Role of lecithin:cholesterol acyltransferase and apolipoprotein A-I in cholesterol esterification in lipoprotein-X in vitro

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Abstract Lipoprotein-X (Lp-X) is an abnormal particle present in the plasma of patients with familial lecithin:cholesterol acyltransferase (LCAT) deficiency syndromes or cholestatic liver disease. Compared to other lipoproteins, Lp-X contains a high content of unesterified cholesterol (30%, w/w) to phosphatidylcholine (60%, w/w). The objective of this study was to evaluate the role of LCAT and apolipoprotein A-I (apoA-I) in Lp-X metabolism in vitro and to elucidate the regulation of cholesterol esterification in this unique lipoprotein. Lp-X isolated from sera of patients with obstructive jaundice had a high content of unesterified cholesterol and phosphatidylcholine and contained apolipoprotein E, apoCs, and albumin. Although human recombinant LCAT used as an enzyme source did bind to isolated Lp-X, no cholesterol esterification was detected. However, addition of human apoA-I in the presence of albumin resulted in significant cholesterol esterification in Lp-X (V_{max} 0.25 ± 0.04 nmol/h per μg LCAT protein). Exogenous apoA-I did not change the size of Lp-X particle as determined by quasi-elastic light scattering analysis. A reduction in Lp-X size was observed when both apoA-I and LCAT were included in the reaction mixture (from 47 nm to 42 nm). Furthermore, addition of apoA-I (but not HDL) dramatically changed the electrophoretic mobility of Lp-X from cathodic to anodic migration. Such changes are not due to displacement of apoC or apoE proteins from Lp-X by apoA-I. While increasing apoA-I concentration (up to 35 μg/ml) in the reaction mixture stimulated cholesterol esterification in Lp-X, addition of apoA-I at the concentration of 8 μg/ml inhibited cholesterol esterification in VLDL, LDL, and HDL by more than 50%. Albumin was required for the LCAT reaction to Lp-X. Our results suggest that while LCAT binds to isolated Lp-X, apoA-I is needed for the LCAT reaction to proceed. The presence of apoA-I does not result in the displacement of apoCs and apoE from Lp-X and addition of apoA-I changes the electrophoretic mobility but not the size of Lp-X.—**O, K., and J. Frohlich.** Role of lecithin:cholesterol acyltransferase and apolipoprotein A-I in cholesterol esterification in lipoprotein-X in vitro. *J. Lipid Res.* 1995. **36:** 2344–2354.

Supplementary key words apoE • apoCs • unesterified cholesterol • phosphatidylcholine

Multiple plasma lipoprotein abnormalities have been observed in patients with lecithin:cholesterol acyltransferase (LCAT, EC2.3.1.43) deficiency syndromes (1, 2). Patients with this autosomal recessive disorder have little or no plasma LCAT activity due to either a total enzyme deficiency or synthesis and secretion of a defective enzyme (2, 3). A number of mutations have been identified in the LCAT gene that are causative for the defect in enzyme synthesis or secretion (3–6). LCAT catalyzes the formation of most cholesteryl esters in plasma via hydrolysis (phospholipase activity) and transfer (acyltransferase activity) of the *sn*-2 fatty acid from phosphatidylcholine to the 3-hydroxy group of cholesterol (7). Under normal conditions, LCAT acts preferentially on high density lipoprotein (HDL) and requires apolipoprotein A-I (apoA-I) for optimal activity (8). One striking feature of LCAT deficiency, either primary or secondary (to liver disease), is the presence in plasma of lipoprotein-X (Lp-X) which is rich in unesterified cholesterol and phosphatidylcholine (2, 9–11).

Lp-X is associated with the low density lipoprotein (LDL) fraction isolated in a density gradient of 1.019–1.063 g/ml (10–13). Under the electron microscope, Lp-X appears as a bilayer vesicle with a diameter of 30–70 nm (10–13). This lipoprotein has a high content of phospholipids (60%, w/w) and unesterified cholesterol (30%, w/w) (10–13). Phosphatidylcholine is the

Abbreviations: Lp-X, lipoprotein-X; LCAT, lecithin:cholesterol acyltransferase; apoA-I, apolipoprotein A-I; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

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major phospholipid in Lp-X and sphingomyelin constitutes the minor fraction. It also contains cholesteryl ester (2%, w/w), triglyceride (2%, w/w), and a small amount of protein (6%, w/w). The major proteins in Lp-X are albumin and C apolipoprotein (apoC-I, C-II and C-III) (10–13). Albumin is trapped in the center of the particle and the apoCs are present on the surface (10–13). Some apoA-I and apolipoprotein E (apoE) may also be associated with Lp-X particles (10–13). This lipoprotein can be separated into three subspecies, Lp-X_1, Lp-X_2, and Lp-X_3 by zonal ultracentrifugation (14). All of the subspecies contain albumin and apoCs as their major protein constituents, but only Lp-X_2 and Lp-X_3 contain apoA-I and apoE (14).

The structure and chemical composition of Lp-X found in LCAT deficiency and cholestatic liver disease appear to be identical (10–12). In familial LCAT deficiency Lp-X is likely derived from the surface of chylomicron remnants that are not further catabolized due to the lack of LCAT activity (2, 10). The mechanism of Lp-X formation in cholestatic liver disease is not entirely clear. It has been postulated that Lp-X may, at least in part, originate from bile lipids regurgitated into the plasma compartment due to obstruction of bile ducts (10, 11). An inverse relationship between plasma concentration of Lp-X and LCAT activity has been observed in patients with familial LCAT deficiency after blood transfusion, indicating that lack of LCAT activity is a prerequisite for Lp-X formation (15). However, there has been some controversy as to whether this lipoprotein is a suitable substrate for the enzyme (16–19). Wengeler and Seidel (16) found that unesterified cholesterol in Lp-X did not serve as substrate for LCAT. In contrast, Patsch et al. (17, 18) showed that incubation of normal plasma with Lp-X resulted in the disappearance of this abnormal lipoprotein and the appearance of high density lipoprotein-like particle. Ritland and Gjone (19) observed that Lp-X was a source of unesterified cholesterol in the LCAT reaction during incubation of Lp-X-containing plasma after blood transfusion. However, the question whether Lp-X was merely a source for transfer of unesterified cholesterol to other lipoproteins for further esterification or whether Lp-X might act as a substrate itself was not answered in these investigations. The objective of this study was to investigate the factors regulating cholesterol esterification in isolated Lp-X and to elucidate the mechanism of interaction between LCAT, Lp-X, and apoA-I.

MATERIALS AND METHODS

Isolation and radiolabeling of Lp-X

Blood samples for the isolation of Lp-X were obtained from patients with obstructive liver diseases, with high bilirubin levels and increased alkaline phosphatase activities. Lp-X-positive sera were identified by 1% agarose gel electrophoresis followed by staining with Sudan Black. Lp-X did not migrate to the anode as opposed to the anodic migration of other plasma lipoproteins (**Fig. 1,** lane 2). Serum was prepared by centrifugation at 1,200 *g* for 20 min. Total bilirubin levels ranged between 200 and 579 μmol/L and the alkaline phosphatase activities were 473–985 U/L. All procedures were approved by the University Ethical Review Committee. The concentrations of unesterified cholesterol, total cholesterol, and triglycerides in sera were determined enzymatically using reagent kits from Boehringer Mannheim GmbH. Quantitation of apoA-I in sera was achieved by ELISA using polyclonal anti-human apoA-I antiserum (Boehringer Mannheim GmbH). LCAT activity was determined using proteoliposome-containing egg yolk phosphatidylcholine–unesterified cholesterol–purified human plasma apoA-I as a substrate as described previously (20). The substrate mixture containing 4.66 nmol [^{3}H]cholesterol (0.03 μCi/nmol), 18.46 nmol phosphatidylcholine, and 5.0 μg purified human plasma apoA-I in 10 mM Tris-HCl (pH 7.4)–150 mM NaCl–5 mM EDTA was preincubated at 37°C for 30 min. Subsequently, 5 mM β-mercaptoethanol and 1.5% bovine serum albumin (essentially fatty acid-free) were added to the substrate mixture. The reaction was initiated by the addition of serum (20 μl) to a final volume of 0.3 ml. The reaction was carried out at 37°C for 1 h. The radioactivity associated with cholesterol and

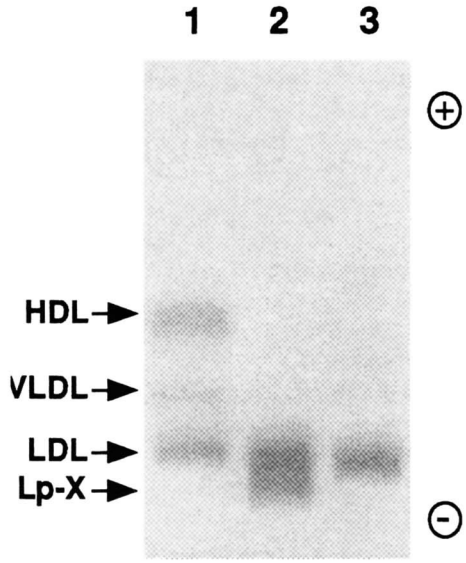

Fig. 1. Lipoprotein electrophoresis. An aliquot of sera from controls (lane 1), pooled sera from obstructive jaundice patients (lane 2), or LDL isolated from normal serum (lane 3) was loaded on a 1% agarose gel and electrophoresed for 35 min. The lipoproteins were stained with Sudan Black. The arrows indicate the position of individual lipoproteins.

TABLE 1. Lipid and apolipoprotein composition and LCAT activity of sera from patients with obstructive jaundice and control

	UC	TC	TG	HDL-C	ApoA-I	LCAT
			mg/dl			nmol/h/ml
Jaundice	344 ± 50	356 ± 61	118 ± 30	20 ± 5	10 ± 2	8.9 ± 1.3
Control	48 ± 8	208 ± 136	385 ± 24	47 ± 12	144 ± 24	31.7 ± 4.5

Lipid and apolipoprotein composition were determined as described in the Materials and Methods. LCAT activity was determined using apoA-I-containing proteoliposome as a substrate. UC, unesterified cholesterol; TC, total cholesterol; TG, triglycerols; HDL-C, HDL cholesterol. Both jaundice and control sera were prepared from eight patients with obstructive jaundice and eight volunteers, respectively. The results are expressed as mean diameter ± standard deviation from Lp-X isolated from eight patients or controls.

cholesteryl ester was determined by thin-layer chromatographic analysis.

Lp-X was isolated by sequential ultracentrifugation followed by gel filtration column chromatography. Briefly, the low density lipoprotein (LDL) fraction was isolated in a density gradient of 1.019–1.063 g/ml by ultracentrifugation (20). Lp-X associated with this fraction was separated from LDL by Superose 6B column chromatography. Lp-X was eluted from the column by 0.15 M NaCl containing 1 mM EDTA and 0.01% NaN_3 (pH 7.4) as a distinct peak. The mobility of the isolated Lp-X was determined by electrophoresis in 1% agarose gel. Lp-X cholesterol and cholesteryl esters were determined enzymatically using reagent kits (Boehringer Mannheim GmbH). Phospholipids and protein were determined by the modified methods of Bartlett (21) and Lowry et al. (22), respectively. After delipidation of Lp-X, apolipoproteins were identified by SDS polyacrylamide gel (7.5%) electrophoresis and isoelectric focusing (pH 3.5–9.5) (23). ApoE was identified by Western immunoblotting with polyclonal goat anti-(human apoE) antibodies. An aliquot of Lp-X was electrophoresed on an SDS polyacrylamide gel (7.5%). After the electrophoretic transfer of proteins from the gel to nitrocellulose membrane (pore size, 0.45 μm), the membrane was incubated with anti-(human apoE) antibodies (Calbiochem) and the blots were developed with Protein G-conjugated horseradish peroxidase.

Production and purification of recombinant LCAT

Transfected baby hamster kidney (BHK) cells that constitutively expressed a significant amount of human recombinant LCAT were incubated in Dulbecco's modified Eagle's medium (DMEM, GIBCO BRL Canada) containing 10% fetal calf serum for 12 h (20). The cells were then washed three times in serum-free medium (Opti-MEM, GIBCO BRL Canada) and then incubated in Opti-MEM for 48 h. Recombinant LCAT secreted into culture medium was purified by phenyl Sepharose CL 4B column chromatography (24). Briefly, 150 ml of culture medium containing LCAT was applied to a phenyl Sepharose column (1.0 × 15 cm) equilibrated with 0.005 M sodium phosphate containing 0.3 M sodium chloride (pH 7.4). The column was washed with the same buffer to remove other proteins and LCAT was eluted from the column by deionized water as a single protein peak. The amount of LCAT protein was determined by solid-phase immunoassay as described previously (24).

Determination of cholesterol esterification in Lp-X

An aliquot of isolated Lp-X was radiolabeled by equilibration with [^{3}H]cholesterol (0.05 μCi/nmol)-coated filter disks at 4°C as described by Dobiasova and Schutzova (25). ApoA-I was purified from human plasma by chromatofocusing column chromatography (23). Briefly,

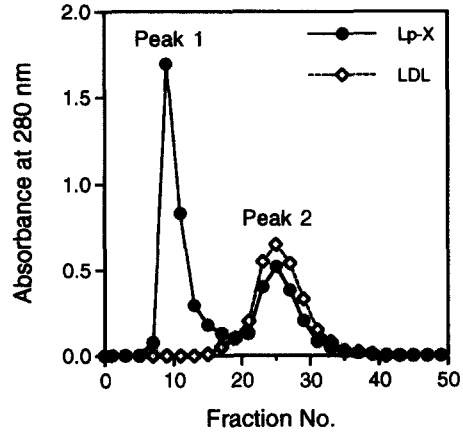

Fig. 2. Isolation of Lp-X by Superose 6B column chromatography. A fraction at a density 1.019–1.063 g/ml was collected after ultracentrifugation of Lp-X-positive sera (●). An aliquot (2 ml) of this fraction was applied to a Superose 6B column (50 × 1.5) equilibrated with 0.15 M NaCl containing 1 mM EDTA and 0.01% NaN_3 (pH 7.4). After the column was washed with 30 ml of the same buffer at a flow rate of 0.7 ml per min, the fractions (1.2 ml per fraction) were collected. As a control, a fraction with the same density was prepared from sera of normal volunteers (◇). An aliquot was applied to a Superose 6B column and the lipoprotein was eluted under the same conditions.

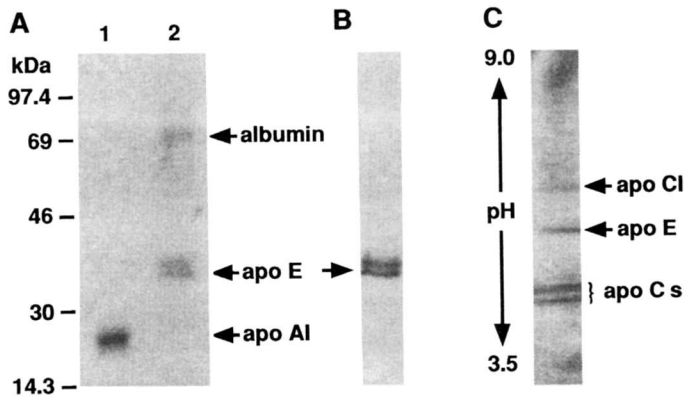

Fig. 3. Identification of proteins in Lp-X. A: An aliquot of purified apoA-I (lane 1) or delipidated Lp-X (lane 2) was loaded on a 7.5% SDS polyacrylamide gel. After electrophoresis, the separated proteins were stained with Coomassie blue. Migration position and molecular mass (in kilodaltons) of protein standards are indicated. B: An aliquot of delipidated Lp-X was loaded on a 7.5% SDS polyacrylamide gel followed by Western immunoblotting using polyclonal goat anti-(human-apoE) antibodies. C: An aliquot of delipidated lipoproteins was loaded on a isoelectric focusing (pH 3.5–9.0 gradient) polyacrylamide gel (7.5%). After electrophoresis for 2 h, the proteins were stained with Coomassie blue.

HDL was isolated by sequential ultracentrifugation at density 1.063–1.210 g/ml. Delipidation of isolated HDL was carried out with ethanol–diethyl ether 3:1 and the final pellet was dried under a stream of nitrogen. The apolipoprotein pellet was solubilized in 10 mM Tris-HCL (pH 7.4) containing 7.2 M urea and 10 mM dithiothreitol. After stirring for 16 h at 4°C, insoluble apoB aggregates were removed by centrifugation. Solubilized apolipoproteins were fractionated on a column (1.5 × 50 cm) of PBE 94 (Pharmacia) equilibrated with 25 mM imidazole-HCL (pH 7.4)–1 mM EDTA–7.2 M urea. The pH gradient was formed by elution with Polybuffer 94 diluted 1:8 with 8 M urea. Polybuffer in purified apoA-I was removed by passage through an hydroxylapatite column. The purity of apoA-I was determined by SDS polyacrylamide gel electrophoresis.

For determination of cholesterol esterification in Lp-X, different amounts of radiolabeled Lp-X were incubated with purified recombinant LCAT (2–6 μg/ml) in the absence or presence of purified human apoA-I (5–35 μg/ml) and fatty acid-free bovine serum albumin (Sigma, St. Louis, MO) at 37°C for 1–6 h. The total volume of incubation mixture was 0.3 ml. The formation of [^{3}H]cholesteryl esters (acyltransferase activity) was measured at different time intervals. The reaction was linear for up to 6 h. The cholesteryl esters were separated from unesterified cholesterol by thin-layer chromatography and the radioactivity associated with these lipids was determined by liquid scintillation counting. The cholesterol esterification rate was calculated and expressed in nmol of Lp-X cholesterol esterified per μg LCAT protein (20).

Phospholipase activity of recombinant LCAT was also determined using Lp-X as a substrate. An aliquot of isolated Lp-X was radiolabeled by equilibration with 1-palmitoyl,2-[9,10-^{3}H(n)]palmitoyl glycerophosphocholine (0.05 μCi/nmol)-coated filter disks at 4°C (25). The reaction was carried out under the same conditions as described above. [^{3}H]fatty acids were separated from radiolabeled phosphatidylcholine by thin-layer chromatography. Phospholipase activity was expressed as nmol of fatty acids released from [^{3}H]phosphatidylcholine per h per μg of LCAT protein.

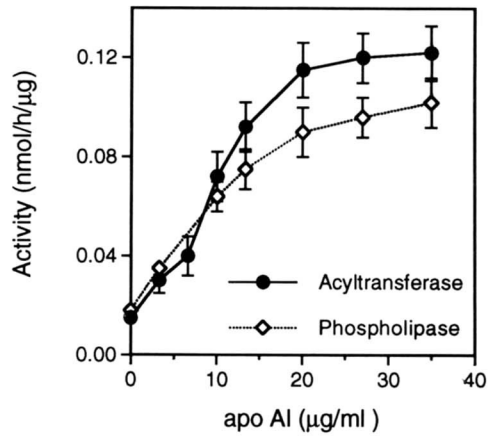

Fig. 4. Effect of apoA-I on the activities of phospholipase and acyltransferase of LCAT. An aliquot of [^{3}H]phosphatidylcholine- or [^{3}H]cholesterol-labeled Lp-X was incubated with recombinant LCAT at 37°C for 5 h in the presence of increasing amounts of apoA-I. Phospholipase and acyltransferase activities were determined and the results were expressed as nmol of fatty acid released from phosphatidylcholine per h per μg of LCAT protein (phospholipase) and nmol of cholesteryl ester formed per h per μg of LCAT protein (acyltransferase). Each point represents the mean of three experiments and standard deviation is indicated as a vertical bar.

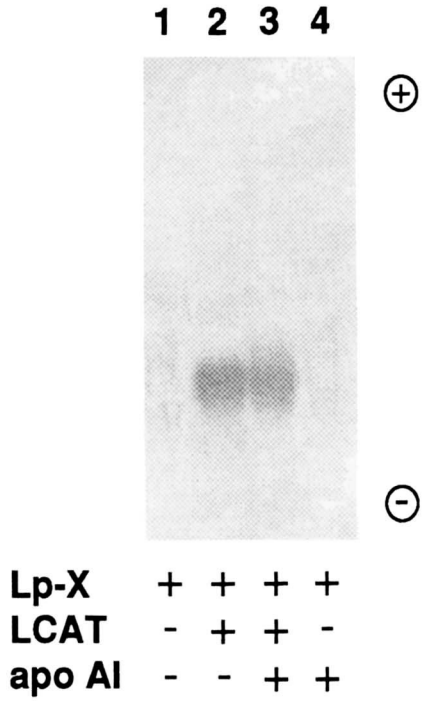

Fig. 5. Interaction of LCAT with isolated Lp-X. Lp-X (10 µl) was incubated with purified recombinant LCAT (3 µg/ml) in the absence or presence of purified human plasma apoA-I (5 µg/ml) at 37°C for 30 min. An aliquot of the reaction mixture was loaded on a 1% agarose gel and electrophoresed for 35 min and proteins were transferred from the gel to a nitrocellulose membrane. The position of LCAT associated with Lp-X was identified by Western immunoblotting with anti-(human LCAT) antibodies.

Determination of Lp-X size

The size of the isolated Lp-X was determined by quasi-elastic light scattering analysis, also referred to as dynamic light scattering or photon correlation spectroscopy (26). The analyses were performed using Nicomp Model 270 Laser Particle Sizer with a 5 mM helium-neon laser at an exciting wavelength of 632.8 nm. The technology used digital autocorrelation to analyze the fluctuations in scattered light intensity generated by the diffusion of vesicles in solution. The diffusion coefficient was then used to calculate the average hydrodynamic radius and hence the mean diameter of the vesicles. Student's *t*-test was used for statistical analysis. The level of significance was set at $P < 0.05$.

Western immunoblotting

Lp-X was preincubated with purified human plasma apoA-I for 30 min or 5 h at 37°C. The mixtures were applied to 1% agarose gel. After electrophoretic transfer of proteins from the gel to nitrocellulose membrane (pore size, 0.45 µm), the membrane was incubated with anti-(human apoC-I) antibodies (Chemicon), anti-(human apoC-II) antibodies (Calbiochem), anti-(human apoC-III) antibodies (raised in rabbits in our laboratory against purified human apoC-III). Blots were developed with horseradish peroxidase conjugate. To determine the presence of apoE, nitrocellulose membrane was incubated with anti-(human apoE) antibodies (Calbiochem) and the blots were developed using Protein-G horseradish peroxidase conjugate.

Interaction of LCAT with Lp-X

Lp-X was preincubated with purified recombinant LCAT and/or purified human plasma apoA-I for 30 min at 37°C. The mixtures were then separated on 1% agarose gel, transferred onto nitrocellulose membrane (pore size, 0.45 µm), and immunoblotted with polyclonal anti-(human LCAT) antibodies (a gift from Dr. A. Lacko, Fort Worth, TX) (20). The antibodies did not cross-react with human plasma apoA-I.

Determination of cholesterol esterification in other lipoproteins

Blood was also collected from eight normal volunteers after a 12-h fast and sera were prepared by low speed centrifugation. Different lipoproteins were isolated by preparative ultracentrifugation based on their densities: $d < 1.006$ g/ml for VLDL, $1.019 < d < 1.063$ g/ml for LDL, and $1.063 < d < 1.21$ g/ml for HDL (20). All lipoprotein preparations were dialyzed extensively at 4°C against 0.01 M Tris-HCl (pH 7.4) containing 0.15 M NaCl and 0.005 M EDTA. Trace amounts of apoA-I in VLDL and LDL preparations were removed by incubating with anti-(human apoA-I) antibodies (Boehringer Mannheim GmbH) followed by immunoadsorption with Gammabind G-agarose. The concentration of unesterified cholesterol in these lipoproteins was determined enzymatically using a reagent kit (Boehringer Mannheim GmbH). The isolated lipoproteins were labeled with [^{3}H]cholesterol (0.05 µCi/nmol) as described above. Cholesterol esterification in isolated VLDL, LDL, or heat-inactivated HDL in the presence of recombinant

TABLE 2. Size of Lp-X before and after incubation with apoA-I and LCAT

	Mean diameter ± SD
	nm
LP-X	47.3 ± 1.1
LP-X + apoA-I	47.2 ± 1.2
LP-X + apoA-I + LCAT	42.3 ± 1.5[a]

Size distribution of Lp-X using quasi-elastic light scattering was determined before and after incubation of Lp-X with apoA-I (20 µg/ml) in the absence or presence of LCAT (6 µg/ml) at 37°C for 5 h. Mean diameter of particles was obtained from Gaussian analysis. The results are expressed as mean diameter ± standard deviation from Lp-X isolated from five patients each measured in duplicate.

[a] $P < 0.05$ when compared to control values.

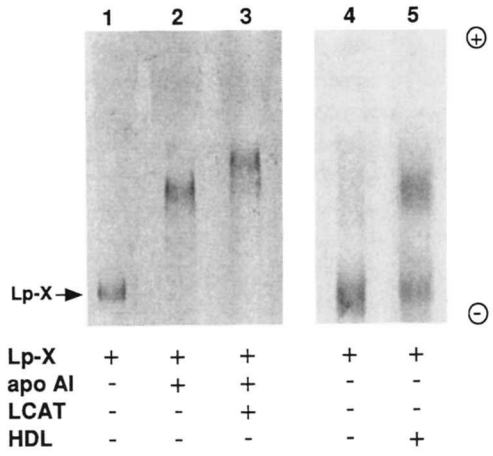

Fig. 6. Effect of apoA-I, LCAT, and HDL on electrophoretic mobility of isolated Lp-X. An aliquot (10 µl) of Lp-X was incubated with apoA-I (5 µg/ml), LCAT (3 µg/ml), or HDL (5 µl) at 37°C for 30 min. An aliquot of the reaction mixture was then loaded on a 1% agarose gel and electrophoresed for 35 min. Lipoproteins were stained with Sudan Black. The arrow indicates the position of native Lp-X.

LCAT was determined as described previously (20). No cholesterol esterification was detected in Lp-X, VLDL, LDL, or heat-inactivated HDL preparations in the absence of recombinant LCAT. The heat-inactivation procedure (56°C for 30 min) to eliminate endogenous enzyme activity associated with HDL has been carried out in a number of laboratories to elucidate the catalytic mechanism of LCAT reaction (20, 27, 28). This procedure did not interfere with the gradient gel pattern of HDL compared to the native preparations (20, 27, 28).

RESULTS

Measurement of cholesterol esterification in Lp-X

The contents of lipids, apolipoproteins, and LCAT activity were determined in Lp-X-positive sera and controls (**Table 1**). The level of apoA-I in Lp-X-positive sera was less than 10% of controls. The cholesterol content was increased, most of it in unesterified form. The levels of HDL cholesterol were also markedly reduced (42% of the control). LCAT activity was about 25% of the normal value when proteoliposomes (exogenous substrate) containing [^{3}H]cholesterol–egg yolk phosphatidylcholine–human apoA-I were used as a substrate. The molar rate of cholesterol esterification in patients' sera was only 30% of the control when LCAT activity was determined, based on the esterification rate of endogenous unesterified cholesterol in sera (data not shown). Lp-X was then isolated with LDL at a density of 1.019–1.063 g/ml by sequential ultracentrifugation and further separated from LDL by a Superose 6B column chromatography. As shown in **Fig. 2,** two distinct protein peaks were eluted from the column. Peak 1 was not seen in control samples and peak 2 corresponded to the LDL fraction. The fractions of peak 1 were pooled and their lipids were analyzed. Unesterified cholesterol (2.5–3.5 nmol/µl) constituted more than 98% of total cholesterol in the pooled fractions. The ratio of unesterified cholesterol to phospholipids was 0.97:1 which was similar to that previously reported for Lp-X (16–19). Peak 1 also displayed cathodal migration in 1% agarose gel electrophoresis. On the basis of its lipid composition and electrophoretic mobility, peak 1 represented the Lp-X fraction. Proteins in the individually isolated Lp-X preparations were identified by Coomassie blue staining after SDS polyacrylamide gel electrophoresis. No apoB was found in the Lp-X preparation (**Fig. 3A**). Albumin and apoE were found to be associated with Lp-X. The protein band with a relative molecular mass of 34–35 kDa was confirmed to be apoE by Western immunoblotting (Fig. 3B). In contrast to a previous report (18), we found no apoA-I in our preparations. ApoCs in Lp-X were identified by analytical isoelectric focusing in polyacrylamide gel electrophoresis (Fig. 3C). At least four protein bands corresponding to the isoelectric point of 6.5 for apoC-I, 5.8 for apoE, and 4.8–5.0 for apoC-III and C-II were observed (Fig. 3C).

To determine cholesterol esterification in Lp-X, an aliquot of isolated Lp-X was first radiolabeled with [^{3}H]cholesterol and various amounts of labeled Lp-X were incubated with purified recombinant LCAT and bovine serum albumin (7 mg/ml) at 37°C for 5 h. In the absence of apoA-I, a very small amount of cholesterol in Lp-X was esterified regardless of the concentration of Lp-X in the reaction mixture. When apoA-I was added to the reaction mixture, the rate of cholesterol esterification increased to 0.12 nmol/h per µg of LCAT protein as the amount of Lp-X increased. As shown in **Fig. 4,** the rate of cholesterol esterification (acyltransferase activity) in Lp-X catalyzed by LCAT increased as a function of apoA-I concentration. Phospholipase activity of recombinant LCAT was determined using [^{3}H]phosphatidylcholine-labeled Lp-X as substrates. In the presence of various amounts of apoA-I, a similar stimulation curve was obtained (Fig. 4).

Interaction of LCAT with isolated Lp-X

To determine whether apoA-I is required for the binding of LCAT to Lp-X, an aliquot of Lp-X was preincubated with purified recombinant LCAT in the absence or presence of purified human plasma apoA-I for 30 min at 37°C. After separation of reaction mixtures in 1% agarose gel followed by electrophoretic

transfer of proteins to nitrocellulose membrane, LCAT was identified by Western immunoblotting with anti-(human LCAT) antibodies. As shown in **Fig. 5,** LCAT was associated with Lp-X in the presence or absence of apoA-I.

Effect of apoA-I on Lp-X size and electrophoretic mobility

Our next objective was to examine the effect of apoA-I on the size and composition of Lp-X. The size of Lp-X particle was measured by quasi-elastic light scattering. The average size of Lp-X particle was 46–48 nm in diameter (**Table 2**). No significant change in Lp-X size occurred during the 5 h of incubation with apoA-I (10–50 μg/ml) at 37°C. A reduction in Lp-X size was observed when both apoA-I and LCAT were added to the incubation mixture. Similar results were obtained whether the mixtures were incubated for 30 min, or 3 or 5 h at 37°C. For comparison, average sizes of LDL (22 nm in diameter) and VLDL (72 nm in diameter) were also determined.

The electrophoretic mobility of Lp-X in agarose gel was examined before and after incubation with apoA-I and LCAT for 30 min at 37°C. Addition of apoA-I (5–20 μg/ml) in the absence or presence of LCAT (3 μg/ml) to the isolated Lp-X resulted in its anodic migration (**Fig. 6**). Similar changes were observed when the mixtures were incubated for 5 h at 37°C (data not shown). Upon incubation with isolated HDL, the electrophoretic mobility of Lp-X remained unchanged (Fig. 6).

In order to elucidate the mechanism by which the addition of apoA-I changed the electrophoretic mobility of Lp-X in agarose gel and the reduction in the size of Lp-X in the presence of both apoA-I and LCAT, the apolipoprotein composition of Lp-X before and after incubation with apoA-I was examined by Western immunoblotting. After the electrophoretic transfer of proteins onto nitrocellulose membrane, the membrane was incubated with anti-(human apoC) antibodies. As shown in **Fig. 7,** apoC-I, C-II, and C-III were present in Lp-X in the absence or presence of added apoA-I (5 μg/ml). Western immunoblotting was also performed with anti-(human apoE) antibodies and the results were shown in Fig. 7. ApoE was associated with Lp-X before and after incubation with apoA-I for 30 min regardless of a change in electrophoretic mobility of Lp-X in agarose gel. These results suggested that changes in the electrophoretic mobility and the size of Lp-X in the presence of apoA-I or LCAT were not due to displacement of apoCs and apoE from Lp-X.

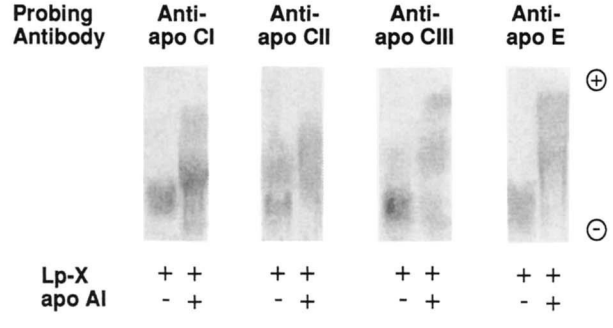

Fig. 7. Western immunoblotting analysis of the influence of apoA-I on Lp-X composition. Aliquots of Lp-X (10 μl) were incubated with or without exogenous apoA-I (5 μg/ml) at 37°C for 30 min. Samples were separated on 1% agarose gel followed by Western immunoblotting with anti-(human apoCI) antibodies, anti-(human apoC-II) antibodies, anti-(human apoC-III) antibodies, and anti-(human apoE) antibodies.

Cholesterol esterification in other lipoproteins

To compare Lp-X with other lipoproteins with regard to their ability to serve as substrates for LCAT, the same amount of purified recombinant LCAT was incubated with [^{3}H]cholesterol-labeled VLDL, LDL, or heat-inactivated HDL. Cholesterol esterification in these lipoproteins catalyzed by recombinant LCAT did not require the addition of apoA-I (**Table 3**). In contrast to the reaction in Lp-X, cholesterol esterification in VLDL, LDL, or HDL was markedly inhibited by exogenously added apoA-I (Table 3). Kinetic analysis was conducted with recombinant LCAT using isolated Lp-X, LDL, or HDL as substrate. The data obtained from the Lineweaver-Burk plot of cholesteryl ester formation versus the concentrations of Lp-X (in the presence of added apoA-I), LDL (in the absence of added apoA-I), or HDL (in the absence of added apoA-I) cholesterol in the assay mixture are summarized in **Table 4.** The apparent K_m and V_{max} values of LCAT for cholesterol were different when different lipoproteins were used as substrates. The rates of cholesterol esterification in LDL and HDL catalyzed by recombinant LCAT were 1.3 and 6.4 times higher than that in Lp-X, respectively.

Effect of serum albumin on cholesterol esterification in Lp-X

In addition to apoA-I, serum albumin may also play an important role in regulating cholesterol esterification in Lp-X. Recombinant LCAT (3 μg/ml) was incubated with isolated Lp-X and an increasing amount of bovine serum albumin (essentially fatty acid-free) in the presence of apoA-I (8 μg/ml). As shown in **Fig. 8,** the rate of cholesterol esterification increased as albumin concentration in the assay mixture was increased. Parallel experiments were carried out with isolated HDL or LDL and VLDL in the absence of exogenous apoA-I. Cholesterol esterification in these lipoproteins was stimulated by albumin at low concentrations (up to 4 μg per ml). However, further increase in albumin concentration in the assay mixture led to no further increase in LCAT activity (Fig. 8).

TABLE 3. Cholesterol esterification in lipoproteins catalyzed by LCAT

	Cholesterol Esterification	
	- ApoA-I	+ ApoA-I
	nmol/h/μg	
Lp-X	0.009 ± 0.001	0.086 ± 0.02
VLDL	0.021 ± 0.008	0.011 ± 0.006
LDL	0.110 ± 0.02	0.051 ± 0.01
HDL	0.490 ± 0.01	0.270 ± 0.01

The isolated Lp-X, VLDL, LDL, and heat-inactivated HDL were radiolabeled with [^{3}H]cholesterol. An aliquot of each labeled lipoprotein containing 12 nmol of unesterified cholesterol was used as substrate for the LCAT reaction in the absence or presence (8 μg/ml) of apoA-I. After incubation at 37°C for 5 h, the rate of cholesterol esterification was determined. The results are expressed as mean ± standard deviation from three separate experiments each performed in duplicate.

DISCUSSION

Although Lp-X consists mostly of unesterified cholesterol and phospholipids, there has been some controversy as to whether this lipoprotein can serve directly as a substrate for LCAT (16–19). In the present study, we investigated the relationship of LCAT and apoA-I to the metabolism of Lp-X. The isolated Lp-X contained apoE and apoCs but no apoA-I or apoB. Very little esterification of cholesterol in the isolated Lp-X occurred during its incubation with recombinant LCAT. However, when exogenous apoA-I was added to the reaction mixture in the presence of albumin, cholesteryl esters were formed at a markedly increased rate. Both phospholipase and acyltransferase activities of the LCAT reaction with Lp-X were apoA-I-dependent. These data indicate that isolated Lp-X, which did not contain apoA-I, was not a suitable substrate for recombinant LCAT. Addition of both apoA-I and albumin to Lp-X resulted in cholesterol esterification when recombinant LCAT, at the concentrations comparable to physiological concentration of LCAT in human plasma, was present in the reaction mixture. ApoA-I has been recognized as the most potent activator for the LCAT reaction in HDL or HDL analogues (proteoliposomes) (5, 29–31). Activation by apoA-I observed in our study might be due to its direct interaction with LCAT or its binding to Lp-X, resulting in a change of its conformation so that lipid substrates are exposed to the enzyme. Alternatively, apoCs or apoE on the surface of Lp-X might prevent the interaction of LCAT with lipid substrates and thus exogenous apoA-I might displace these apolipoproteins from the surface of Lp-X. However, the results from agarose gel electrophoresis followed by Western immunoblotting indicated that there was no displacement of either apoC or apoE from Lp-X by exogenously added apoA-I.

Interestingly, the electrophoretic mobility of Lp-X was markedly altered upon incubation with apoA-I. Such change was not observed upon incubation of Lp-X with HDL. As the charge of lipoprotein particles is the primary determinant of their electrophoretic mobility (32), the increased anodic migration indicates the presence of increased number of negative charges on the surface of the particle. Anodic migration of Lp-X due to a change in the electrophoretic properties rather than size after incubation of postheparin plasma has also been reported by other investigators (33). The change in the electrophoretic mobility observed in our study, therefore, suggests a change in surface charge in Lp-X in the presence of apoA-I. Such change caused by apoA-I might be one of the regulatory factors for the LCAT reaction in this lipoprotein. We also tested the possibility that apoA-I might be required for the interaction of LCAT with Lp-X. However, our results demonstrate that the interaction of LCAT with isolated Lp-X takes place in the presence or absence of apoA-I. Given the fact that there was a change in electrophoretic mobility of Lp-X with no change in particle size and apolipoprotein composition in the presence of apoA-I, and LCAT by itself was able to interact with Lp-X, it is tempting to suggest that addition of apoA-I to Lp-X may modify the local surface structure, which in turn makes the lipid substrates available for LCAT. Further study will be needed to make more definitive interpretation of these findings.

In the present study, addition of apoA-I might result in dissociation of phosphatidylcholine and cholesterol from the surface of Lp-X and the formation of transitory HDL-like particles which could then serve as a substrate for LCAT. The results obtained from our study suggested that this was not the case. The average size of the isolated Lp-X particle was 46–48 nm in diameter as determined by quasi-elastic light scattering analysis. It was not changed after incubation with apoA-I. However, a reduction in Lp-X size was observed when both apoA-I and LCAT were present. The change in the size of Lp-X upon LCAT reaction may be explained by the loss of phospholipid and unesterified cholesterol as well as the formation of cholesteryl esters.

Previously, Patsch et al. (18) demonstrated that Lp-X could serve as a substrate for partially purified plasma LCAT. Unlike Lp-X isolated by others (12) the Lp-X used by Patsch et al. (18) contained substantial amounts of apoA-I and their partially purified LCAT also contained serum apoA-I. Their results suggest that the activation by apoA-I might have been due to its binding or association with Lp-X (18). In contrast, Lp-X isolated in our laboratory contained no apoA-I and our purified recombinant LCAT was free of other proteins. Thus, one of the

TABLE 4. Apparent kinetic parameters of LCAT activity with different lipoproteins

	K_m	V_{max}
	μM	*nmol/h/per μg*
Lp-X	65 ± 7.50	0.25 ± 0.04
LDL	84 ± 8.20	0.32 ± 0.05
HDL	28 ± 2.50	1.60 ± 0.15

The values of the apparent K_m and V_{max} were determined from linear regression analysis of three separate experiments each performed in duplicate. The low LCAT activities detected when VLDL was used as substrate did not permit us to determine accurately the apparent K_m and V_{max}.

reasons for the different findings in our laboratory and others could be the difference in preparation of Lp-X, content of apolipoproteins, and the presence of other lipoproteins.

The present study has also demonstrated that cholesterol esterification by LCAT is in the order of HDL > LDL > Lp-X (in the presence of exogenous apoA-I) > VLDL. However, the size of these lipoproteins is in the order of HDL < LDL < Lp-X < VLDL. These results suggest that particle size may also play a role in regulating the LCAT reaction. The smaller the lipoprotein particles, the better they serve as substrates. Although apoA-I has been shown to be a primary activator of LCAT in reconstituted HDL or HDL analogues (4, 5, 29–31), it is not required for LCAT reaction with LDL (20). In the present study, cholesterol esterification by recombinant LCAT in isolated VLDL or LDL did not require exogenous apoA-I. Addition of apoA-I resulted in approximately 50% decrease in cholesteryl ester formation in LDL and VLDL. The mechanism by which added apoA-I inhibits LCAT reaction in lower density lipoproteins remains to be determined. Chen and Albers (34) demonstrated that LCAT was also activated by apoA-IV or apoE when these apolipoproteins were incorporated into egg yolk phosphatidylcholine–cholesterol. Subbaiah, Norum, and Bagdade (35) also observed that the absence of apoA-I did not significantly affect either the activity or the specificity of LCAT, and the other apolipoproteins could substitute adequately for it. Our present results are consistent with those reported by other investigators (34, 35) that the presence of apoA-I is not an absolute requirement for LCAT reaction in some lipoproteins. Moreover, exogenous apoA-I was also found to have an inhibitory effect on LCAT activity on HDL. This inhibitory effect might be explained by the competition of exogenous apoA-I and LCAT for the limited lipid surface of HDL.

Albumin is known to stimulate the LCAT reaction in proteoliposomes (HDL analogue), LDL, and HDL (29, 30). Although Lp-X contains albumin, it is probably trapped in the center of Lp-X particle as albumin can only be detected by denaturing or delipidating the particle (36). Addition of albumin stimulated the LCAT reaction in isolated Lp-X in the presence of apoA-I. The stimulatory effect of albumin has also been observed by other investigators and our laboratory in the reaction of LCAT with HDL and with synthetic substrates (20, 29, 30, 37). Thus, the level of serum albumin may be an important factor regulating cholesterol esterification by LCAT. It has been suggested that this stimulatory effect might be mediated via binding of lysophosphatidylcholine to albumin which prevents product inhibition of LCAT reaction (29, 30, 38). However, low levels of serum albumin are not always linked to a reduction in LCAT activity (39, 40). Normal cholesterol esterification in analbuminemia may be due to the ability of HDL to store and transport a limited amount of lysophosphatidylcholine. The patients in our study had low HDL levels. Dullaart et al. (39) reported that low albumin levels did not impair LCAT activity in proteinuric patients. Joles et al. (40) also observed that plasma cholesterol esterification in hypoalbuminemic rats was not decreased. Thus, albumin is not always an obligatory acceptor of lysophosphatidylcholine. It has been suggested that under hypoalbuminemic conditions, lysophosphatidylcholine may be transported via HDL or LDL (39, 40). It is tempting to speculate that accumulation of serum Lp-X in patients with obstructive jaundice may be caused by combined

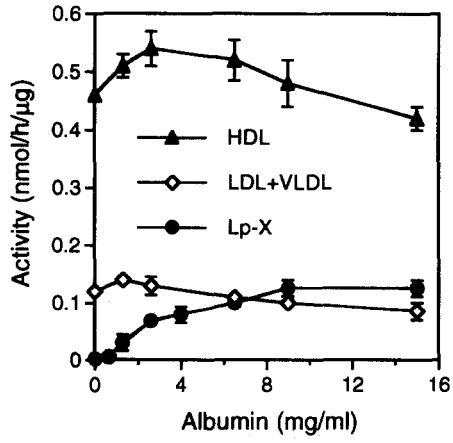

Fig. 8. Effect of albumin on cholesterol esterification in Lp-X, HDL, LDL, and VLDL. An aliquot of [^{3}H]cholesterol-labeled Lp-X, heat-inactivated HDL, or LDL and VLDL was incubated with recombinant LCAT (3 μg/ml) for 5 h at 37°C in the presence of various amounts of bovine serum albumin. ApoA-I (8 μg/ml) was included only in the Lp-X reaction mixture. LCAT activity was determined and expressed as nmol of cholesteryl ester formed per h per μg of LCAT protein. Each point represents the mean of three experiments and standard deviation is indicated as a vertical bar. As a control, cholesterol esterification was also determined in lipoproteins in the absence of recombinant LCAT and albumin alone did not result in cholesterol esterification in lipoproteins.

effects of the following factors such as *1)* a decrease in the serum concentration of apoA-I, a cofactor for cholesterol esterification by LCAT; *2)* deficiency in LCAT activity secondary to liver disease; *3)* a decrease in levels of serum HDL, which has been suggested to have an ability to store and transport lysophosphatidylcholine (39, 40); and *4)* a decrease in serum level of albumin due to reduced hepatic synthesis and increased concentration of serum bilirubin (formation of conjugated bilirubin). This, in turn, interferes with the binding of albumin to lysophosphatidylcholine which is inhibitory to the LCAT reaction. However, the physiological role of albumin in plasma lipoprotein metabolism remains to be further studied.

In summary, our results clearly demonstrate that, in the presence of apoA-I and albumin, unesterified cholesterol and phosphatidylcholine in Lp-X can be used for LCAT reaction. Although LCAT can bind to Lp-X, apoA-I is required for cholesterol esterification to proceed. Addition of apoA-I changes the electrophoretic mobility but not the size of the Lp-X particle. Such changes are not due to displacement of apoC or apoE from the surface of Lp-X by apoA-I. Investigations into the physiological significance of these findings are in progress.

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REFERENCES

1. Norum, K. R., and E. Gjone. 1967. Familial plasma lecithin:cholesterol acyltransferase deficiency. Biochemical study of a new inborn error of metabolism. *Scand. J. Clin. Lab. Invest.* **20:**231–243.
2. Norum, K. R., E. Gjone, and J. A. Glomset. 1989. Familial lecithin:cholesterol acyltransferase deficiency, including fish eye disease. *In* The Metabolic Basis of Inherited Disease. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw-Hill, Inc., New York. 1181–1194.
3. McLean, J. W. 1992. Molecular defects in the lecithin:cholesterol acyltransferase gene. *In* High Density Lipoproteins and Atherosclerosis III. N. E. Miller and A. R. Tall, editors. Elsevier Science Publishers B. V. 59–65.
4. Klein, H-G., P. Lohse, N. Duverger, J. J. Albers, D. J. Rader, L. A. Zech, S. Santamarina-Fojo, and H. B. Brewer, Jr. 1993. Two different allelic mutations in the lecithin:cholesterol acyltransferase (LCAT) gene resulting in classic LCAT deficiency: LCAT(tyr^{83} → stop) and LCAT(tyr^{156} → asn). *J. Lipid Res.* **34:**49–58.
5. Funke, H., A. von Eckardstein, P. H. Pritchard, A. E. Hornby, H. Wiebusch, C. Motti, M. R. Hayden, C. Dachet, B. Jacotot, U. Gerdes, O. Faergeman, J. J. Albers, N. Colleoni, A. Catapano, J. Frohlich, and G. Assmann. 1993. Genetic and phenotypic heterogeneity in familial lecithin:cholesterol acyltransferase (LCAT) deficiency. Six newly identified defective alleles further contribute to the structural heterogeneity in this disease. *J. Clin. Invest.* **91:** 677–683.
6. Klein, H-G., P. Lohse, P. H. Pritchard, D. Bojanovski, H. Schmidt, and H. B. Brewer, Jr. 1992. Two different allelic mutations in the lecithin:cholesterol acyltransferase gene associated with the fish eye syndrome: lecithin:cholesterol acyltransferase (Thr_{123} → Ile) and lecithin:cholesterol acyltransferase (Thr_{347} → Met). *J. Clin. Invest.* **89:**499–506.
7. Fielding, C. J. 1990. Lecithin:cholesterol acyltransferase. *In* Advances in Cholesterol Research. M. Esfahani and J. Swaney, editors. Telford Press, Caldwell, NJ. 270–314.
8. Jonas, A. 1991. Lecithin:cholesterol acyltransferase in the metabolism of high density lipoproteins. *Biochim. Biophys. Acta.* **1084:**205–220.
9. Torsvik, H., K. Berg, H. N. Magnani, W. J. McConathy, P. Alaupovic, and E. Gjone. 1972. Identification of the abnormal cholestatic lipoprotein (LP-X) in familial lecithin:cholesterol acyltransferase deficiency. *FEBS Lett.* **24:** 165–167.
10. Sabesin, S. M. 1982. Cholestatic lipoproteins—their pathogenesis and significance. *Gastroenterology.* **83:**704–709.
11. Miller, J. P. 1990. Dyslipoproteinaemia of liver disease. *Bailliere's Clin. Endocrinol. Metab.* **4:**807–833.
12. Seidel, D., E. Gjone, J. P. Blomhoff, and H. P. Geisen. 1974. Plasma lipoproteins in patients with familial plasma lecithin:cholesterol acyltransferase (LCAT) deficiency—studies on the apolipoprotein composition of isolated fractions with identification of Lp-X. Horm. *Metab. Res.* **Suppl. 4:**6–11.
13. Narayanan, S. 1984. Biochemistry and clinical relevance of lipoprotein X. *Ann. Clin. Lab. Sci.* **14:**371–374.
14. Patsch, J. R., K. C. Aune, A. M. Gotto, Jr., and J. D. Morrisett. 1977. Isolation, chemical characterization, and biophysical properties of three different abnormal lipoproteins: Lp-X_1, Lp-X_2, and Lp-X_3. *J. Biol. Chem.* **252:**2113–2120.
15. Norum, K. R., and E. Gjone. 1968. The effect of plasma transfusion on the plasma cholesterol esters in patients with familial plasma lecithin:cholesterol acyltransferase deficiency. *Scand. J. Clin. Lab. Invest.* **22:**339–342.
16. Wengeler, H., and D. Seidel. 1973. Does lipoprotein-X (Lp-X) act as a substrate for the lecithin:cholesterol acyltransferase (LCAT)? *Clin. Chim. Acta.* **45:**429–432.
17. Patsch, J. R., A. K. Soutar, J. D. Morrissett, A. M. Gotto, Jr., and L. C. Smith. 1977. Lipoprotein-X: a substrate for lecithin:cholesterol acyltransferase. *Eur. J. Clin. Invest.* **7:**213–217.
18. Patsch, W., J. R. Patsch, F. Kunz, S. Sailer, and H. Braunsteiner. 1977. Studies on the degradation of lipoprotein-X. *Eur. J. Clin. Invest.* **7:**523–530.
19. Ritland, S., and E. Gjone. 1975. Quantitative studies of lipoprotein-X in familial lecithin:cholesterol acyltransferase deficiency and during cholesterol esterification. *Clin. Chim. Acta.* **59:**109–119.
20. O, K., J. S. Hill, X. Wang, and P. H. Pritchard. 1993. Recombinant lecithin:cholesterol acyltransferase contain-

ing a Thr_{123} → Ile mutation esterifies cholesterol in low density lipoprotein but not in high density lipoprotein. *J. Lipid Res.* **34:** 81–88.
21. Anderson, R. L., and S. Davis. 1982. An organic phosphorus assay which avoids the use of hazardous perchloric acid. *Clin. Chim. Acta.* **121:** 111–116.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
23. McLeod, R., A. D. Lacko, P. H. Pritchard, and J. Frohlich. 1986. Purification of biological active apolipoproteins by chromatofocussing. *J. Chromatogr.* **381:** 271–283.
24. Hill, J. S., K. O, X. Wang, S. Paranjape, D. Dimitrijevich, A. G. Lacko, and P. H. Pritchard. 1993. Expression and characterization of recombinant human lecithin:cholesterol acyltransferase. *J. Lipid Res.* **34:** 1245–1251.
25. Dobiasova, M., and M. Schutzova. 1986. Cold labelled substrate and estimation of cholesterol esterification rate in lecithin:cholesterol acyltransferase radioassay. *Physiol. Bohemoslov.* **35:** 319–327.
26. Mayer, L. D., M. J. Hope, and P. R. Cullis. 1986. Vesicles of variable sizes produced by a rapid extrusion procedure. *Biochim. Biophys. Acta.* **858:** 161–168.
27. Carlson, L. A., and L. Holmquist. 1985. Evidence for the presence in human plasma of lecithin:cholesterol acyltransferase activity (β-LCAT) specifically esterifying free cholesterol of combined pre-β and β-lipoproteins. Studies of fish eye disease patients and control subjects. *Acta Med. Scand.* **218:** 197–205.
28. Karpe, F., J. Johansson, and L. A. Carlson. 1990. Studies on lecithin:cholesterol acyltransferase substrate properties of HDL as determined by its subclass distribution analyzed by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **1042:** 310–314.
29. Liu, M., and P. V. Subbaiah. 1993. Activation of plasma lysolecithin acyltransferase reaction by apolipoproteins A-I, C-I and E. *Biochim. Biophys. Acta.* **1168:** 144–152.
30. Jonas, A., J. L. Daehler, and E. R. Wilson. 1985. Kinetics of lecithin:cholesterol acyltransferase reaction with discoidal complexes of apolipoprotein A-I • phosphatidylcholine • ether phospholipid • cholesterol. *J. Biol. Chem.* **260:** 2757–2762.
31. Ohta, T., R. Nakamura, Y. Ikeda, M. Shinohara, A. Miyazaki, S. Horiuchi, and I. Matsuda. 1992. Differential effect of subspecies of lipoprotein containing apolipoprotein A-I on cholesterol efflux from cholesterol-loaded macrophages: functional correlation with lecithin:cholesterol acyltransferase. *Biochim. Biophys. Acta.* **1165:** 119–128.
32. Sparks, D. L., and M. C. Philips. 1992. Quantitative measurement of lipoprotein surface charge by agarose gel electrophoresis. *J. Lipid Res.* **33:** 123–130.
33. Ritland, S., J. Sauar, R. Holme, and J. P. Blomhoff. 1977. The electrophoretic mobility of lipoprotein X in postheparin plasma. *Clin. Chim. Acta.* **75:** 129–135.
34. Chen, C. H., and J. J. Albers. 1985. Activation of lecithin:cholesterol acyltransferase by apolipoproteins E-2, E-3, and A-IV isolated from human plasma. *Biochim. Biophys. Acta.* **836:** 279–285.
35. Subbaiah, P. V., R. A. Norum, and J. D. Bagdade. 1991. Effect of apolipoprotein activators on the specificity of lecithin:cholesterol acyltransferase: determination of cholesteryl esters formed in A-I/C-III deficiency. *J. Lipid Res.* **32:** 1601–1609.
36. Seidel, D., P. Alaupovic, R. H. Furman, and W. J. McConathy. 1970. A lipoprotein characterizing obstructive jaundice. II. Isolation and partial characterization of the protein moieties of low density lipoproteins. *J. Clin. Invest.* **49:** 2396–2407.
37. Czarnecka, H., and S. Yokoyama. 1993. Regulation of lecithin:cholesterol acyltransferase reaction by acyl acceptors and demonstration of its "idling" reaction. *J. Biol. Chem.* **268:** 19334–19340.
38. Switzer, S., and H. A. Eder. 1965. Transport of lysolecithin by albumin in human and rat plasma. *J. Lipid Res.* **6:** 506–511.
39. Dullaart, R. P. F., R. T. Gansevoort, B. D. Dikkeschei, D. De Zeeuw, P. E. De Jong, and A. Van Tol. 1993. Role of elevated lecithin:cholesterol acyltransferase and cholesterol ester transfer protein activities in abnormal lipoproteins from proteinuric patients. *Kidney Int.* **44:** 91–97.
40. Joles, J. A., N. Willekes-Koolschijn, L. M. Scheek, H. A. Koomans, T. J. Rabelink, and A. Van Tol. 1994. Lipoprotein phospholipid composition and LCAT activity in nephrotic and analbuminemic rats. *Kidney Int.* **46:** 97–104.