High density lipoprotein particle size distribution in subjects with obstructive jaundice

Peter M. Clifton, Philip J. Barter,[1,*] and A. Malcolm Mackinnon

Department of Medicine and Unit of Clinical Biochemistry,* Flinders University of South Australia, Bedford Park, South Australia, 5042

Abstract High density lipoproteins (HDL) from 14 patients with obstructive jaundice were examined by gradient gel electrophoresis to determine the effect of obstruction on particle size distribution. HDL from 7 of these patients were fractionated by gel permeation chromatography and further characterized by electron microscopy, SDS gel electrophoresis, apolipoprotein A-I and apolipoprotein A-II immunoturbidimetry, and analysis of chemical composition. In addition, lecithin:cholesterol acyltransferase (LCAT) activity was measured and correlated with plasma apolipoprotein A-I concentration and particle size distribution. HDL were abnormal in all patients regardless of severity, cause, or duration of obstruction. The major HDL subfraction in normal subjects, HDL_{3a} (radius 4.1–4.3 nm) was either absent or considerably diminished, and HDL_{2b} (radius 5.3 nm) was also frequently absent. Very small particles comparable in size to normal HDL_{3c} (radius 3.8 nm) were prominent. In patients with a bilirubin concentration >250 μmol/l, normal HDL had totally disappeared and were replaced by large discoidal particles of radius 8.5 nm and small spherical particles of radius 3.6–3.7 nm. Both populations of particles were markedly depleted of cholesteryl ester and enriched in free cholesterol and phospholipid. The discoidal particles were rich in apolipoproteins E, A-I, A-II, and C, while the small spherical particles contained predominantly apolipoprotein A-I. LCAT activity was diminished in all subjects to 8–54% of normal, and was strongly positively correlated (r = 0.91 $P < 0.05$) with plasma apolipoprotein A-I levels.—**Clifton, P. M., P. J. Barter, and A. M. Mackinnon.** High density lipoprotein particle size distribution in subjects with obstructive jaundice. *J. Lipid Res.* 1988. **29:** 121–135.

Supplementary key words LCAT deficiency • apolipoprotein A-I • purified LCAT • gel permeation chromatography

Major disturbances of plasma lipoprotein metabolism occur in both obstructive and parenchymal liver disease (1–5). In patients with prolonged obstructive jaundice, the most striking change is the appearance of a novel discoidal lipoprotein, lipoprotein X (LPX) in the plasma (6–10). However, in addition, high density lipoproteins (HDL) are significantly reduced in amount and consist of large, discoidal, particles (11–18) and very small spherical particles (13, 15, 17) as well as normal-sized particles. The main technique used for assessment of particle size, electron microscopy, is relatively insensitive, so that more subtle disturbances of HDL particle size have not been detected, especially early after the onset of biliary obstruction. There are no data available on the evolution of changes in HDL particle size and morphology. Although characterization of the abnormal HDL subfractions is not complete, all fractions are depleted in cholesteryl ester and enriched in free cholesterol and phospholipid, and the large discoidal HDL particle contains apolipoproteins (apo) E, A-I, A-II, and C (14–19). Plasma apoA-I and apoA-II concentrations are low and apoE concentration is high (20, 21).

Since the liver is the site of synthesis of lecithin: cholesterol acyltransferase (LCAT) (22, 23), the enzyme responsible for cholesterol esterification in the plasma, obstructive liver disease could be anticipated to disturb LCAT activity. However published data are contradictory with LCAT activity being reported as low, normal, or even high (17, 18, 24–28). Unfortunately, most of the LCAT assays have been unable to eliminate interference from autologous substrate. Lipoproteins from patients with obstructive jaundice are better substrates for LCAT than liposomes or normal HDL. Consequently no clear relationship has been found between LCAT activity and changes in HDL composition and size. In order to clarify this question we have examined in detail HDL from patients with varying degrees of biliary obstruction and correlated their HDL particle size distribution, as assessed by the sensitive technique of gradient gel electrophoresis, with their plasma LCAT activity. LCAT was assayed using HDL_3 from a normal subject in a test system where the influence of autologous substrate was minimized. We have also utilized our recently developed technique of separating normal HDL subpopulations using gel permeation chromatography to separate HDL subpopulations in obstructive jaundice. These subpopulations were

Abbreviations: apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; LPX, lipoprotein X; LCAT, lecithin: cholesterol acyltransferase; VLDL, very low density lipoproteins.

[1]Current address: Baker Medical Research Institute, Prahran, Victoria, 3181.

then fully characterized by gradient gel electrophoresis, SDS polyacrylamide gel electrophoresis, and analysis of chemical composition. Finally, in order to confirm the role of LCAT deficiency in the genesis of the abnormal HDL particle size distribution, we incubated cholestatic plasma with purified LCAT and examined the changes in HDL particle size.

METHODS

Subjects

Subjects were entered into the study over a 6-month period from September 1985 to March 1986. They were clinically jaundiced at the time of entry and were subsequently shown to have an obstructive cause for their hyperbilirubinemia. Patients with septicemia, renal or hepatic failure were excluded. All were hospital inpatients at the time of blood sampling but were ambulatory prior to admission. Fourteen patients were entered. Further limited data was obtained from eight patients as the serum bilirubin rose or fell. Only one patient (D) was discharged with a normal bilirubin; the remainder either died or were discharged still jaundiced. Plasma from all patients was assayed for free and total cholesterol and for apoA-I and apoA-II. Lipoproteins were submitted to gradient gel electrophoresis. Seven patients were studied in greater detail: lipoproteins were separated by gel permeation chromatography and selected HDL fractions were further characterized by gradient gel electrophoresis, electron microscopy, SDS gel electrophoresis, and chemical composition.

Lipoprotein separation

Fifty ml of blood was collected on ice into tubes containing Na_2 EDTA (at a final concentration of 1 mg/ml) and plasma was separated immediately by low speed centrifugation at 4°C. Plasma was adjusted to a density of 1.25 g/ml with solid KBr (29) and the lipoproteins were separated by ultracentrifugation at 55,000 rpm for 28 hr in a Ti 55.2 rotor (Beckman). Ultracentrifugation was performed at 4°C in a Beckman L5-65 or L8-70M ultracentrifuge. The supernatant (total volume 5 ml) was collected by tube slicing and immediately filtered through a 0.22-μm filter (Gelman).

Gel permeation chromatography

Gel permeation chromatography was performed using a 1.6 × 75 cm column of Superose 6B (Pharmacia Uppsala, Sweden) attached to a Pharmacia Fast Protein Liquid Chromatography System. Equilibration of the column and elution of lipoproteins were performed with a solution of 0.15 M NaCl, 0.001 M Na_2EDTA and 0.02% NaN_3, at a standard flow rate of 45 ml/hr. All solutions were filtered with a 0.22-μm Gelman filter and degassed prior to use. A continuous absorbance reading of the column eluate was obtained with a UVI cell (Pharmacia). Fractions of 0.5 ml were collected on a Pharmacia FRAC 100 fraction collector.

Polyacrylamide gradient gel electrophoresis of HDL

Polyacrylamide gradient gel electrophoresis of HDL was performed using slab gradient gels (2.5–27% acrylamide, Gradipore Ltd., Sydney, Australia). Samples containing 30 μg of HDL protein were applied in a volume of 15–200 μl (5–190 μl of sample and 10 μl of a solution containing 40% sucrose and 0.01% Bromophenol Blue) and subjected to electrophoresis at 160 V for 17 hr in a Tris-borate buffer (pH 8.35). The diameter of the HDL particles was calculated by reference to coelectrophoresed standards of thyroglobulin (radius 8.5 nm), ferritin (radius 6.1 nm), lactate dehydrogenase (radius 4.08 nm), and bovine serum albumin (radius 3.55 nm) from a high molecular weight electrophoresis calibration kit (Pharmacia Fine Chemicals, Uppsala, Sweden). Gels were fixed in 10% sulfosalicylic acid for 1 hr, stained for 4 hr in 0.04% Coomassie G-250 in 3.5% perchloric acid, and destained in 5% acetic acid (30). The gels were then scanned in a laser densitometer (2202 Ultrascan, LKB, Bromma, Sweden) and quantitated using a Hewlett Packard 3390A integrator.

In some of the experiments, plasma samples were prestained for 1 min with a concentrated solution of Sudan Black in ethylene glycol (20 μl of plasma and 20 μl of stain) and then applied directly to the gradient gel. Lanes containing protein standards were separately stained as above. This technique was used in patients with a bilirubin of >250 μmol/l to demonstrate that the very small particles contained lipid.

SDS polyacrylamide gel electrophoresis

SDS polyacrylamide gel electrophoresis was carried out in a Laemmli buffer system on a 15% polyacrylamide gel (31).

Chemical composition

Total cholesterol and free cholesterol were measured using Boehringer kits (CHOD-PAP Method Kits, numbers 290319 and 310328, respectively). Triglycerides were measured using Boehringer Perodichrom Kit number 701904. Phospholipids were estimated using a Wako Kit 279 54009 (Wako Pure Chemicals, Osaka, Japan) which measures the choline-containing phospholipids. The standard solution used contained 54 mg/dl of choline chloride corresponding to 300 mg/dl of phosphatidylcholine. Choline is found in 95–97% of human HDL phospholipids. Protein levels were estimated using the method of Lowry et al. (32) adapted for use on a centrifugal analyzer (Cobas-Bio, Roche). The test was modified by scaling all volumes down to a total volume of 0.4 ml and reducing the total incubation time to 20 min. No loss of linearity or sensitivity was noted within the concentration range 50–1000 μg/ml. Bovine serum albumin was used as a standard.

All assays were performed in duplicate on a Cobas-Bio (Roche) centrifugal analyzer. The coefficients of variation within a single run were: cholesterol (free and total) <0.5%, triglyceride <1%, phospholipid <0.5%, and protein 4% in the concentration range 0–250 μg/ml, and 2% in the range 250–1000 μg/ml. Day-to-day and run-to-run coefficients of variation were total cholesterol <0.5%, free cholesterol 5%, triglyceride 3%, phospholipids 1%, and protein 5% (0–250 μg/ml) and 3% (250–1000 μg/ml).

ApoA-I and apoA-II were estimated by immunoturbidimetry on a Cobas-Bio centrifugal analyzer using Boehringer antisera as described by Austin and Maznicki (33) and Rifai, King, and Malekpour (34). Standards were prepared from a Hyland Omega lipid control serum. The coefficient of variation for both apoA-I and apoA-II within a single run was 6%. The method was validated with 'rocket' electroimmunoassay using the same antisera and standards.

Electron microscopy

Lipoprotein samples were brought to a protein concentration of 500 μg/ml and dialyzed against 5 mM $(NH_4)CO_3$ (pH 8.0). After negative staining with 0.5% phosphotungstic acid, pH 7.4, the samples were examined at a magnification of 100,000 (35).

LCAT purification and incubation schedules

LCAT was purified exactly as previously described (36). Plasma samples (0.5 ml) from jaundiced patients were incubated for 24 hr at 37°C with 0.5 ml of a solution of purified LCAT (60–85 units), in 5 mM Tris buffer, 150 mM NaCl, pH 7.4. One unit of LCAT esterifies 1 nmol/ml per hr of free cholesterol. Free and total cholesterol concentrations were measured before and after incubation. Lipoproteins were separated by a single ultracentrifugation at d 1.25 g/ml (see Lipoprotein separation) and submitted to gradient gel electrophoresis to assess HDL particle size distribution. In addition to whole plasma, isolated HDL fractions containing predominantly small spheres were also incubated with LCAT. HDL fractions containing 20 nmol of free cholesterol were supplemented with 50 nmol of free cholesterol (as autologous LDL) and 20 μg of bovine serum albumin. The incubation contained 20 units of purified LCAT in a total volume of 200 μl. Incubation was performed at 37°C for 1 hr and 24 hr.

LCAT assay

Activity of LCAT was measured using normal HDL_3 prelabeled with [^{3}H]cholesterol as the substrate, as previously described (37). [1,2-^{3}H]Cholesterol (47 Ci/mmol) and cholesteryl [1-^{14}C]oleate (58 mCi/mmol) were obtained from the Radiochemical Centre, Amersham, U. K.; each was reported to have a radiochemical purity of more than 97%. A further check, using thin-layer silicic acid chromatography, was made of the [^{3}H]cholesterol at the time of each experiment when more than 97% of the ^{3}H was recovered in the free cholesterol fraction and less than 0.2% was recovered in the esterified cholesterol fraction. Fifty to 100 μl of the labeled HDL_3, containing 55 nmol of free cholesterol, was incubated with 10 μl of test plasma (containing a maximum of 3 nmol of HDL free cholesterol) for 5 hr at 37°C. Fractional rate of esterification was determined as previously described (37) using thin-layer silicic acid chromatography, and compared to the activity of four normal plasmas assayed at the same time. Values were corrected for recovery by reference to an internal standard of cholesteryl [1-^{14}C]oleate which had been added to the solution in which the samples were extracted. All samples were snap-frozen in liquid nitrogen and stored at −70°C for between 1 and 6 months. Normal plasmas were snap-frozen and stored for 6 months prior to being assayed. All samples were assayed in quadruplicate with a coefficient of variation of less than 5%.

RESULTS

Patients studied

Table 1 lists the ages, clinical diagnoses, levels of serum bilirubin, and duration of jaundice in the 14 patients included in this study. It is apparent that duration of jaundice varied from 2 days to 3 months. Four patients had acute, nonmalignant biliary obstruction, 2 from gallstones, 1 from pancreatitis, and 1 from phenothiazine-induced intrahepatic cholestasis. The latter patient became jaundiced within 3 days of commencing the drug.

Lipid and apoprotein levels

Table 2 contains the total cholesterol, free cholesterol, apoA-I, and apoA-II levels for all subjects. HDL cholesterol levels were estimated in 11 patients. Eight subjects were studied on more than one occasion: subjects F and G were restudied as the bilirubin rose while subjects L, B, K, A, C, and I were restudied as the bilirubin level fell. The subjects are arrayed in order of increasing bilirubin. With increasing levels of serum bilirubin there was a significant trend toward a decrease in the proportion of esterified cholesterol ($r = -0.80$, $P < 0.001$) and a decrease in concentrations of both apoA-I and apoA-II ($r = -0.84$, $P < 0.001$). The apoA-I and HDL cholesterol concentration and the percentage of plasma cholesterol in the esterified form were highly positively correlated ($r = 0.90$, $P < 0.001$). There was no significant difference between patients with acute nonmalignant obstruction and those with malignant obstruction, despite the fact that malignant obstruction may have been present subclinically for many weeks. In most cases the serum bilirubin level was the best predictor of the degree of lipoprotein abnormalities. Subject A had more marked abnormalities of cholesterol esterification and apolipoprotein concentration than predicted

TABLE 1. Clinical details of subjects with obstructive jaundice

Subject	Sex	Age	Diagnosis	Bilirubin	Duration of Jaundice
				μmol/l	
A	F	71	Choledocholithiasis	(a) 102	2 weeks
				(b) 40	3 weeks
B	F	81	Pancreatitis	(a) 118	2 days
				(b) 60	5 days
C	F	69	Carcinoma of pancreas (stented)	(a) 146	3 weeks
				(b) 105	4 weeks
D	M	72	Choledocholithiasis	146	7 days
E	F	82	Carcinoma gallbladder (external drain)	148	> 1 month
F	F	87	Carcinoma of pancreas	(a) 161	2 weeks
				(d) 440	3 weeks
				(f) 340	4 weeks
				(g) 380	5 weeks
G	M	69	Nodes in porta hepatis	(a) 175	2 weeks
				(b) 270	3 weeks
H	M	83	Carcinoma pancreas	210	7 days
I	M	32	Carcinoma bile duct (choledocho jejunostomy)	(a) 240	4 weeks
				(b) 172	5 weeks
J	M	70	Lymphoma nodes in porta hepatis	250	2 weeks
K	M	72	Carcinoma gallbladder (external drain)	(a) 295	1 month
				(b) 220	4 months
L	F	78	Carcinoma gallbladder (external drain)	(a) 340	10 days
				(b) 181	19 days
				(c) 120	25 days
M	M	80	Intrahepatic cholestasis (drug induced)	350	2 weeks
N	F	52	Nodes in porta hepatis	440	> 3 months

by the level of bilirubin; this subject, however, had been mildly jaundiced for at least 2 weeks prior to being studied. Similarly both C and I had been partially obstructed, despite surgical bypass, for at least 1 month so their apoA-I and apoA-II levels were quite low. The plasma bilirubin reached a plateau of 440 μmol/l after 3 weeks in subject F, but the concentration of apoA-I and cholesteryl esters continued to fall until the apoA-I level was less than 5% of normal after 5 weeks of obstruction and only 15% of the plasma cholesterol was in the esterified form.

Lipid distribution

Fig. 1 shows the total cholesterol, triglyceride, and apoA-I distribution of subject C after separation of 1 ml of whole plasma by gel permeation chromatography. This distribution was quite typical of many of the patients with obstructive jaundice. The bulk of the cholesterol, mostly unesterified, eluted in the void volume in discoidal particles (normally where VLDL elutes) and only 5% of the total cholesterol eluted with apoA-I. The LDL (2nd peak) was abnormally triglyceride-enriched. The profile was essentially unchanged when the lipoproteins were separated from plasma by ultracentrifugation prior to gel permeation chromatography.

HDL particle size distribution

HDL is polydisperse and can be separated on gradient gel electrophoresis into at least five subpopulations. We have utilized the terminology devised by Blanche and associates (30) to describe these subfractions, viz HDL_{2b} (radius 5.28 nm), HDL_{2a} (radius 4.58 nm), HDL_{3a} (radius 4.22 nm), HDL_{3b} (radius 3.98 nm), and HDL_{3c} (radius 3.81 nm). The normal HDL profile shown in **Fig. 2** is very similar in particle size and distribution to that of the normal males described by Blanche. Fig. 2 illustrates the gradient gel electrophoretic profiles of HDL from all 14 subjects studied. All the profiles are clearly abnormal. The major subpopulation of HDL in normal subjects, HDL_{3a}, containing particles of radius 4.1–4.3 nm, was either greatly diminished in quantity (subjects A and B) or completely absent. This subpopulation was replaced by both smaller particles and larger particles. With increasing serum bilirubin concentration or increased duration of obstruction when serum bilirubin was maximally elevated, the small particles became progressively smaller and particles of radii 3.6 nm and 3.7 nm became prominent. These particles are rarely seen in normal subjects. Similarly, very large particles, greater than radius 5.3 nm became more prominent, such that in subjects F, M, and N at least half of the HDL had an apparent radius 8.5–10 nm. As shown later, these particles were predominantly discoidal in shape. The size of these discoidal particles as measured by electron microscopy (radius 7.5 nm) was compatible with the estimates obtained by gradient gel electrophoresis. In those subjects whose HDL consisted predominantly of spherical particles (e.g., D and E) there were subtle changes in the normal HDL_2

TABLE 2. Cholesterol and apoprotein levels of subjects with obstructive jaundice

Subject		Bilirubin	Total Cholesterol	Free Cholesterol	% Esterified	HDL Cholesterol	ApoA-I	ApoA-II
		μmol/l	*mmol/l*	*mmol/l*		*mmol/l*	*g/l*	
'Normal' plasma[a]		<20	4.9	0.83	83	1.37	1.32	0.39
A	(a)	102	4.0	2.2	45	nd[b]	0.41	0.13
	(b)	40	4.7	1.4	69		1.10	0.25
B	(a)	118	6.2	1.2	81	nd	1.06	0.31
	(b)	60	6.6	2.0	70		0.88	0.27
C	(a)	146	14.1	8.8	38	0.19	0.29	0.09
	(b)	105	13.1	8.5	35	0.26	0.28	0.08
D		146	6.1	2.8	55	0.43	0.62	0.12
E		148	5.2	2.7	48	0.49	0.48	0.14
F	(a)	161	5.4	2.8	48		0.55	0.18
	(b)	nd	5.8	3.2	44		0.49	0.16
	(c)	nd	3.2	1.8	44	nd	0.32	0.10
	(d)	440	2.9	1.9	35		0.16	0.05
	(e)	340	2.7	2.0	27		0.09	0.03
	(f)	nd	3.7	2.8	23		0.09	0.01
	(g)	380	4.5	3.8	15		0.05	0.02
G	(a)	175	6.1	1.6	74	0.34	0.91	0.26
	(b)	250	6.0	2.9	53	0.10	0.30	0.09
H		210	5.3	2.8	47	0.32	0.51	0.18
I	(a)	240	4.4	2.6	41	0.08	0.26	0.08
	(b)	172	4.8	1.8	63	0.10	0.24	0.07
J		250	5.8	3.7	35	0.27	0.44	0.17
K	(a)	295	4.3	2.2	49	nd	0.25	0.09
	(b)	220	2.7	1.7	62	0.15	0.06	0.01
L	(a)	340	5.8	3.8	35	0.09	0.13	0.05
	(b)	181	4.7	2.2	53	0.18	0.32	0.02
	(c)	120	4.2	1.7	60	0.43	0.51	0.07
M		350	4.7	2.7	43	0.03	0.21	0.07
N		440	4.6	3.6	21	0.05	0.20	0.07

[a] 'Normal' plasma consisted of a pool of plasma from eight normal laboratory workers. A sample of this pool was run in duplicate with the patient plasmas.
[b] Not determined, nd.

size range. Particles corresponding to normal HDL_{2b} (radius 5.3 nm) were absent while there was an increase in the proportion of particles corresponding to HDL_{2a} (radius 4.6–4.8 nm). There was no significant difference in the type of HDL particle size change between the four patients with acute, nonmalignant obstruction and those with slower onset malignant obstruction. In the former group, in whom the exact onset of obstruction was known, it could be seen that significant changes in the HDL profile occurred in 5 days while marked abnormalities, with large discoidal and very small spherical particles, were present after 2 weeks. As noted in a later section, LCAT activity had fallen to 9% of normal after 2 weeks.

Protein-stained gels severely understimated the contribution of the large particles to the HDL fraction, especially when the particle had a radius of 8.5 nm or greater. Lipid-stained gels (not shown) demonstrated that 90% of the HDL lipid was in particles of radius >6.1 nm in patients with a bilirubin level of >250 μmol/l. The small particles contained such a low proportion of lipid that they were barely visible. However, the gels demonstrated that the plasma of these patients contained lipoprotein particles almost as small as an albumin molecule.

The changes in HDL particle size distribution related both to the degree and the duration of obstruction. Thus, the level of the serum bilirubin at the time of blood sampling did not always correlate with the changes seen in HDL particle size. For instance, subject F with a serum bilirubin of 160 μmol/l had a much more abnormal profile than subject D with a comparable bilirubin of 146 μmol/l. The latter subject had complete biliary obstruction (due to a bile duct stone) of less than 1 week duration at the time the HDL profile was obtained, whereas subject F had been jaundiced for at least 2 weeks prior to blood sampling. In subject K, despite a moderate fall in bilirubin over 3 months, the HDL profile had become more abnormal with an increase in large particles.

A clear lag period is seen with subject B, whose peak bilirubin of 118 μmol/l was observed 2 days after the onset of acute pancreatitis. Cholesterol esterification was not demonstrably abnormal until 3 days later, when the bilirubin had fallen to 60 μmol/l (Table 1). At this time the HDL particle size distribution was also abnormal (Fig. 2). This lag period before changes are seen in the HDL particle size distribution is consistent with the relatively slow turnover of HDL, with a residence time of 4–6 days (38).

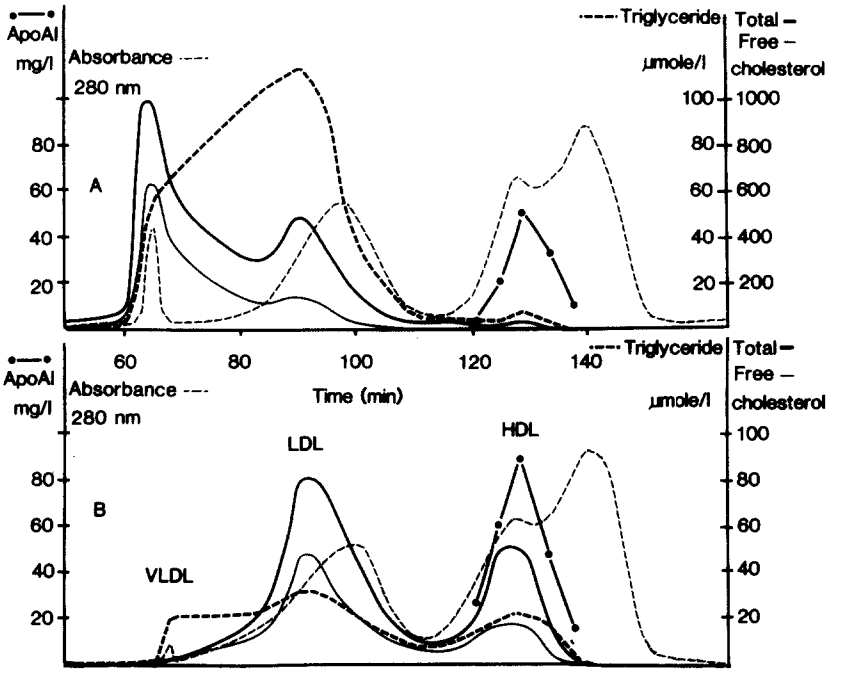

Fig. 1. Cholesterol and triglyceride profiles after separation of lipoproteins by gel permeation chromatography from (a) subject C, bilirubin 146 μmol/l (top), and (b) a normal male subject (bottom). One ml of plasma was applied to a 75-cm Superose 6B column and eluted with 0.15 M NaCl, 0.001 M Na_2 EDTA, pH 7.4, at 45 ml/hr. Fractions were collected and assayed for total cholesterol (solid line), free cholesterol (fine solid line), triglyceride (broken line), and apoA-I (graph points). Absorbance at 280 nm is also shown (fine broken line). The elution time of normal VLDL, LDL and HDL is marked on the figure.

Fig. 3(A) shows sequential gradient gel profiles of HDL from subject F as the bilirubin rose from 160 μmol/l to 440 μmol/l. A period of 2 weeks separated the first and last sample. Despite the serum bilirubin concentration reaching a pleateau after 11 days, changes in the HDL particle size distribution progressed. The major difference between the two samples is a further decrease in particle radius in the small pore region of the gel and an increase in size and amount of the larger particles. Fig. 3(B) shows sequential gradient gel profiles of subject G as the bilirubin increased from 175 μmol/l to 300 μmol/l. The essentially unimodal distribution of radius 3.9–4.1 nm particles became bimodal with large particles of radius 8.5 nm and small particles of radius 3.8 nm. The changes that occurred within these individuals were analogous to the changes seen in the 14 subjects with varying degrees of obstruction.

Changes occurring in the HDL particle size distribution as obstruction is relieved are shown in **Fig. 4**. Subject L had a T-tube inserted to bypass a carcinoma of the gallbladder. The bilirubin fell from 340 μmol/l to 120 μmol/l over 14 days. Within 9 days the HDL profile (Fig. 4A) had dramatically changed: all the abnormal large and small particles had disappeared and were replaced by a single population of radius 4.1 nm. As recovery continued the particles increased in size further to radius 4.7 nm, while other normal subpopulations were beginning to appear. In subject C (Fig. 4B), the bilirubin fell from 146 μmol/l to 105 μmol/l over 5 days; over this period of time the small HDL particles became less prominent and a population of normal-sized particles of radius 4.2 nm appeared. Similarly, in subject I (Fig. 4C), as the bilirubin fell from 240 μmol/l to 172 μmol/l over 1 week, the proportion of small particles of radius 3.9 nm decreased. Thus, despite the continued presence of the malignancy, the HDL profile became more normal with relief of the obstruction.

Separation of small and large particles

Major lipoprotein classes can be quickly and easily separated from each other by gel permeation chromatography with Superose 6B of the plasma d < 1.25 g/ml supernatant. **Fig. 5** (solid line) shows the elution profile of lipoproteins from a normal subject while Fig. 5 (dotted line) shows the elution profile of lipoproteins from subject J with a serum bilirubin of 250 μmol/l. Two changes were apparent: *i*) a new population of particles appeared between the LDL peak and the major HDL peak, and *ii*) the major HDL peak eluted later than normal. Aliquots from each peak were submitted to gradient gel electrophoresis and clear separation of the two HDL subpopulations of subject J was apparent. The first HDL peak contained particles of radius 5.6–8.6 nm, while the second peak contained particles of radius 3.8 nm. Both peaks contained apoA-I and apoA-II

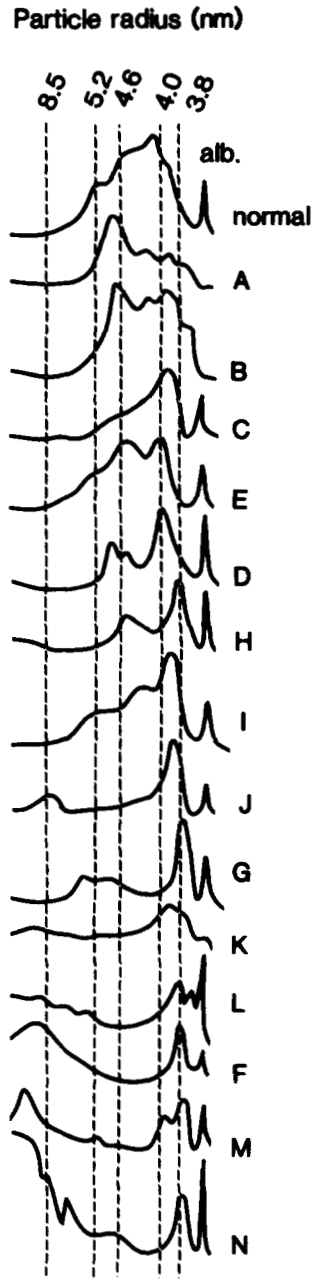

Fig. 2. Gradient gel profiles of HDL from subjects with obstructive jaundice. The profiles are arrayed in order of increasing serum bilirubin. Lipoproteins were isolated after a single ultracentrifugation at a density of 1.25 g/ml. Thirty μg of HDL was applied to a 2.5–27% polyacrylamide gradient gel and electrophoresed for 2,700 v-hr in a Tris-borate buffer (pH 8.35). The gels were fixed in 10% sulfosalicylic acid, stained with Coomassie Blue G250 in 3.5% perchloric acid and destained in 5% acetic acid. The gels were then scanned in a laser densitometer and particle size was calculated by reference to coelectrophoresed high molecular weight standards.

when analyzed by immunoturbidimetry. Four of the seven subjects studied in detail had a clear new peak between LDL and the normal HDL peak. ApoA-containing particles could be detected in small amounts up to the peak of the LDL fraction.

Electron microscopy of small and large particles

In all seven subjects examined the fractions eluting between LDL and the elution position of normal HDL contained stacked discs of radius 8.2 ± 1.5 nm (mean ± SD, 50 discs) although in subjects E and D these consisted of <10% of all particles. In the four subjects with a clear new subpopulation of particles between LDL and HDL on gel permeation chromatography, the peak fractions of this subpopulation consisted predominantly of discoidal particles (**Fig. 6a**). In addition there were some round particles of radius 8.7 ± 0.1 nm (mean ± SD, 50 particles) and larger round particles of radius 15–20 nm. Fractions of HDL that eluted later contained progressively fewer discoidal particles and more small spherical particles. The second HDL peak which eluted later than normal HDL contained only small spherical particles (Fig. 6b) of about 2.7 ± 0.1 nm (mean ± SD, 50 particles).

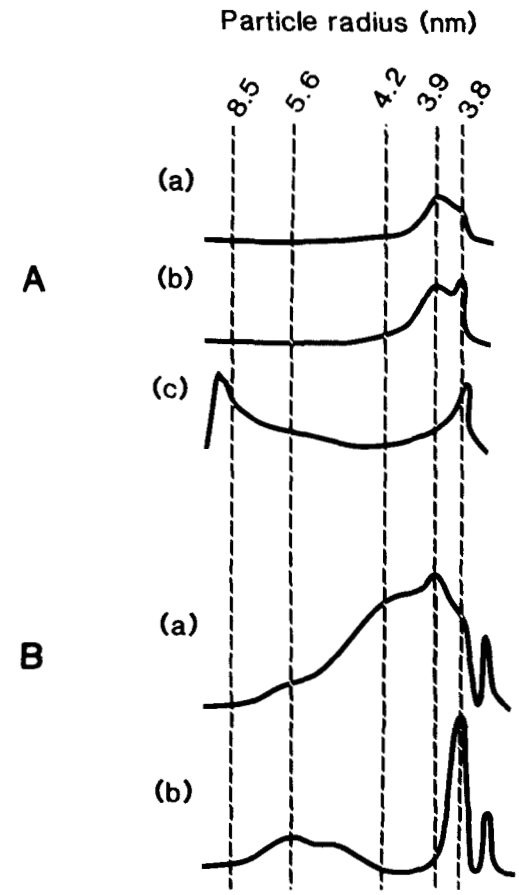

Fig. 3. A: Gradient gel profiles of HDL subject F. Electrophoresis was performed as described in the legend to Fig. 2. Thirty μg of HDL protein was applied to each track. Profile (a) was obtained on admission, 2 weeks after jaundice was first noted. Plasma bilirubin was 161 μmol/l. Profile (b) was obtained 3 days later and profile (c) 2 weeks later. Plasma bilirubin had risen to 340 μmol/l. B: Gradient gel profiles of HDL from subject G. Profile (a) was obtained on admission 2 weeks after jaundice was noted. Plasma bilirubin was 175 μmol/l. Profile (b) was obtained 1 week later, when the bilirubin had risen to 270 μmol/l.

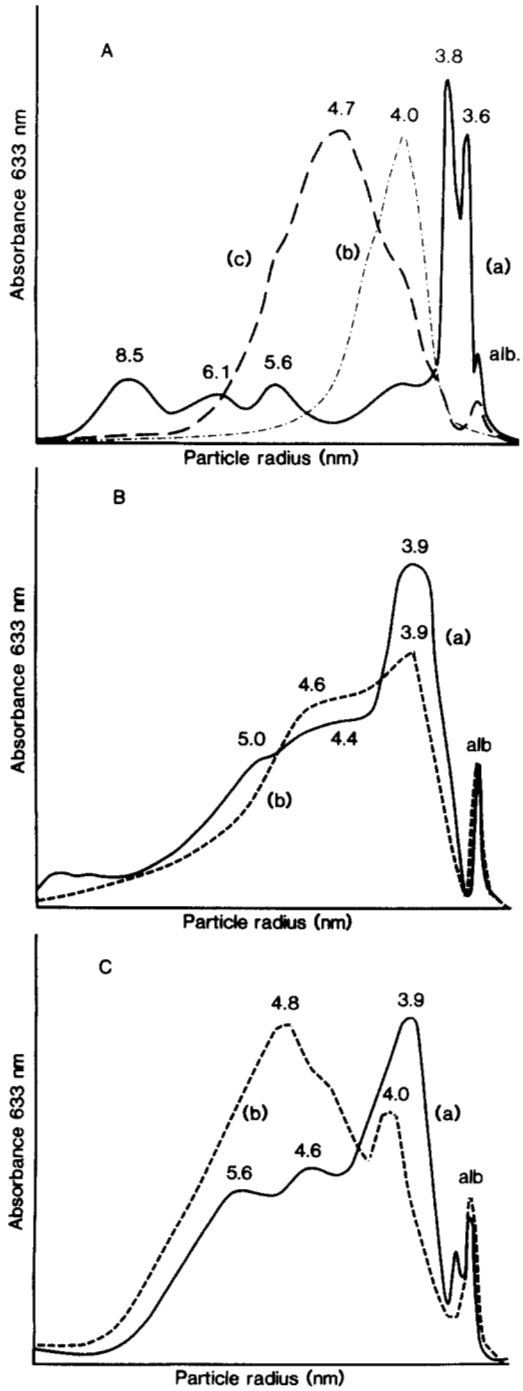

Fig. 4. A: Gradient gel profiles of HDL from subject L. Electrophoresis was performed as described in the legend to Fig. 2. Profile (a) (solid line) was obtained on admission (serum bilirubin was 340 μmol/l); profile (b) (dotted line) was obtained 9 days later when the serum bilirubin had fallen to 181 μmol/l. Profile (c) (broken line) was obtained 15 days after admission when the serum bilirubin had fallen further to 120 μmol/l. B: Gradient gel profile of HDL from subject C. Profile (a) (solid line) was obtained 8 days after internal stenting and 3 weeks after jaundice was first noted. Plasma bilirubin was 146 μmol/l having fallen from 390 μmol/l on admission. Profile (b) (dotted line) was obtained 5 days later when the plasma bilirubin was 105 μmol/l. C: Gradient gel profiles of HDL from subject I. Profile (a) (solid line) was obtained 4 weeks after the onset of jaundice, when the plasma bilirubin was 240 μmol/l, having fallen from a preoperative peak of 360 μmol/l. Profile (b) (dotted line) was obtained 1 week later when the plasma bilirubin was 172 μmol/l.

Composition of small and large particles

Fractions recovered after gel permeation chromatography that were reasonably homogeneous on both electron microscopy and gradient gel electrophoresis were subjected to chemical analysis (**Table 3**). All fractions were severely depleted in cholesteryl ester and enriched in phospholipid and free cholesterol. The triglyceride and the esterified cholesterol in the fraction containing the stacked discs was presumably present in the contaminating large round particles of LDL size, as none of the three preparations containing discoidal particles were completely homogeneous. The small spheres were severely depleted of core materials, although the triglyceride content was greater than normal. Even in subject D who was jaundiced for less than 1 week, the cholesteryl ester content of the small spherical particles was less than 40% of normal.

Apolipoprotein content was assessed using SDS gel electrophoresis and apoA-I and apo-II immunoturbidimetry. ApoA was detectable from the peak of the LDL fraction (90 min elution time) onwards. The apoA-I/apoA-II ratio differed from normal (**Fig. 7**) in that the highest ratio was found in the smallest particles, rather than in the HDL_{2b} fraction. No patient showed a significant rise in the ratio of apoA-I/apoA-II in the HDL_2 fractions. The major apolipoproteins of the discoidal fractions were apoE, apoA-I, apoA-II, apoC, apoA-IV, and albumin and several unidentified high molecular weight proteins (**Table 4**). This fraction was not separated further and it is not known whether all apolipoproteins coexisted on one particle or whether there were specific apoE-containing discs or spheres. The albumin may be contributed by small LPX_3 (39) discs which were not readily separable in size from large HDL discs. The small spherical particles contained predominantly apoA-I and apoA-II and were depleted of apoC. As the particles became smaller the relative content of apoA-I increased. Particles of radius 3.6 nm contained predominantly apoA-I.

LCAT activity

Plasma from six subjects and four normal controls was deep frozen at −70°C within 1 hr of the blood being drawn from the subject. All seven samples (two from subject L) from the jaundiced patients and four normal samples were assayed together. LCAT activity (**Table 5**) was reduced in all subjects, ranging from 8 to 54% of normal, and was negatively correlated with serum bilirubin ($r = -0.91$, $P < 0.01$). There was a strong correlation between the plasma apoA-I level and the LCAT activity ($r = 0.91$, $P < 0.01$) suggesting that LCAT activity may be one determinant of the plasma concentration of HDL apolipoprotein. In the one subject (L) retested, as the bilirubin dropped from 340 μmol/l to 120 μmol/l over 2 weeks, the activity of LCAT rose from 11% to 53% of normal. In the two subjects with acute nonmalignant obstruction, in whom it was possible to pinpoint exactly the onset of obstruction, LCAT activity was reduced to 40% of normal in 7 days and 9% of normal

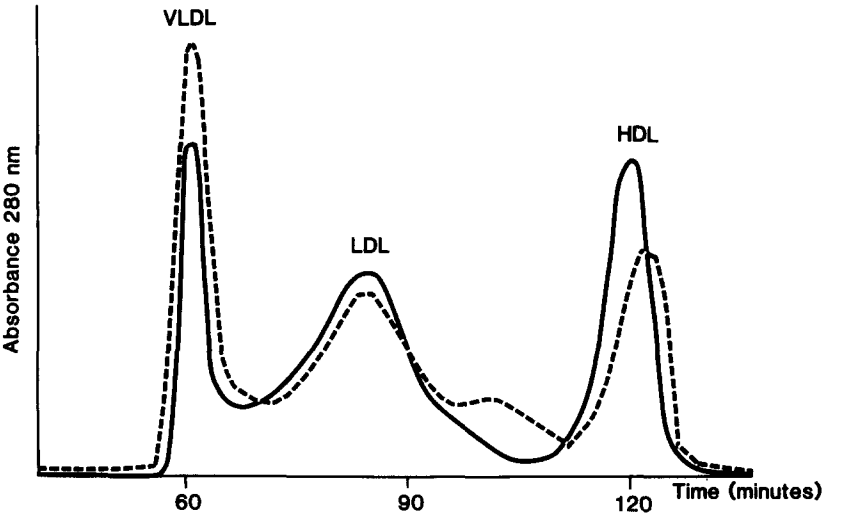

Fig. 5. Elution profile of lipoproteins separated by Superose 6B gel permeation chromatography. The solid line is the profile of a normal subject, while the broken line is the profile of subject J (serum bilirubin, 250 μmol/l). Lipoproteins from 30 ml of plasma were separated by a single ultracentrifugation at a density of 1.25 g/ml. The supernatant (3–5 ml) was filtered with a 0.22-μm filter and applied to a 1.6×75 cm column of Superose 6B. Equilibration and elution were performed with 0.15 M NaCl, 0.001 M Na_2 EDTA, pH 7.4, at a flow rate of 45 ml/hr. One-ml fractions were collected.

in 14 days. There was no difference between malignant and nonmalignant obstruction in the reduction of LCAT activity. Albumin concentrations were normal in short-term or treated obstruction, and lower in prolonged (>2 weeks) obstruction, but there was no statistically significant correlation between LCAT activity and serum albumin concentration. LCAT mass was not measured.

Incubations with purified LCAT

Seven plasma samples from six patients were incubated with purified LCAT. Overall, 51% of the available free cholesterol was esterified in 24 hr (range 27–64%), with an average of 1.2 mmol/l per 24 hr of free cholesterol esterified (range 0.8–1.8). Gradient gel electrophoresis of the lipoproteins isolated from the incubation mixture demonstrated a marked increase in HDL particle size compared to the unincubated HDL. In three subjects (**Fig. 8b**), the HDL particle size distribution after incubation with purified LCAT was approaching the profile of normal, unincubated HDL (Fig. 8c). In the other three subjects (Fig. 8a), the HDL had become even larger and were in the normal HDL_{2a} size range. This HDL profile resembles that of normal HDL after incubation with purified LCAT. The reason for the difference in behavior of the various HDL preparations is not clear; it does not relate to either the absolute or fractional rate of esterification or to the initial HDL particle distribution.

Incubation of isolated fractions of HDL from two subjects with purified LCAT demonstrated similar changes to the whole plasma incubations. Particles of radius 3.9 nm were converted to particles of radius 4.3 nm and 4.8 nm after 24 hr incubations with LCAT and LDL as a source of free cholesterol. At least 75% of the 3.9-nm particles had been converted into 4.3-nm radius particles within the first hour of incubation **(Fig. 9)**. The cholesterol in the resultant HDL was 74% esterified which was within the normal range.

DISCUSSION

In this report we have confirmed previous findings that HDL in patients with obstructive jaundice are abnormal in particle size, morphology, and composition (11–18), and that some of the observed abnormalities are due to LCAT deficiency (12, 18, 24) and may be reversed by incubation of cholestatic plasma with purified LCAT (40, 41). The novel aspects of this study are twofold. Firstly, the use of gradient gel electrophoresis allowed the evolution of changes in HDL particle size to be charted far more accurately than could be achieved with electron microscopy, and secondly, the inclusion of patients with acute obstruction (gallstones and pancreatitis) and incomplete obstruction demonstrated that the changes in LCAT activity and HDL particle size distribution occurred relatively early in the course of the illness and were not related to frank liver damage.

In the patients with acute, extrahepatic obstruction, three changes were evident in the HDL particle size distribution. *i*) The major HDL_3 subpopulation (normally 4.1–4.3 nm in radius) became smaller than usual and was reduced in amount; *ii*) a normal HDL_{2b} subpopulation (5.2–5.4 nm in radius) was absent; and *iii*) particles of HDL_{2a} size

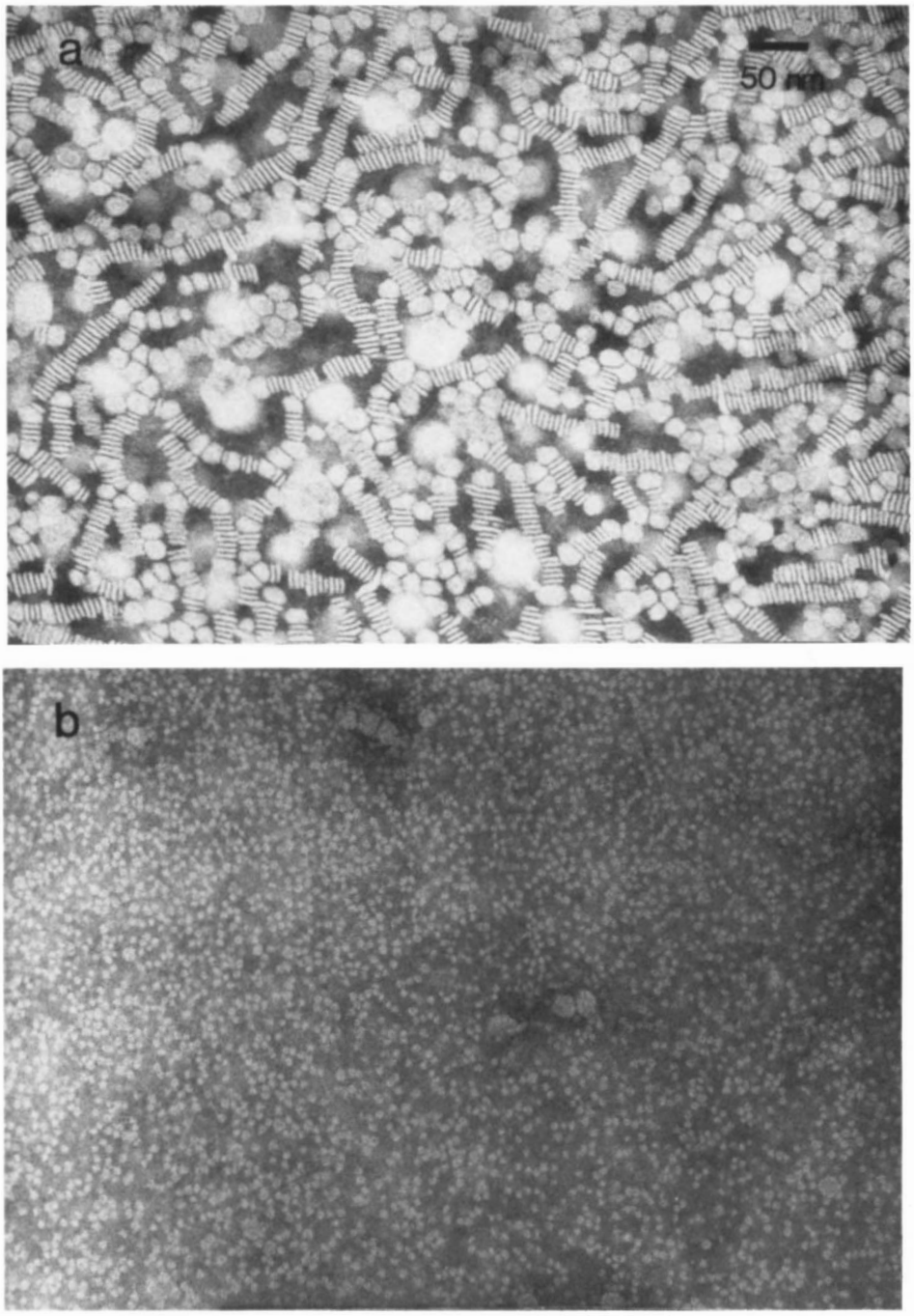

Fig. 6. Electron micrographs of lipoproteins from subject N (plasma bilirubin, 440 μmol/l); (a) lipoproteins from the first HDL peak eluted from the column (see Fig. 4); (b) lipoproteins from the second HDL peak.

(4.6–4.8 nm in radius) were prominent. Patients with malignant partial obstruction with bilirubin levels similar to those of the three patients with acute extrahepatic obstruction had very similar HDL profiles. ApoA-I levels were depressed in both groups, although to a greater extent in the group with malignancy. LCAT activity assayed in two subjects in this group with partial obstruction (bilirubin <150 μmol/l) was 40–43% of normal.

In higher grades of obstruction the LCAT activity was reduced further to 8–11% of normal while the HDL particle size distribution was very abnormal. The HDL fraction contained very small spherical particles of radius 3.6–3.8 nm and very large discoidal particles of >6.0 nm in radius. ApoA-I and apoA-II levels were significantly reduced. There was no significant difference in HDL particle size distribution and LCAT activity between the four pa-

TABLE 3. Chemical composition of HDL (percentage weight, mean ± SD)

Fraction	Protein	Esterified Cholesterol	Free Cholesterol	Phospholipid	Triglyceride
Discs[a]	28.7	2.8	17.0	43.7	7.7
Small spheres[b] (3.7–3.9 nm radius)	50.5 ± 1.8	5.6 ± 2.7	5.1 ± 3.1	33.1 ± 2.5	6.3 ± 2.2
Normal particles[c] (3.9 nm radius)	51.0 ± 0.4	19.6 ± 1.9	2.5 ± 0.4	23.3 ± 1.27	3.1 ± 1.2

The normal particles of radius 3.9 nm were isolated by gel filtration chromatography and their size was determined by gradient gel electrophoresis.
[a]Three subjects.
[b]Seven subjects.
[c]Five subjects.

tients with acute nonmalignant obstructions and the patients with malignant obstruction and similar levels of serum bilirubin. Although a matched control group was not recruited, several lines of evidence support the contention that the HDL abnormalities were probably due to LCAT deficiency secondary to obstructive jaundice and not related to malignancy per se or to major illness. Although patients with major illnesses, such as large myocardial infarctions, have reductions in HDL cholesterol and apoA-I and apoA-II concentrations, the reductions are not as marked as in biliary obstruction; nor do these patients have an HDL particle size distribution that resembles the distribution in the obstructed patients (42). Thirdly, the HDL in familial LCAT deficiency closely resemble, in morphology, composition, and particle size distribution, the HDL from patients with a bilirubin of >250 μmol/l (11, 35, 43–45). In addition, the HDL produced by cultured Hep G2 cells (46) and perfused primate livers (47) are very similar in size and morphology, to the HDL in these patients. In both of these in vitro situations, LCAT activity is apparently low (47, 48). Finally, the rapid evolution of changes in HDL particle size and the recovery in LCAT activity as obstruction is relieved support the contention that biliary obstruction per se produces both LCAT deficiency and the HDL abnormalities. Normalization of HDL size morphology and composition with purified LCAT provides further evidence.

This report also provides detailed information on the changes in HDL particle size following incubation of cholestatic plasma with purified LCAT. In all patients the majority of the particles after incubation had a radius of between 4.1 and 4.9 nm. In three patients, despite 24 hr incubation with a relatively high concentration of LCAT, a certain proportion of the small particles failed to be transformed. Thus, in these three patients, the HDL particle distribution after LCAT incubation is very similar to the particle size distribution of normal HDL with one exception, the absence of any HDL_{2b} subfraction. In the other subjects, no small particles were present and the major peak lay between radius 4.9 and 5.4 nm. These HDL profiles resembled that of subject L during the recovery phase and were quite similar to those seen when normal HDL was incubated with purified LCAT. The reason for the difference in behavior between these two groups is not clear; it does not relate to severity of illness, initial HDL profile, or concentration of LCAT used. However, deficient hepatic lipase activity, as has been demonstrated in patients with primary biliary cirrhosis (49), may be a factor. Forte et al. (11), Norum et al. (40), and Utermann et al. (41) found that incubation of HDL from familial LCAT deficiency patients (and from a patient with obstructive jaundice) with purified LCAT produced an HDL identical to that from normal subjects, although the incubated HDL tended to be of HDL_2 size.

LCAT activity was found to be low in all six patients tested, with no difference noted between acute, nonmalignant

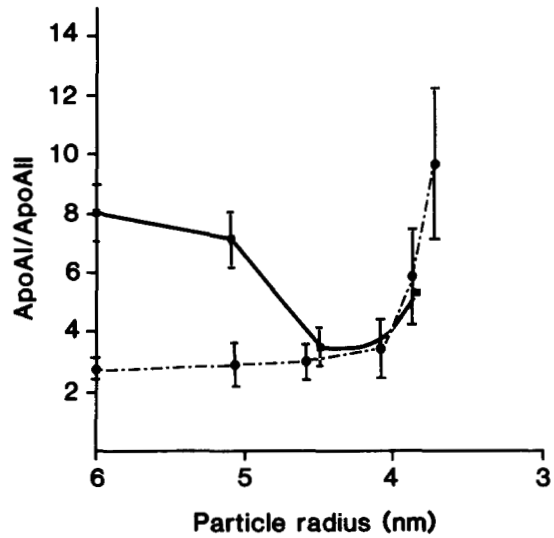

Fig. 7. ApoA-I to apoA-II ratio plotted as a function of particle size. The solid line shows the mean apoA-I to apoA-II ratio of five normal subjects, while the dotted line shows the mean apoA-I to apoA-II ratio of seven subjects with obstructive jaundice. ApoA-I and apoA-II levels were assessed by immunoturbidimetry in alternate HDL fractions eluted from the Superose 6B column. Particle size of the major component of each fraction was assessed by gradient gel electrophoresis performed as described in the legend to Fig. 2.

TABLE 4. HDL apolipoprotein composition (percentage by weight)

Fraction	High Mol. Wt. Proteins[a]	Alb.	A-IV	E	A-I	A-II	C
Discs[b]	14	13	5	22	22	9	15
Spheres[c]					81	17	2.0
Normal HDL					75	20	5

One hundred μg of delipidated HDL protein was electrophoresed on a 10% SDS gel under non-reducing conditions. Gels were stained with Coomassie Brilliant Blue R and scanned on a laser densitometer. No correction was made for differing chromogenicity of the individual apoproteins.

[a] Unidentified high molecular weight proteins (mol wt 70–90,000).

[b] Mean of four patients.

[c] Mean of seven patients.

obstruction and malignant obstruction. Following relief of malignant obstruction in one patient, LCAT activity rose fivefold in 2 weeks. Numerous investigators have examined LCAT activity in obstructive jaundice with conflicting results (12, 17, 18, 24–28). Most have found diminished LCAT activity in over half of the patients tested, although Wengeler, Greten, and Seidel (26) found no reduction in LCAT activity in ten patients tested. The most common explanation for the reduction in LCAT activity is liver damage secondary to prolonged obstruction. However, in this group of patients, LCAT was reduced to 40% of normal within 7 days and to 8% of normal in 14 days following acute nonmalignant obstruction. Although it is clearly difficult to date the onset of obstruction in malignancy, two of the patients were jaundiced for only 7–10 days and had no evidence of secondary hepatic damage. Conceivably the synthesis and/or release of LCAT is sensitive to intraductal pressure, the converse of the stimulation of alkaline phosphatase synthesis in biliary obstruction (50). However, with biliary ductule destruction, as in primary biliary cirrhosis (41), LCAT mass and activity do not fall until liver function is severely compromised.

TABLE 5. LCAT activity and apoA-I levels

Subject	ApoA-I[a]	LCAT Activity[b]	Serum Bilirubin	Serum Albumin[c]
	% of normal		*μmol/l*	*g/l*
L(a)	11	11	340	35
N	15	8	440	30
M	16	9	350	34
E	36	43	148	41
L(b)	38	53	120	33
H	39	54	210	41
D	46	40	146	42

[a] Correlation coefficient $r = 0.91$ ($P < 0.01$) between apoA-I and LCAT activity.

[b] 'Normal' activity was determined by calculating the fractional esterification rate of plasma from four normal subjects. Mean esterification rate was 1.78 ± 0.38% over 5 hr.

[c] Correlation coefficient $r = 0.61$ ($P > 0.05$) between serum albumin and LCAT activity.

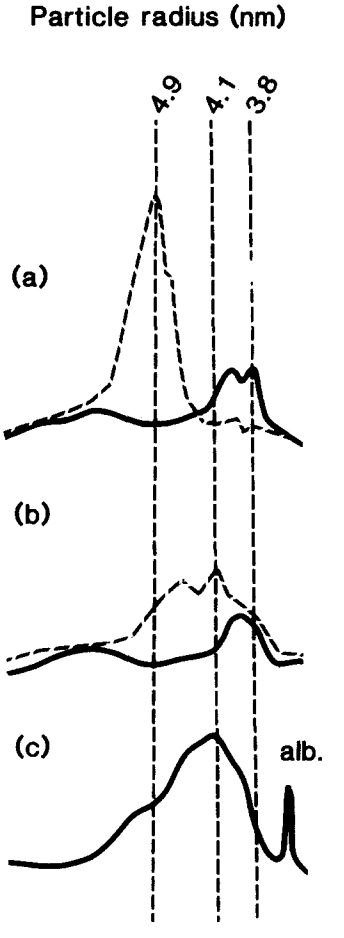

Fig. 8. Gradient gel profiles of HDL isolated from cholestatic plasma before and after LCAT incubations. Plasma (0.5 ml) was incubated for 24 hr at 37°C with 85 units of purified LCAT in a total volume of 1 ml. The incubation mixture was then brought to a density of 1.25 g/ml and subjected to ultracentrifugation for 40 hr at 50,000 rpm. The lipoproteins were then directly applied to a gradient gel as described in the legend to Fig. 2. The solid lines are the profiles of the HDL prior to incubation while the broken lines are the profiles of the LCAT-modified HDL. Two types of changes occurred: (a) enlargement to a mean radius of 4.9 nm with no residual population of small particles, or (b) enlargement to two discrete subpopulations of radius 4.7 nm and 4.1 nm, with 15% of the HDL as persistent 3.9 nm particles. This profile is very similar to the normal HDL profile shown in panel C.

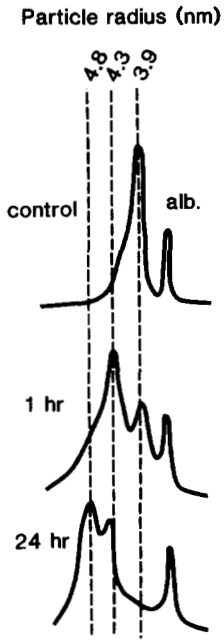

Fig. 9. Time course of reaction of small HDL with purified LCAT. A subpopulation of small spherical HDL from subject J was isolated by gel filtration. Twenty units of purified LCAT was added to a mixture of HDL containing 20 nmol of free cholesterol, autologous LDL containing 50 nmol of free cholesterol, and 20 μg of bovine serum albumin in a total volume of 200 μl. After reaction at 37°C for 1 hr and 24 hr, aliquots of the incubation mixture were subjected to gradient gel electrophoresis as described in the legend to Fig. 2. The first panel is the profile of the 4°C control HDL. Similar results were obtained with small spherical HDL from subject L.

Published crossed plasma incubation studies (25, 28) as well as similar studies performed on these patients (data not shown) have demonstrated that cholestatic plasma does not inhibit LCAT in normal plasma. In fact, stimulation of esterification can occur as cholestatic lipoproteins are better substrates for LCAT than normal lipoproteins. The level of bile acids reached in prolonged obstruction (approximately 150 μg/ml) will inhibit normal LCAT by 5% only (24). In addition, since the activity of LCAT had fallen to 9% of normal after 14 days of obstruction in patient M, it follows that the half-life of LCAT must be considerably less than 7 days. Blomhoff (51) found the half-life of LCAT infused in plasma into a patient with familial LCAT deficiency to be 4–8 days.

In patients with a serum bilirubin of >250 μmol/l, the large apoE-enriched discoidal particles and the small apoA-I-enriched spherical particles seen in this study and previously reported by Arnesjö et al. (15) and Agorastos et al. (17) are very similar to the HDL particles seen in familial LCAT deficiency (44, 45). In both situations the particles present in plasma may represent nascent spheres and discs from liver and intestine not acted on by LCAT (52–55).

The strong correlation between LCAT activity and plasma apoA-I level suggests that the level of LCAT activity may in part determine the plasma apoA-I level by controlling the total core volume of HDL. The reverse hypothesis is unlikely as LCAT requires only a very small amount of apoA-I for full activation. The intestinal contribution of apoA-I (both on chylomicrons and HDL), although it may be reduced by impaired fat absorption, is likely to be adequate for LCAT activation. Nestel, Tada, and Fidge (56) have found from kinetic studies in humans that apoA-I production in severe alcoholic hepatitis is normal while apoA-I catabolism is increased. The increased catabolism is probably secondary to lack of HDL core material (due to LCAT deficiency) to which apoA-I can bind.

In conclusion, we have demonstrated a considerable reduction in LCAT activity in both malignant and non-malignant biliary obstruction. The fall in LCAT activity is associated with a decrease in plasma apoA-I and apoA-II concentrations and marked abnormalities of the HDL particle distribution, in particular an increase in the proportion of spherical particles comparable in size to the normal HDL_{2a} and HDL_{3c} subfractions. Large discoidal HDL particles of radius 7.5–8.5 nm were detected in all subjects, but were present in abundance only when the bilirubin was >250 μmol/l. Incubation of cholestatic plasma with purified LCAT partially normalized the HDL particle size distribution.

Many thanks to Ludmilla Gorjatschko for performing the electron microscopy and to Chris Holt for typing the manuscript. This work was supported by the NH & MRC of Australia and the National Heart Foundation of Australia.

Manuscript received 14 April 1987 and in revised form 29 July 1987.

REFERENCES

1. Epstein, E. Z. 1932. Cholesterol in the blood in hepatic and biliary disease. *Arch. Intern. Med.* **50:** 203–222.
2. Sabesin, S. M., H. L. Hawkins, L. Kuikin, and J. B. Ragland. 1977. Abnormal plasma lipoproteins and lecithin: cholesterol acyltransferase deficiency in alcoholic liver disease. *Gastroenterology.* **72:** 510–518.
3. Eder, H. A., E. M. Russ, R. A. Rees Pritchett, M. Wilber, and D. P. Barr. 1955. Protein-lipid relationships in human plasma in biliary cirrhosis, obstructive jaundice and acute hepatitis. *J. Clin. Invest.* **34:** 1147–1162.
4. Russ, E. M., J. Raymunt, and D. P. Barr. 1956. Lipoproteins in primary biliary cirrhosis. *J. Clin. Invest.* **35:** 133–144.
5. Furman, R. H., and L. L. Conrad. 1957. Ultracentrifugal characterization of the lipoprotein spectrum in obstructive jaundice: studies of serum lipid relationships in intra- and extra-hepatic biliary obstruction. *J. Clin. Invest.* **36:** 713–722.
6. Switzer, S. 1967. Immunologically distinct low density lipoproteins in patients with biliary obstruction. *J. Clin. Invest.* **46:** 1855–1866.
7. Seidel, D., P. Alaupovic, and R. H. Furman. 1969. A lipoprotein characterizing obstructive jaundice. I. Method for quantitative separation and identification of lipoproteins in jaundiced subjects. *J. Clin. Invest.* **48:** 1211–1223.

8. 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.
9. Hamilton, R. L., R. J. Havel, J. P. Kane, A. E. Blannock, and T. Sata. 1971. Cholestasis: lamellar structure of the abnormal human serum lipoproteins. *Science.* **172:** 475–478.
10. Seidel, D., S. Agostini, and P. Muller. 1972. Structure of an abnormal plasma lipoprotein LP-X characterizing obstructive jaundice. *Biochim. Biophys. Acta.* **260:** 146–152.
11. Forte, T., A. Nichols, J. Glomset, and K. Norum. 1974. The ultrastructure of plasma lipoproteins in lecithin: cholesterol acyl transferase deficiency. *Scand. J. Clin. Lab. Invest.* **33 Suppl. 137:** 121–132.
12. Utermann, G., W. Schoenborn, K. H. Langer, and P. Dieker. 1972. Lipoproteins in LCAT deficiency. *Humangenetick.* **16:** 295–306.
13. Blomhoff, J. P. 1974. High density lipoprotein in cholestasis. *Scand. J. Gastroenterol.* **9:** 591–596.
14. Danielsson, B., R. Ekman, and B. G. Petersson. 1975. An abnormal high density lipoprotein in cholestatic plasma isolated by zonal ultracentrifugation. *FEBS Lett.* **50:** 180–184.
15. Arnesjö, B., B. Danielsson, R. Ekman, B. G. Johansson, and B. G. Petersson. 1977. Characterization of high density lipoproteins in human cholestasis. *Scand. J. Clin. Lab. Invest.* **37:** 587–597.
16. Coulhon, M. P., F. Tallet, J. Yonger, J. Agneray, and D. Raichvarg. 1985. Changes in human high density lipoproteins in patients with extrahepatic biliary obstruction. *Clin. Chim. Acta.* **145:** 163–172.
17. Agorastos, J., C. Fox, D. S. Harry, and N. McIntyre. 1978. Lecithin:cholesterol acyltransferase and the lipoprotein abnormalities of obstructive jaundice. *Clin. Sci. Mol. Med.* **54:** 369–379.
18. Utermann, G., H. J. Menzel, and K. H. Langer. 1974. On the polypeptide composition of an abnormal high density lipoprotein (LP-E) occurring in LCAT-deficient plasma. *FEBS Lett.* **45:** 29–32.
19. Teramoto, T., H. Kato, Y. Hashimoto, M. Kinoshita, G. Toda, and H. Oka. 1985. Abnormal high density lipoprotein of primary biliary cirrhosis analyzed by high performance liquid chromatography. *Clin. Chim. Acta.* **149:** 135–148.
20. Fujii, S., S. Koga, T. Shono, K. Yamamoto, and H. Ibayashi. 1981. Serum apoprotein A-I and A-II levels in liver disease and cholestasis. *Clin. Chim. Acta.* **115:** 321–331.
21. Floren, C. H., and A. Gustafson. 1985. Apolipoproteins A-I, A-II and E in cholestatic liver disease. *Scand. J. Clin. Lab. Invest.* **45:** 103–108.
22. Simon, J. B., and J. L. Boyer. 1970. Production of lecithin:cholesterol acyltransferase by the isolated perfused rat liver. *Biochim. Biophys. Acta.* **218:** 549–551.
23. Osuga, T., and O. W. Portman. 1971. Origin and disappearance of plasma lecithin:cholesterol acyltransferase. *Am. J. Physiol.* **220:** 735–741.
24. Gjone, E., and I. P. Blomhoff. 1970. Plasma lecithin: cholesterol acyltransferase in obstructive jaundice. *Scand. J. Gastroenterol.* **5:** 305–308.
25. Calandra, S., M. J. Martin, and N. McIntyre. 1971. Plasma lecithin:cholesterol acyltransferase activity in liver disease. *Eur. J. Clin. Invest.* **1:** 352–360.
26. Wengeler, H., H. Greten, and D. Seidel. 1972. Serum cholesterol esterification in liver disease. Combined determinations of lecithin:cholesterol acyltransferase and lipoprotein-X. *Eur. J. Clin. Invest.* **2:** 372–378.
27. Ritland, S., J. P. Blomhoff, and E. Gjone. 1973. Lecithin: cholesterol acyltransferase and lipoprotein X in liver disease. *Clin. Chim. Acta.* **49:** 251–259.
28. Kepkay, D. L., R. Poon, and J. B. Simon. 1973. Lecithin: cholesterol acyltransferase and serum cholesterol esterification in obstructive jaundice. *J. Lab. Clin. Med.* **81:** 172–181.
29. Hatch, F. T., and R. S. Lees. 1968. Practical methods for plasma lipoprotein analysis. *Adv. Lipid Res.* **6:** 1–68.
30. Blanche, P. J., E. L. Gong, T. M. Forte, and A. V. Nichols. 1981. Characterization of human high density lipoproteins by gradient gel electrophoresis. *Biochim. Biophys. Acta.* **665:** 408–419.
31. Laemmli, U. K. 1970. Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
32. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
33. Austin, G. E., and E. Maznicki. 1983. Automated turbidimetric assay of serum apolipoprotein A-I using the Cobas-Bio centrifugal analyzer. *Clin. Biochem.* **16:** 338–340.
34. Rifai, N., M. E. King, and A. Malekpour. 1984. Apolipoprotein profile on the Cobas-Bio. *Clin. Chem.* **30:** 992.
35. Forte, T., K. R. Norum, J. A. Glomset, and A. V. Nichols. 1971. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: structure of low and high density lipoproteins as revealed by electron microscopy. *J. Clin. Invest.* **50:** 1141–1148.
36. Rajaram, O. V., and P. J. Barter. 1985. Reactivity of human lipoproteins with purified lecithin:cholesterol acyltransferase during incubations in vitro. *Biochim. Biophys. Acta.* **835:** 41–49.
37. Barter, P. J., G. J. Hopkins, and L. Gorjatschko. 1985. Lipoprotein substrates for plasma cholesterol esterification. Influence of particle size and composition of the high density lipoprotein subfraction 3. *Atherosclerosis.* **58:** 97–107.
38. Blum, C. B., R. I. Levy, S. Eisenberg, M. Hall III, R. H. Goebel, and M. Berman. 1977. High density lipoprotein metabolism in man. *J. Clin. Invest.* **60:** 795–807.
39. Patsch, J. R., C. A. Kirk, 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, LP-X_3. *J. Biol. Chem.* **232:** 2113–2120.
40. Norum, K. R., J. A. Glomset, A. V. Nichols, T. Forte, J. J. Albers, W. C. King, C. D. Mitchell, K. R. Applegate, E. L. Gong, V. Cabana, and E. Gjone. 1975. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: effects of incubation with lecithin:cholesterol acyltransferase in vitro. *Scand. J. Clin. Lab. Invest.* **35 Suppl. 142:** 31–55.
41. Utermann, G., H-J. Menzel, G. Adler, P. Dieker, and W. Weber. 1980. Substitution in vitro of lecithin:cholesterol acyltransferase. Analysis of changes in plasma lipoproteins. *Eur. J. Biochem.* **107:** 225–241.
42. Clifton, P. M., A. M. Mackinnon, and P. J. Barter. 1985. Effects of serum amyloid A protein (SAA) on composition, size, and density of high density lipoproteins in subjects with myocardial infarction. *J. Lipid Res.* **26:** 1389–1398.
43. Torsvik, H., M. H. Solass, and E. Gjone. 1970. Serum lipoproteins in plasma lecithin:cholesterol acyltransferase deficiency, studied by electron microscopy. *Clin. Genet.* **1:** 139–150.
44. Chen, C., K. Applegate, W. C. King, J. A. Glomset, K. R. Norum, and E. Gjone. 1984. A study of the small spherical high density lipoproteins of patients afflicted with familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **25:** 269–282.

45. Mitchell, C. D., W. C. King, K. R. Applegate, T. Forte, J. A. Glomset, K. R. Norum, and E. Gjone. 1980. Characterization of apolipoprotein E-rich high density lipoproteins in familial lecithin:cholesterol acyltransferase deficiency. *J. Lipid Res.* **21:** 625–634.
46. Thrift, R. N., T. M. Forte, B. E. Cahoon, and V. G. Shore. 1986. Characterization of lipoproteins produced by the human liver cell line, Hep G2, under defined conditions. *J. Lipid Res.* **27:** 236–250.
47. Johnson, F. L., R. W. St. Clair, and L. L. Rudel. 1983. Studies on the production of low density lipoproteins by perfused livers from nonhuman primates: effect of dietary cholesterol. *J. Clin. Invest.* **72:** 221–236.
48. Chen, C. H., T. M. Forte, B. E. Cahoon, R. N., Thrift, and J. J. Albers. 1986. Synthesis and secretion of lecithin: cholesterol acyltransferase by the human hepatoma cell line Hep G2. *Biochim. Biophys. Acta.* **877:** 433–439.
49. Jahn, C. E., E. J. Schaefer, L. A. Taam, J. H. Hoofnagle, F. T. Lindgren, J. J. Albers, E. A. Jones, and H. B. Brewer, Jr. 1985. Lipoprotein abnormalities in primary biliary cirrhosis. Association with hepatic lipase inhibition as well as altered cholesterol esterification. *Gastroenterology.* **89:** 1266–1278.
50. Kaplan, M. M., and A. Righetti. 1970. Induction of rat liver alkaline phosphatase: the mechanism of the serum elevation in bile duct obstruction. *J. Clin. Invest.* **49:** 508–516.
51. Blomhoff, J. 1974. In vitro determination of lecithin: cholesterol acyltransferase in plasma. *Scand. J. Clin. Lab. Invest.* **33 Suppl. 137:** 35–43.
52. Hamilton, R. L., M. C. Williams, C. J. Fielding, and R. J. Havel. 1976. Discoidal bilayer structure of nascent high density lipoproteins from perfused rat liver. *J. Clin. Invest.* **58:** 667–680.
53. Green, P. H. R., A. R. Tall, and R. M. Glickman. 1978. Rat intestine secretes discoidal high density lipoprotein. *J. Clin. Invest.* **61:** 528–534.
54. Forester, G. P., A. R. Tall, C. L. Bisgaier, and R. M. Glickman. 1983. Rat intestine secretes spherical high density lipoproteins. *J. Biol. Chem.* **258:** 5938–5943.
55. Hoffman, J. S., and E. P. Benditt. 1982. Secretion of serum amyloid protein and assembly of serum amyloid protein-rich high density lipoprotein in primary mouse hepatocyte culture. *J. Biol. Chem.* **257:** 10518–10522.
56. Nestel, P. J., N. Tada, and N. H. Fidge. 1980. Increased catabolism of high density lipoprotein in alcoholic hepatitis. *Metabolism.* **23:** 101–104.