Familial lecithin:cholesterol acyltransferase deficiency. Biochemistry of the cornea

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Abstract Opacification of the cornea from lipid accumulation is an early and characteristic feature of familial lecithin:cholesterol acyltransferase (LCAT) deficiency. Visual impairment in a female age 48 years led to keratoplasty and the first detailed analysis of cornea in this disorder. Multilaminar figures were present, and total lipid extracts were enriched with phospholipid and cholesterol; cholesteryl esters were reduced, and accounted for about 12% of the cholesterol. Linoleate C18:2 was the predominant residue in the cholesteryl ester fatty acid fraction, with a C18:1/18.2 ratio of 1:6.5. This ratio differs from that in normal cornea, and from that in plasma and in other tissue deposits in LCAT deficiency. Various disorders of the HDL/LCAT system in plasma can lead to corneal lipid accumulation and opacification. These disorders may share general defects of lipid clearance from the cornea, but this study of LCAT cornea indicates that the character of the accumulating lipid is significantly influenced by active local metabolism, irrespective of the defect in the HDL/LCAT system also present. —**Winder, A. F., A. Garner, G. A. Sheraidah, and P. Barry.** Familial lecithin: cholesterol acyltransferase deficiency. Biochemistry of the cornea. *J. Lipid Res.* 1985. **26:** 283–287.

Supplementary key words high density lipoprotein • fish-eye disease • linoleate • lipoproteins

Lecithin:cholesterol acyltransferase (LCAT) deficiency occurs as a rare autosomal recessive disorder concerning which about 26 cases have been reported (1). Esterification and transport of cholesterol and maturation of high density lipoprotein (HDL) components in plasma is impaired, and tissue deposits enriched with free cholesterol accumulate. In the presentation of the disorder, corneal opacification due to the presence of a diffuse punctate panstromal deposit is a characteristic feature, which may be observed in childhood, and can lead to presentation and diagnosis (2, 3). Three patients have proceeded to keratoplasty (4–6). Morphological and limited biochemical studies have demonstrated numerous predominantly extracellular membranous and other vesicular inclusions associated with the presence in corneal extracts of excess phospholipid and unesterified cholesterol (5), as is found with lipid accumulating at other sites with this disorder (7–10). Keratoplasty has now been performed on a further patient. The morphology of this corneal material is described, and, in particular, the first extensive biochemical study of the lipid accumulating in the cornea in familial LCAT deficiency is now presented.

CASE REPORT

The patient was a female, aged 48 years, of Irish extraction. The diagnosis was made during an admission for assessment of renal failure and hypertension; renal grafting was subsequently performed. A familial background, lack of severe visual impairment, and artifactual plasma lipids as a result of transfusion to correct anemia initially led to a diagnosis of fish-eye disease (11–13). Corneal clouding had been noticed 19 years previously on treatment of a corneal abrasion, but no further action had then been taken. Documented levels of lipids and lipoproteins in plasma had fluctuated over a period of 13 years, in association with nephrotic syndrome and peritoneal dialysis.

It is probable that the progression of renal disease in this patient had been accelerated by previous toxemia of pregnancy, and by a throat infection, with evidence of streptococcal involvement. On presentation shortly after peritoneal dialysis, lipids in plasma were: triglycerides, 3.4 mmol/l; cholesterol, 3.6 mmol/l and rising; high density lipoprotein (as cholesterol), 0.13 mmol/l. Low density lipoprotein (LDL) was enriched with triglycerides and large molecular weight LDL (14) was present. Owing to recent transfusion, the proportion of cholesterol in plasma in ester form was then 40%, but absence of intrinsic LCAT activity in plasma was later confirmed. Initial studies on this patient and her family have been previously reported (15, 16). The appearance of the cornea is shown in **Fig. 1.**

Abbreviations: LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoprotein.

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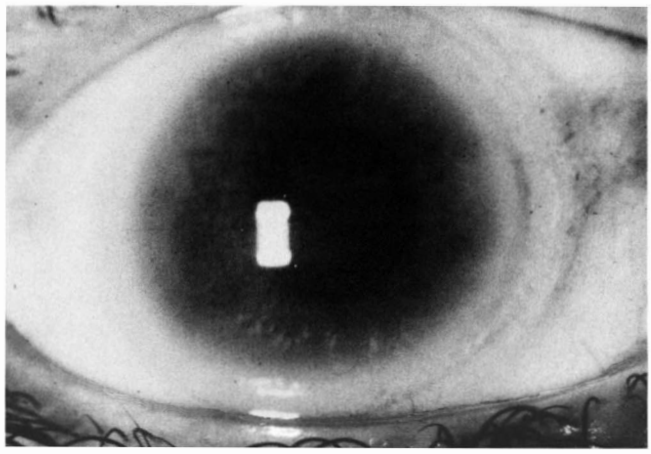

Fig. 1 Gross appearance of the cornea in familial lecithin:cholesterol acyltransferase deficiency.

STUDIES ON THE CORNEA

Microscopy

Portions of the corneal button obtained by full thickness keratoplasty were prepared the same day for light and electron microscopy. The staining reactions applied to cryostat and paraffin-embedded sections are shown in **Table 1;** material for electron microscopy was fixed in glutaraldehyde and osmic acid.

Analytical studies

The residual corneal button was not freeze-dried, in an attempt to reduce the subsequent extraction of normal structural corneal lipid in addition to the extracellular stromal material found in familial LCAT deficiency. The button, weight dry but not dehydrated of 2.0 mg, was stored at −70°C under nitrogen for several weeks prior to analysis.

The button was then thawed, rehydrated, and finely minced using a blade before lipid extraction by the Folch procedure as described previously (5). The sample was insufficient to consider removal of epithelium prior to analysis; epithelium may contain lipoprotein material differing from that present in stroma (17). After homogenization in 4 ml of chloroform–methanol 2:1 (v/v) in a Dounce all-glass hand-held homogenizer, the organic phase was washed three times with 0.25 ml of 0.88% potassium chloride, dried over sodium sulfate, evaporated under nitrogen, and dissolved in 100 μl of dichloromethane at 4°C. Lipid class separation was performed on silica gel TLC plates developed in benzene–diethylether–ethanol–glacial acetic acid 50:40:10:2 (v/v) with standards incorporating free cholesterol, cholesterol ester as acetate, and phospholipid as phosphatidylcholine. Quantitation was by densitometry after plate-charring with sulfuric acid at 110°C. Other plates allowed determination of the proportion of cholesterol present in ester form in the corneal extracts, and recovery of the corneal cholesteryl ester fraction by plate scraping and elution with hexane. After evaporation under nitrogen and hydrolysis with alcoholic KOH at 110°C for 60 min, cholesterol was removed by three extractions with hexane, and the acyl residues were extracted into hexane after neutralization of the aqueous phase with HCl. The dry residue was methylated using 5% acetyl chloride in methanol with recovery of the acyl fraction by triple extraction with hexane and evaporation under nitrogen. Analysis of chain length and configuration was performed with a Perkin-Elmer F.11 dual channel gas chromatograph with flame ionization detector, and a 6 foot × 3 mm I.D. column of 10% EGS PZ run at 160°C. Sigma lipid standards contained acyl residues of C16:0, 18:0, 18:1, 18:2, 18:3, and 20:4.

RESULTS

Light microscopy

Sections of the paraffin-embedded tissue showed a full thickness corneal disc. The epithelium was normal, with an intact basement membrane. Bowman's membrane was also intact, and there was no recognizable abnormality in the deeper tissues. Cryostat sections, however, revealed patchy infiltration of the entire stroma with lipids (staining as indicated in Table 1). Taken together, these results are evidence of the presence of excess lipid, consistent

TABLE 1. Staining reaction of sections of the cornea in familial LCAT deficiency

Staining Reaction	Result	Inference
Sudan black	Patchy grey	Phospholipid, cholesteryl ester, and triglycerides
Nile Blue sulfate	Pale blue patches	Phospholipid
Oil Red O	Pale pink (equivocal)	?Phospholipid
Bromine-Oil Red O	Patchy red staining	Lecithin, free fatty acid, and cholesterol
Acid hematin	Blue-black	Lecithin and sphingomyelin
NaOH-acid hematin	Staining prevented	Absence of sphingomyelin
Okamoto	Weak reaction	Cholesterol
Digitonin-Okamoto	Weak reaction	Free cholesterol

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with phospholipid, but with little cholesterol other than a small amount of cholesteryl ester in Bowman's layer.

Electron microscopy

Both between and within the stromal lamellae, there were round and/or oval spaces varying in size from 5 to 200 nm, with a preponderance of smaller spaces. The boundaries were delimited by a concentration of amorphous material or, alternatively, by a more definite electron-dense membrane. In some cases there were small membranous rings within the spaces, and exceptionally these were multilaminar (**Fig. 2** and **Fig. 3**). The keratocytes and epithelium were apparently normal and free from lipid inclusions.

Analytical studies

The mean results of lipid analysis by thin-layer chromatography of three applications of corneal lipid extract, each assessed by repeat densitometry, are shown in **Table 2.** An elution profile obtained by separation of the methylated acyl residues from the cholesteryl ester fraction by gas–liquid chromatography is shown in **Fig. 4.** Average data from these experiments are shown in **Table 3.** Recovery of defined acyl residues was approximately 13 nmol/mg of cornea, in good agreement with estimates of this cholesteryl ester fraction as determined by thin-layer chromatography and calibrating with cholesteryl acetate standards as 15.8 nmol/mg cornea. The C18:1/18:2 acyl ratio for this fraction was 1:6.5. The cholesterol/cholesteryl ester ratio in the corneal extracts was calculated from the thin-layer chromatographic data directly, assuming an average acyl residue molecular weight of 660, or after substitution of data obtained by gas–liquid chromatography. Thus the proportion of cholesterol present in ester form was 10–14%.

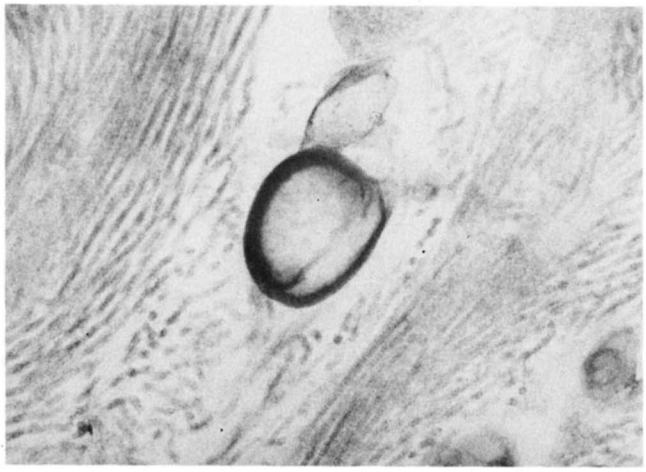

Fig. 2 Appearance by electron microscopy of the corneal stroma in familial lecithin:cholesterol acyltransferase deficiency, showing an inclusion body. Magnification × 104K.

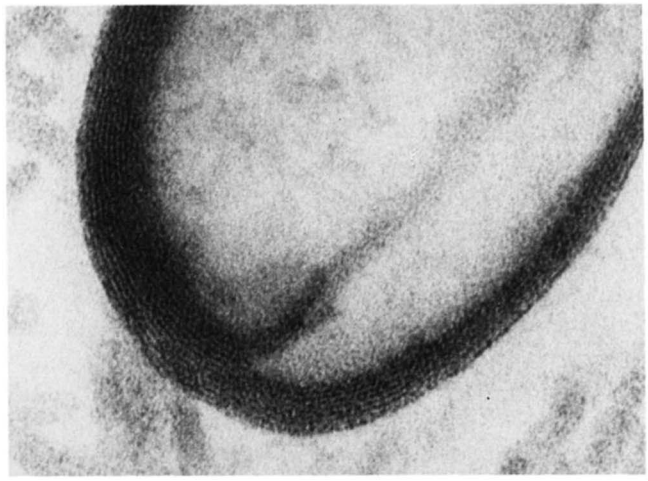

Fig. 3 Corneal inclusion bodies in familial lecithin:cholesterol acyltransferase deficiency, showing the multilaminar structure. Magnification × 468K.

DISCUSSION

Information on the lipid composition of normal human cornea is scanty, but the structural and analytical data obtained for the LCAT cornea, together with control studies and preliminary observations on an earlier LCAT cornea (5), are consistent with the accumulation of excess lipid mainly as phospholipid and as cholesterol, of which the proportion in ester form is reduced. Complications for the present and the former patient led to keratoplasty and analysis of the cornea. The former younger patient additionally showed superficial corneal scarring from trachoma (4, 5); the present patient entered the hospital with renal failure and hypertension. Major generalization on corneal biochemistry in familial LCAT deficiency is therefore speculative, but it is of interest that the results of the outline studies in the first case are closely in accord with the detailed studies on the second case now reported, even though differing additional features were involved.

The discrepancy between the weak histochemical reaction for cholesterol and the considerable accumulation demonstrated by direct assay is not readily explained. Possibilities include insensitivity of the staining method and incorporation of the cholesterol within phospholipid micelles, evident as multilaminar structures, such that it

TABLE 2. Lipid composition of corneal extracts in familial LCAT deficiency

Component	ng/mg of Tissue	% of Lipid Extracted
Total phospholipids	45	63
Free cholesterol	25	28
Cholesteryl esters	7	9
Triglycerides	traces only — not quantifiable	

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was effectively masked. However, the material resembled, in most respects, that accumulating at other sites in this disorder—liver, spleen, renal glomeruli, erythrocyte membranes, and arterial atheroma (1, 7-10)—consistent with the experience that corneal accumulation is an early, evident, characteristic and occasionally diagnostic feature of familial LCAT deficiency. Insudation of material from plasma may contribute to accumulation of lipid-rich material at these sites, together with phagocytosis perhaps involving remnant particles and abnormal components such as large molecular weight LDL (9). It has been suggested that tissue reactions to the accumulating material provoke further tissue damage (9). In the cornea there is no evidence, from the present or earlier specimens examined, of phagocytosis or any such tissue reaction, excluding the anomalous features seen in the patient with evidence of trachoma (4, 5).

Normal subjects and those with familial LCAT deficiency show differences in the acyl composition of the cholesteryl ester fatty acid fraction in plasma and in the various tissue deposits, and differences are also evident in cornea. Thus the C18:1/18:2 ratio is about 4:1 in atheroma from LCAT patients, and about 1:1 to 2:1 in normal subjects (1, 10). This difference is in parallel with that in plasma. The C18:1/18:2 ratio in LCAT plasma is about 3:1, while in normal plasma it is about 1:2 (1, 10). The ratio in the LCAT cornea examined was about 1:6.5. Tschetter (18) had reported ratios in the range 1.7-4.6:1 in total lipid extracts of human cornea with and without corneal arcus. We had also found that the C18:1/18:2 ratio in saline extracts of minced de-epithelialized human cornea was about 3.5:1 (19) with similar ratios in total lipid extracts of adult human cornea (G. A. Sheraidah and A. F. Winder, unpublished results). The data on acyl residues recorded here for LCAT cornea, and the evident differences in composition from plasma and deposits at other sites, strongly suggest that the processes of accumulation are significantly affected by re-esterification and active metabolism of lipid and lipoprotein in the cornea.

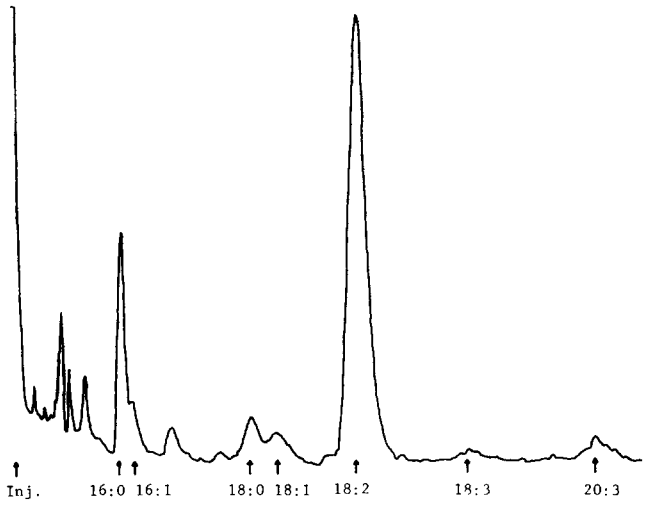

Fig. 4 Gas-liquid chromatographic profile of methyl ester derivatives of acyl residues from the cholesteryl ester fatty acid fraction prepared from cornea in familial lecithin:cholesterol acyltransferase deficiency. Elution positions of authentic methyl esters are also shown.

TABLE 3. Cholesteryl ester fatty acid composition of lipid extracts of the cornea in familial LCAT deficiency

Residue		nmol/mg Cornea	as % of Total FA Extracted
Palmitate	C16:0	3.90	29.0
Stearate	C18:0	1.04	8.5
Oleate	C18:1	1.04	8.5
Linoleate	C18:2	6.75	54.0
Linolenate	C18:3	Detected, but traces only	
Arachidonate	C20:4	Detected, but traces only	

Thus, although differences in acyl ratios arise between plasma and cornea in both LCAT-deficient and normal subjects, the ratios involved are not similar. Any proposed modification of plasma-derived material in LCAT cornea also does not obviously involve corneal acyl:cholesterol acyl transferases (ACAT) for which C18:1 is preferred substrate.

Diffuse opacification of the cornea is associated with at least four disorders of the HDL/LCAT system in plasma—familial apolipoprotein A deficiency or Tangier disease, familial LCAT deficiency, fish-eye disease, and variant apolipoprotein A deficiency, which may also be familial (16, 20). Some differences arise in the distribution and composition of the accumulating lipid, but the feature which these disorders have in common is probably defective clearance of lipid from the cornea (16). The relationship between fish-eye disease and LCAT deficiency is of special interest. The abnormalities of plasma in these disorders are clearly different (13), and in fish-eye disease reduced but apparently significant levels of HDL are present. However, the biological findings recorded for cornea in fish-eye disease (12) are clearly similar to those now recorded for familial LCAT deficiency. There is no direct evidence of localized LCAT deficiency or other ophthalmological metabolic disturbance in fish-eye disease, and the origins of the ophthalmological changes remain to be determined. In this general group of disorders associated with corneal opacification owing to lipid accumulation, the contributions of intrinsic corneal lipid metabolism, in addition to the HDL/LCAT transport system, are not defined, but from data obtained in the present study such contributions appear to be significant.

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