (22, 33), and therefore appear primarily to affect LCAT reaction velocity by regulating the availability of surface cholesterol. TG also affects the accessibility of LCAT to substrate cholesterol molecules, but in addition, TG appears to reduce the binding affinity of LCAT to the lipoprotein as well. Substrate accessibility may be affected by the partitioning of cholesterol into different domains within the lipoprotein. Because cholesterol can exist both at the surface and core, some of the surface cholesterol may migrate into the TG core and become less available to the enzyme. Because TG has quite the opposite effect on apoA-I, potentially displacing and spreading out

the molecule on the surface, it may be that this expanded comformation of apoA-I reduces the affinity with which LCAT binds to the lipoprotein particle (22, 34). It is of note, however, that a reduced affinity usually parallels an increased reactivity (V_{max}) with HDL (10, 18, 22). This may suggest that the movement and solubility of cholesterol in the lipoprotein core is primarily responsible for inhibition by TG.

In contrast to that observed with TG, DG significantly stimulates cholesterol esterification by LCAT. DG has a similar effect as TG and increases apparent K_m values, but has quite the opposite effect on the enzyme maximum velocity. Consequently, increases in DG content appear to have little effect on the catalytic efficiency (V_{max}/K_m) of the enzyme. This shows that this estimate of enzyme function is inappropriate for interfacially active enzymes, such as LCAT and HL, because their activity appears to be enhanced by a reduced binding affinity for lipoprotein particles (9, 19, 22). DG may reduce LCAT substrate affinity in a manner similar to TG, by promoting an expanded apoA-I conformation, which may compete with LCAT for binding to the particle surface. Increases in DG, in the presence of a TG core, are also able to stimulate the esterification of cholesterol by LCAT. This may suggest that DG is able to competitively prevent the partitioning of cholesterol into the lipoprotein core and maintain its accessibility to LCAT at the lipoprotein surface.

Different acylglycerides may affect LCAT by controlling the substrate accessibility and/or by domain-specific changes in the conformation of apoA-I. Studies in a variety of laboratories have shown that both central (residues 100–122) and C-terminal (residues 148–186) domains in apoA-I may be critical to the activation of LCAT (22, 35–39). The role of this central region of apoA-I in LCAT activation, however, appears to be more specific to discoidal HDL particles; no such relationship between the exposure of this region and LCAT activity has been identified with sonicated/spherical LpA-I complexes. Further, the critical importance of the central domain is now in doubt, as more recent studies have shown that a complete deletion of this region in apoA-I has little effect on LCAT (40). This is not the case with the more C-terminal domain of apoA-I. Epitope exposure measurements in this study show that while DG and TG have similar conformational effects on the N terminus of apoA-I, the lipids have quite opposite effects on the conformation of the domain comprising residues 148–186. A reduced exposure of this region directly parallels decreased rates of cholesterol esterification (Fig. 4). It therefore appears that, as proposed by Fielding et al. (39–42), this region may indeed be central to the activation of LCAT by apoA-I. In addition, a decreased exposure of this region is also directly associated with an increased apoA-I charge (Fig. 4). Because previous investigations have shown that the rate of cholesterol esterification by LCAT is related to the conformation-dependent charge on apoA-I (18, 22), it appears possible that LCAT may indeed interact with this region of apoA-I and that this interaction may be electrostatic in nature.

Physiological significance

New studies in this laboratory show that the DG-to-TG ratio is altered in the HDL isolated from hypertriglyceridemic patients (T. A. Ramsamy and D. L. Sparks, unpublished data). These compositional abnormalities would be expected to significantly modify both the structure and function of these lipoproteins. While we have shown that DG will affect the actions of both LCAT and HL, it appears likely that this lipid may affect other metabolic processes as well.

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REFERENCES

1. Gordon, T., W. P. Castelli, M. C. Hjortland, W. B. Kannel, and T. R. Dawber. 1977. High density lipoprotein as a protective factor against coronary heart disease. The Framingham Study. *Am. J. Med.* **62:** 707–714.
2. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein—the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
3. Morton, R. E. 1985. Binding of plasma-derived lipid transfer protein to lipoprotein substrates. The role of binding in the lipid transfer process. *J. Biol. Chem.* **260:** 12593–12599.
4. Sparks, D. L., and P. H. Pritchard, 1989. Transfer of cholesteryl ester into high density lipoprotein by cholesteryl ester transfer protein: effect of HDL lipid and apoprotein content. *J. Lipid Res.* **30:** 1491–1498.
5. Jonas, A. 1986. Reconstitution of high-density lipoproteins. *Methods Enzymol.* **128:** 553–582.
6. Davidson, W. S., K. L. Gillotte, S. Lund-Katz, W. J. Johnson, G. H. Rothblat, and M. C. Phillips. 1995. The effect of high density lipoprotein phospholipid acyl chain composition on the efflux of cellular free cholesterol. *J. Biol. Chem.* **270:** 5882–5890.
7. Mowri, H. O., W. Patsch, L. C. Smith, A. M. J. Gotto, and J. R. Patsch. 1992. Different reactivities of high density lipoprotein 2 subfractions with hepatic lipase. *J. Lipid Res.* **33:** 1269–1279.
8. Zhao, Y., D. L. Sparks, and Y. L. Marcel. 1996. Specific phospholipid association with apolipoprotein A-I stimulates cholesterol efflux from human fibroblasts. Studies with reconstituted sonicated lipoproteins. *J. Biol. Chem.* **271:** 25145–25151.
9. Coffill, C. R., T. A. Ramsamy, D. M. Hutt, J. R. Schultz, and D. L. Sparks. 1997. Diacylglycerol is the preferred substrate in high density lipoproteins for human hepatic lipase. *J. Lipid Res.* **38:** 2224–2231.
10. Sparks, D. L., P. G. Frank, S. Braschi, T. A. Neville, and Y. L. Marcel. 1999. Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. *Biochemistry.* **38:** 1727–1735.
11. Braschi, S., T. A. Neville, M. C. Vohl, and D. L. Sparks. 1999. Apolipoprotein A-I charge and conformation regulate the clearance of reconstituted high density lipoprotein in vivo. *J. Lipid Res.* **40:** 522–532.
12. Lamarche, B., K. D. Uffelman, A. Carpentier, J. S. Cohn, G. Steiner, P. H. Barrett, and G. F. Lewis. 1999. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. *J. Clin. Invest.* **103:** 1191–1199.
13. Eisenberg, S. 1984. High density lipoprotein metabolism [review; 398 refs]. *J. Lipid Res.* **25:** 1017–1058.
14. Vieu, C., B. Jaspard, R. Barbaras, J. Manent, H. Chap, B. Perret, and X. Collet. 1996. Identification and quantification of diacylglycerols in HDL and accessibility to lipase. *J. Lipid Res.* **37:** 1153–1161.
15. Lalanne, F., V. Pruneta, S. Bernard, and G. Ponsin. 1999. Distribution of diacylglycerols among plasma lipoproteins in control subjects and in patients with non-insulin-dependent diabetes. *Eur. J. Clin. Invest.* **29:** 139–144.

16. Sparks, D. L., W. S. Davidson, S. Lund-Katz, and M. C. Phillips. 1995. Effects of the neutral lipid content of high density lipoprotein on apolipoprotein A-I structure and particle stability. *J. Biol. Chem.* **270:** 26910–26917.
17. Sparks, D. L., S. Lund-Katz, and M. C. Phillips. 1992. The charge and structural stability of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. *J. Biol. Chem.* **267:** 25839–25847.
18. Sparks, D. L., W. S. Davidson, S. Lund-Katz, and M. C. Phillips. 1993. Effect of cholesterol on the charge and structure of apolipoprotein A-I in recombinant high density lipoprotein particles. *J. Biol. Chem.* **268:** 23250–23257.
19. Sparks, D. L., G. M. Anantharamaiah, J. P. Segrest, and M. C. Phillips. 1995. Effect of the cholesterol content of reconstituted LpA-I on lecithin:cholesterol acyltransferase activity. *J. Biol. Chem.* **270:** 5151–5157.
20. Calabresi, L., Q. H. Meng, G. R. Castro, and Y. L. Marcel. 1993. Apolipoprotein A-I conformation in discoidal particles: evidence for alternate structures. *Biochemistry.* **32:** 6477–6484.
21. Marcel, Y. L., P. R. Provost, H. Koa, E. Raffai, N. V. Dac, J. C. Fruchart, and E. Rassart. 1991. The epitopes of apolipoprotein A-I define distinct structural domains including a mobile middle region. *J. Biol. Chem.* **266:** 3644–3653.
22. Sparks, D. L., P. G. Frank, and T. A. Neville. 1998. Effect of the surface lipid composition of reconstituted LPA-I on apolipoprotein A-I structure and lecithin:cholesterol acyltransferase activity. *Biochim. Biophys. Acta.* **1390:** 160–172.
23. Wang, J., H. Liu, B. D. Sykes, and R. O. Ryan. 1995. Identification and localization of two distinct microenvironments for the diacylglycerol component of lipophorin particles by ^{13}C NMR. *Biochemistry.* **34:** 6755–6761.
24. Davidson, W. S., D. L. Sparks, S. Lund-Katz, and M. C. Phillips. 1994. The molecular basis for the difference in charge between pre-beta- and alpha-migrating high density lipoproteins. *J. Biol. Chem.* **269:** 8959–8965.
25. Horowitz, B. S., I. J. Goldberg, J. Merab, T. M. Vanni, R. Ramakrishnan, and H. N. Ginsberg. 1993. Increased plasma and renal clearance of an exchangeable pool of apolipoprotein A-I in subjects with low levels of high density lipoprotein cholesterol. *J. Clin. Invest.* **91:** 1743–1752.
26. Edelstein, C., and A. M. Scanu. 1980. Effect of guanidine hydrochloride on the hydrodynamic and thermodynamic properties of human apolipoprotein A-I in solution. *J. Biol. Chem.* **255:** 5747–5754.
27. Collet, X., B. Perret, G. Simard, E. Raffai, and Y. L. Marcel. 1991. Differential effects of lecithin and cholesterol on the immunoreactivity and conformation of apolipoprotein A-I in high density lipoproteins. *J. Biol. Chem.* **266:** 9145–9152.
28. Sparks, D. L., M. C. Phillips, and S. Lund-Katz. 1992. The conformation of apolipoprotein A-I in discoidal and spherical recombinant high density lipoprotein particles. ^{13}C NMR studies of lysine ionization behavior. *J. Biol. Chem.* **267:** 25830–25838.
29. Breiter, D. R., M. R. Kanost, M. M. Benning, G. Wesenberg, J. H. Law, M. A. Wells, I. Rayment, and H. M. Holden. 1991. Molecular structure of an apolipoprotein determined at 2.5-Å resolution. *Biochemistry.* **30:** 603–608.
30. Lund-Katz, S., K. H. Weisgraber, R. W. Mahley, and M. C. Phillips. 1993. Conformation of apolipoprotein E in lipoproteins. *J. Biol. Chem.* **268:** 23008–23015
31. Hamilton, J. A., S. P. Bhamidipati, D. R. Kodali, and D. M. Small. 1991. The interfacial conformation and transbilayer movement of diacylglycerols in phospholipid bilayers. *J. Biol. Chem.* **266:** 1177–1186.
32. Lund-Katz, S., and M. C. Phillips. 1986. Packing of cholesterol molecules in human low-density lipoprotein. *Biochemistry.* **25:** 1562–1568.
33. Subbaiah, P. V., and M. Liu. 1993. Role of sphingomyelin in the regulation of cholesterol esterification in the plasma lipoproteins. Inhibition of lecithin-cholesterol acyltransferase reaction. *J. Biol. Chem.* **268:** 20156–20163.
34. Bolin, D. J., and A. Jonas. 1994. Binding of lecithin:cholesterol acyltransferase to reconstituted high density lipoproteins is affected by their lipid but not apolipoprotein composition. *J. Biol. Chem.* **269:** 7429–7434.
35. Rall, S. C., Jr., K. H. Weisgraber, R. W. Mahley, C. Ehnholm, O. Schamaun, B. Olaisen, J. P. Blomhoff, and P. Teisberg. 1986. Identification of homozygosity for a human apolipoprotein A-I variant. *J. Lipid Res.* **27:** 436–441.
36. Banka, C. L., D. J. Bonnet, A. S. Black, R. S. Smith, and L. K. Curtiss. 1991. Localization of an apolipoprotein A-I epitope critical for activation of lecithin-cholesterol acyltransferase. *J. Biol. Chem.* **266:** 23886–23892.
37. Minnich, A., X. Collet, A. Roghani, C. Cladaras, R. L. Hamilton, C. J. Fielding, and V. I. Zannis. 1992. Site-directed mutagenesis and structure-function analysis of the human apolipoprotein A-I. Relation between lecithin-cholesterol acyltransferase activation and lipid binding. *J. Biol. Chem.* **267:** 16553–16560.
38. Sorci-Thomas, M., M. W. Kearns, and J. P. Lee. 1993. Apolipoprotein A-I domains involved in lecithin-cholesterol acyltransferase activation. Structure:function relationships. *J. Biol. Chem.* **268:** 21403–21409.
39. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
40. Frank, P. G., D. N'Guyen, V. Franklin, T. Neville, M. Desforges, E. Rassart, D. L. Sparks, and Y. L. Marcel. 1998. Importance of central alpha-helices of human apolipoprotein A-I in the maturation of high-density lipoproteins [in process citation]. *Biochemistry.* **37:** 13902–13909.
41. Sorci-Thomas, M. G., L. Curtiss, J. S. Parks, M. J. Thomas, and M. W. Kearns. 1997. Alteration in apolipoprotein A-I 22-mer repeat order results in a decrease in lecithin:cholesterol acyltransferase reactivity. *J. Biol. Chem.* **272:** 7278–7284.
42. Dhoest, A., Z. A. Zhao, B. De Geest, E. Deridder, A. Sillen, Y. Engelborghs, D. Collen, and P. Holvoet. 1997. Role of the Arg^{123}-Tyr^{166} paired helix of apolipoprotein A-I in lecithin:cholesterol acyltransferase activation. *J. Biol. Chem.* **272:** 15967–15972.