Abnormal High Density Lipoproteins in Cerebrotendinous Xanthomatosis

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ABSTRACT The plasma lipoprotein profiles and high density lipoproteins (HDL) were characterized in patients with the genetic disease cerebrotendinous xanthomatosis (CTX). Abnormalities in the HDL may contribute to their increased atherogenesis and excessive deposits of tissue sterols in the presence of low or low-normal concentrations of plasma cholesterol (165 ± 25 mg/dl) and low density lipoproteins (LDL). The mean HDL-cholesterol concentration in the CTX plasmas was 14.5 ± 3.2 mg/dl, about one-third the normal value. The low HDL-cholesterol reflects a low concentration and an abnormal lipid composition of the plasma HDL. Relative to normal HDL, the cholesteryl esters are low, free cholesterol and phospholipids essentially normal, and triglycerides increased. The ratio of apoprotein (apo) to total cholesterol in the HDL of CTX was two to three times greater than normal. In the CTX HDL, the ratio of apoAI to apoAII was high, the proportion of apoC low, and a normally minor form of apoAI increased relative to other forms. The HDL in electron micrographs appeared normal morphologically and in particle size. The abnormalities in lipoprotein distribution profile and composition of the plasma HDL result from metabolic defects that are not understood but may be linked to the genetic defect in bile acid synthesis in CTX. As a consequence, it is probable that the normal functions of the HDL, possibly including modulation of LDL-cholesterol uptake and the removal of excess cholesterol from peripheral tissues, are perturbed significantly in this disease.

INTRODUCTION

Cerebrotendinous xanthomatosis (CTX)[1] is a rare, inherited lipid-storage disease characterized clinically by progressive neurologic dysfunction, premature atherosclerosis, and cataracts (1). Large deposits of cholesterol and cholesterol are present in virtually every tissue, particularly the Achilles tendons, brain, and lungs. Cholestanol, the 5-α-dihydro derivative of cholesterol, is enriched relative to cholesterol in all tissues (1–3). In contrast to type IIa hyperlipoproteinemia in which tendon xanthomas are common and plasma cholesterol levels exceed 300 mg/dl, plasma cholesterol concentrations are low normal in CTX patients (1–3). We have recently presented evidence that the major biochemical defect in CTX is abnormal bile acid synthesis, specifically, impaired oxidation of the cholesterol side chain in the formation of cholic acid (4, 5).

The transport system for sterol influx and efflux of tissues comprise the plasma lipoproteins, including low density lipoproteins (LDL), levels of which are positively correlated with atherosclerosis, and high density lipoproteins, levels of which are inversely correlated (6). The HDL may have major roles in inhibiting uptake and accumulation of LDL-cholesterol (7–9) and in transporting cholesterol from peripheral tissues to liver for excretion (10). Hence, we examined the plasma lipoprotein profiles and the properties of the

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[1] *Abbreviations used in this paper*: CTX, cerebrotendinous xanthomatosis; HDL, high density lipoproteins; LDL low density lipoproteins; IDL, intermediate density lipoproteins; VLDL, very low density lipoproteins; SDS, sodium dodecyl sulfate.

HDL of CTX patients for abnormalities that might promote excessive deposition of sterols in their peripheral tissues.

METHODS

Clinical characteristics of CTX patients. Clinical descriptions of the nine CTX patients (five women and four men) are given in Table I. None was on medication or other treatment. Patients J.C., E.E., and E.S. were the subjects of previous metabolic studies (1, 4, 5). The patients were compared with a control group of 10 normolipidemic subjects (3 women and 7 men) of an average age of 50 yr (range: 20–65). Blood samples were taken at ~8:00 a.m. after a fast of 12–14 h, and EDTA was added as an anticoagulant. Plasma triglyceride levels were determined by a Technicon Auto Analyzer (Technicon Instruments Corp. Tarrytown, N. Y. [11]). Plasma cholesterol and HDL cholesterol (after removal of lipoproteins of lower density by preparative centrifugation) were determined colorimetrically by the cholesterol oxidase method of Allain et al. (12). Plasma cholestanol was measured in a separate aliquot according to the gas-liquid chromatographic method of Salen (1).

Isolation of lipoproteins. Plasma lipoproteins were isolated by preparative ultracentrifugation. For analytical ultracentrifugal analysis, the low and high density lipoproteins were isolated as described by Ewing et al. (13). The isolated HDL (d = 1.063–1.21 g/ml) were subfractionated into HDL$_2$ (d = 1.063–1.125 g/ml) and HDL$_3$ (d = 1.125–1.21 g/ml) (14) or into narrower density ranges by density gradient centrifugation. In the latter, 2 ml undialyzed HDL was layered atop 0.5 ml 50% sucrose (wt:wt) in 9/16 × 3 1/2-in. tubes followed by 7 ml of a d = 1.110 solution (0.196 M NaCl, 1.390 M NaBr) and finally 3 ml of a d = 1.080 solution (0.196 M NaCl, 0.973 M NaBr). The tubes were centrifuged in a Beckman SW 41 rotor (Beckman Instruments, Inc., Fullerton,

Calif.) for 65 h at 40,000 rpm and 16°C. Twelve 1-ml fractions were pipetted sequentially down the tube. HDL$_2$ (d = 1.08–1.125 g/ml) and HDL$_3$ (d = 1.125–1.21 g/ml) were also isolated after density adjustment with NaCl and D$_2$O as described by Shore (15). All solutions contained 0.1 mg EDTA/ml.

The HDL were dialyzed against a cold solution containing 0.1 or 0.15 M NaCl, 0.0008 M EDTA, and in some cases Tris buffer at pH 7.2 before analyzing their lipid and protein moieties. Protein concentration was determined by the colorimetric method of Lowry et al. (16). Phospholipids were estimated from the phosphorus content, determined by a modified colorimetric method of Chen et al. (17), in which hydrolysis was performed as described by Nelson (18). Free and total cholesterol were quantitated by the method of Allain et al. (12), and triglyceride, by Worthington Biochemical Corp. Freehold, N. J. enzymatic method.

Analytical ultracentrifugation of serum lipoproteins. The concentrations of the serum lipoproteins and their distribution as a function of flotation rate (S$_f^0$ or F$_{1.20}^0$) were determined by computerized analysis of data from the schlieren patterns of analytical ultracentrifuge runs at 52,640 rpm of the isolated LDL (LDL, IDL, and VLDL) in a baseline solution of density 1.063 g/ml and the isolated HDL in a solution of density 1.20 g/ml (13, 19). The flotation rates, determined by an "η F^0 vs. ρ" technique (19), are expressed in Svedbergs (10^{-13} cm·S^{-1}·dyn^{-1}·g^{-1}) and are corrected for concentration dependence and to standard conditions (T = 26°C). The apparently bimodal schlieren pattern for HDL is analyzed for concentrations of subfractions, the HDL$_2$ and HDL$_3$ (19). The HDL$_2$ (F$_{1.20}^0$ = 3.5–9.0) and HDL$_3$ (F$_{1.20}^0$ = 0–3.5) correspond closely but not exactly to the subfractions defined, as is generally the case, in terms of the density intervals used in their isolation by preparative ultracentrifugation (20).

Electron microscopy of isolated HDL$_2$ and HDL$_3$. After dialysis against 0.13 M ammonium acetate buffer, pH 7.4, containing 345 μM EDTA and 124 μM Merthiolate, the lipoproteins were negatively stained with 2% sodium phosphotungstate, pH 7.4 (21). Diameters of 100–200 free standing particles were obtained from each fraction by means of a computerized program using a sonic digitizer (Graf-Pen of Science Accessories Corporation, Southport, Conn.) which records X-Y coordinates and translates them into particle diameters. The precision of the method is 5–10%. Values from electron microscopy for particle diameters are ~10% higher than those calculated from analytical ultracentrifugal data and very similar to those from gradient gel electrophoresis (20). In the latter two methods, the lipoproteins were assumed to be Stokes' spheres. The electron microscopic method gives information on particle shape, morphology, and heterogeneity in both size and shape as well as the average particle size.

Lipid analysis. Total lipids of isolated HDL$_3$ were determined gravimetrically after extraction into 3:2 and 3:1 (vol/vol) mixtures of chloroform and methanol. Phospholipid content of the lipids was estimated from the phosphorus content (17). The major classes of lipids (phospholipids, cholesterol, triglycerides, and cholesteryl esters) were separated and quantitated by thin-layer chromatography combined with flame ionization detection (22). The lipids were also separated as described by Nelson (18) by one-dimensional, thin-layer chromatography on a Pyrex plate coated with silica gel. The solvent was petroleum ether/diethyl ether/acetic acid (85:15:2 vol/vol). Cholesterol and the individual cholesteryl esters were quantitated by a high-pressure liquid-chromatographic procedure similar to that of Duncan et al. (23). A Waters μBondapak C$_{18}$ column (0.39

TABLE I
Data on CTX Patients

Patient	Sex	Age	Clinical symptoms
		yr	
W.H.	M	59	Dementia, spinal cord paresis, Achilles tendon xanthomas, xanthelasma
J.C.	M	44	Achilles tendon xanthomas, tuberous xanthomas of forearm
G.A.	F	60	Dementia, cataracts, spinal cord paresis, Achilles tendon xanthomas, tuberous xanthomas dorsum of hand
V.R.	F	60	Achilles tendon xanthomas, spinal cord paresis
E.E.	F	52	Achilles tendon xanthomas, spinal cord paresis
E.S.	F	54	Achilles tendon xanthomas, spinal cord paresis
T.G.	M	21	Achilles tendon xanthomas
N.P.	F	23	Cataracts, Achilles tendon xanthomas
L.R.	M	28	Achilles tendon xanthomas

× 30 cm) was used isocratically with a solvent of isopropyl alcohol/acetonitrile (25:75 vol/vol) at a flow rate of 1 ml/min.

Amino acid analysis. The apolipoproteins (~50–150 μg) were hydrolyzed *in vacuo* for 40 h at 110°C with 0.25 ml of 4 N methanesulfonic acid containing 0.2% 3-(2-aminoethyl) indole. Amino acids were analyzed by the procedure of Liu and Chang (24).

Polyacrylamide gel electrophoresis. Electrophoresis of delipidated HDL apolipoproteins (30 μg protein/sample) was carried out in the presence of sodium dodecyl sulfate (SDS) in 4% and 10% gels (25). Standards of pure apoAI (15, 18, and 21 μg) and apoAII (6, 8, and 10 μg) were run in 10% gels. After electrophoresis, the gels were fixed, stained, and then destained as described previously. (25). The gels were photographed with Polaroid film (Polaroid Corp., Cambridge, Mass.) and scanned in a Gilford recording spectrophotometer (Gilford Instrument Laboratories Inc., Oberlin, Ohio), which was equipped with a linear transport system and attached to a computing integrator. This electrophoretic system separated apoB, apoD, apoE, apoAI, apoAII, apoCIII, apoCII, and apoCI and gave information on the relative amounts of these apolipoproteins in CTX HDL as compared with normal HDL.

Polyacrylamide gel isoelectric focusing. The apolipoproteins of HDL (100 μg/sample) in 0.05 M NaCl were separated in the pH ranges 4–6 and 4–8 and stained with Coomassie Blue R-250 (25). Gels were photographed and scanned by a densitometer as described above for SDS gels. This method does not detect apoB, but it resolves the variant forms of the other apolipoproteins.

RESULTS

Plasma lipids and HDL-cholesterol. Plasma triglyceride, cholesterol, cholestanol, and HDL-cholesterol concentrations are listed in Table II. All these CTX subjects had low to normal levels of plasma cholesterol and elevated cholestanol concentrations (2.9±0.9 vs. 0.6±0.2 mg/dl) as compared with 10 normolipidemic subjects who served as controls. The relative abundance of cholestanol in the HDL sterols was comparable to that in the plasma sterols (26). The proportions of

cholestanol in esterified form in plasma and isolated HDL are similar to the proportions of esterified cholesterol to total cholesterol in normal and CTX subjects, as expected from previous measurements of the rates of esterification of both sterols in two of our CTX patients and a normal subject (26). The combination of the clinical findings (Table I) with the plasma-sterol measurements established the diagnosis of cerebrotendinous xanthomatosis (CTX). In these subjects, plasma HDL-cholesterol levels were markedly lower than in the control subjects (14.5±3.2 mg/dl vs. 48.0±9.0 mg/dl). Furthermore, the ratio of total plasma cholesterol to HDL-cholesterol was substantially higher in the CTX subjects than in the control groups (11.4 vs. 4.8). Thus, the HDL-cholesterol is reduced out of proportion to the total plasma cholesterol, which is also low in this disease. Fasting triglyceride levels in the CTX and control subjects were similar, although elevated values were detected in one CTX subject (W.H.).

The total protein and cholesterol concentrations of the isolated HDL₂ and HDL₃ preparations are given in Table III. In the HDL₂ and HDL₃ of CTX patients, the protein to cholesterol ratios were two to three times higher than in normal HDL. Thus, the low concentration of HDL-cholesterol in the plasma of CTX patients (Table II) results in part from the abnormal composition of the HDL, indicated also by the results of lipid analyses described in a later section.

Plasma lipoprotein profiles. Plasma lipoproteins of seven CTX individuals (three males and four females) were analyzed by analytical ultracentrifugation. Samples from two of these (W.H., male and G.A., female) were examined twice at intervals of 1.9 and 2.5 yr, respectively. Fig. 1 compares the lipoprotein profile of

TABLE II
Plasma Triglyceride, Cholesterol, Cholestanol, and HDL-Cholesterol Levels in CTX Patients and Normal Subjects*

Group	Triglyceride	Cholesterol	Cholestanol	HDL-Cholesterol	Total cholesterol / HDL cholesterol
Normolipidemic subjects‡	115±33	230±25	0.6±0.015	48.0±9.0	4.8
Patients					
W.H.	273	149	2.1	15.9	9.4
J.C.	115	151	2.1	12.6	12.0
G.A.	122	201	3.2	15.0	13.4
V.R.	105	185	4.4	16.1	11.5
T.G.	61	149	3.0	15.7	9.5
N.P.	81	197	2.0	9.9	19.9
E.E.	116	136	3.0	11.0	12.4
E.S.	111	154	3.7	20.0	7.7
Mean±SD	123±64	165±25	2.9±0.9	14.5±3.2	11.4

* Concentrations in milligrams per deciliter.
‡ Mean±SD of seven male and three female subjects.

TABLE III
Protein and Cholesterol Concentrations* in HDL Fractions from CTX Patients and Normolipidemic Subjects

	HDL_2			HDL_3		
	Protein (P)	Cholesterol (C)	P/C	Protein (P)	Cholesterol (C)	P/C
Normolipidemic subjects	49.4±4.8‡	17.6±2.0‡	2.8	65.6±4.3‡	24.8±1.2‡	2.6
Patients						
W.H.	31.9	6.0	5.3	59.6	9.9	6.0
J.C.	59.0	7.1	8.3	89.4	5.5	16.3
G.A.	36.0	7.9	4.6	42.4	7.1	6.0
V.R.	41.4	7.3	5.7	58.6	8.8	6.7
T.G.	39.8	7.3	5.5	51.2	8.4	6.1
N.P.	36.8	4.0	9.2	55.2	5.9	9.4
Mean±SD	40.8±9.5	6.6±1.4	6.2	59.4±16.0	7.6±1.7	7.8

* Concentrations in milligrams per deciliter.
‡ Mean±SD of seven male and three female subjects.
P/C in the normolipidemic female and male subjects was identical. Cholestanol accompanied cholesterol in the same proportion in each subfraction as in total plasma and HDL.

a normal, age-matched male with that of W.H. at the time the initial sample of Table II was taken and almost 2 yr later, before initiation of treatment. At the latter time, W.H.'s plasma triglycerides and cholesterol were 125 mg/dl and 104 mg/dl, respectively, and his disease had progressed. The plasma levels of LDL and HDL, which increase gradually with age in normal subjects

FIGURE 1 Serum lipoprotein profiles of CTX patient W. H. initially (— -) and almost 2 yr later (——) and a normal, age-matched male (– – –) from analytical ultracentrifugal analysis. Patterns are computer-reconstructed diagrams of the schlieren patterns indicating the distribution of lipoproteins as a function of flotation rate (S_f^0 or $F_{1.20}^0$). Peak area is proportional to concentration.

(27), declined significantly in W.H. (Fig. 1) and to a lesser extent in G.A. over the period of study. However, the plasma lipoprotein levels of L.R., a relatively young patient with a plasma cholestanol level of 1.9 mg/dl and no apparent neurological disease, were also below those of age- and sex-matched controls and close to the average for male CTX patients. Table IV compares the average plasma lipoprotein concentrations determined by analytical ultracentrifugation for the male and female CTX patients and normal age- and sex-matched controls. Our control values are comparable to those obtained by an analytical ultracentrifugation study of 160 normal adults, 20 from each decade and sex (27). Values in Table IV from the latter study of normal subjects in different age groups were averaged to match the age distribution of the CTX patients. The normal HDL_2 and HDL_3 levels in milligrams per deciliter (not reported for the Modesto populations (27)) of our controls were similar to those reported previously (28) for 35–50-yr-old subjects: 172±81 and 264±59, respectively, for 16 normal females and 53±48 and 222±31, respectively, for 16 normal males. The LDL levels of the CTX patients were considerably below average except in G.A. The levels of HDL_2 and total HDL were below average in two of the male CTX patients and normal (79 and 287 mg/dl, respectively) in J.C. In the female patients, the HDL_2 levels were low (23–84 mg/dl vs. 195 mg/dl for normal) in all and the HDL_3 levels were low or low normal in three and high (312 mg/dl) in E.S. Since the peak $F_{1.20}^0$ rates (1.7–1.8) of the CTX HDL_3 were in the normal range and the lipoprotein size and shape appeared normal (see below), analysis of the schlieren peak for HDL_2 and HDL_3 seems valid. The ratio of LDL to HDL (wt:wt) varied considerably in both male pa-
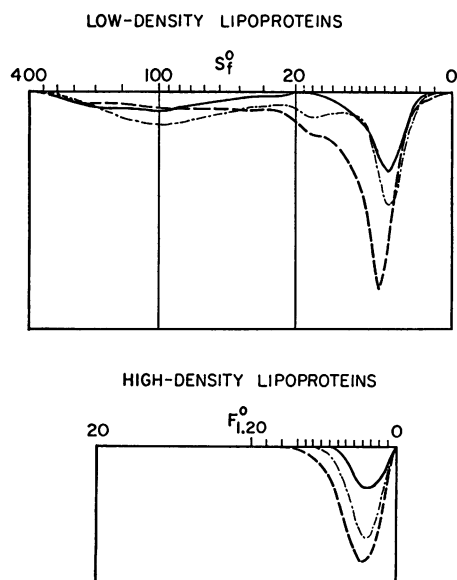
TABLE IV

Plasma Lipoprotein Concentrations* of CTX Patients and Normal Controls

| | Lipoprotein fraction | | | | | | | | |
| | VLDL | | | LDL | | | HDL | | |
Subjects	Sf 100–400	Sf 20–100	Sf 20–400	Sf 12–20	Sf 0–12	Sf 0–20	HDL₂	HDL₃	HDL
Male									
CTX (n = 3)	62±36	96±43	158±73	18±18	210±32	228±48	29±34	160±60	187±77
Normal	59	117	176	77	412	489	36	236	272
Normal‡			102±68			402±85			266±73
Female									
CTX (n = 4)	33±15	84±22	117±36	23±16	206±39	229±44	66±30	206±55	272±68
Normal		7	7	43	408	451	195	237	432
Normal‡			114±110			398±100			408±96

* Concentrations in milligrams per deciliter±SD.
‡ From Lindgren et al. (27).

tients (0.9–2.2 vs. 1.5–1.8 for the normal averages) and female patients (0.5–1.5 vs. 0.7–1.0 for normal), although the ratio of LDL-cholesterol to HDL-cholesterol was well above normal in all CTX subjects (Table II). The HDL concentrations are considerably higher than one might assume from the plasma HDL-cholesterol values (Table II). The latter values reflect, in addition to low HDL concentrations in CTX plasma (Table IV), an abnormal composition (Table III).

Morphology and particle size of CTX HDL. The HDL₂ and HDL₃ fractions from CTX patients (Fig. 2) were morphologically indistinguishable from those of normal subjects and consisted primarily of spherical particles. In the HDL₂, 1–2% of the total particles are larger (22–24 nm in Diam) than HDL and probably represent contaminating LDL or HDLc. The large particles frequently are seen as a minor species in normal HDL₂ isolated by conventional preparative ultracentrifugation. The diameter of HDL₂ from a representative patient W.H. was 11.4±1.3 nm, which is very similar to that (11.5±1.8 nm) from the age-matched, normal male. The HDL₃ diameters in both these subjects were also similar, 9.7±1.0 nm for the former and 9.2±1.6 nm for the latter. The abnormal composition of HDL in CTX patients does not seem to perturb the lipoprotein morphology, at least within the limits of detection by electron microscopy.

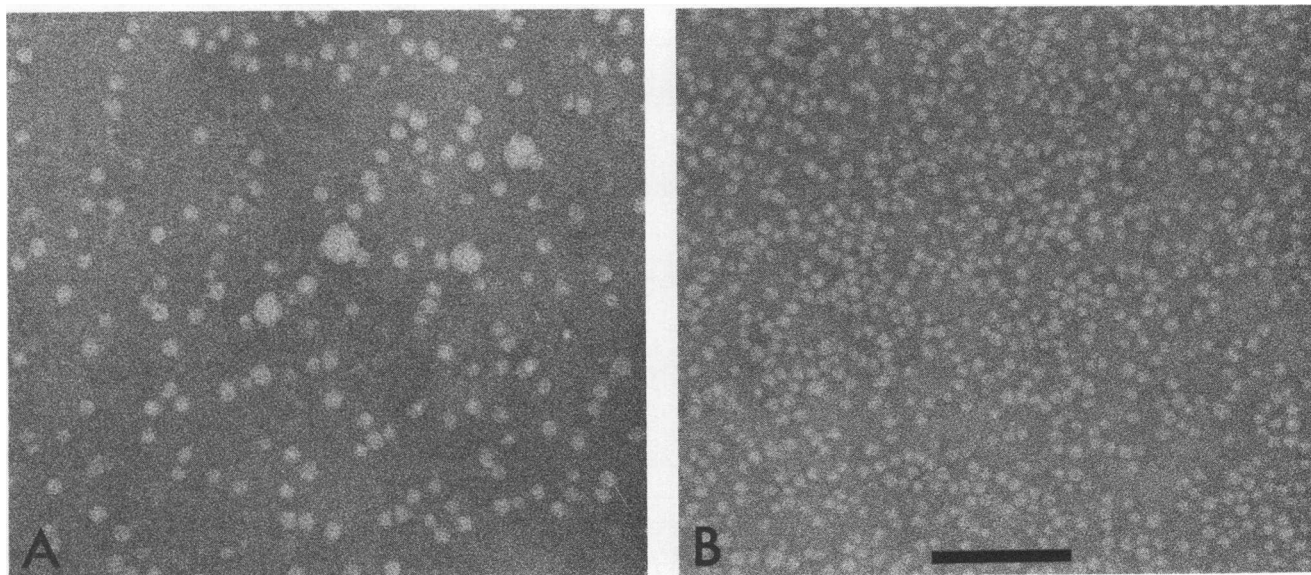*Lipid composition of the HDL of CTX patients.*

FIGURE 2   Electron micrographs of negatively stained HDL₂ (A) and HDL₃ (B) from CTX patient W.H. The bar marker represents 100 nm and applies to both micrographs.

The HDL_2 and HDL_3 of 4 CTX patients (1 female and 3 males) and 10 normal subjects were analyzed quantitatively by enzymatic assays and thin-layer chromatography for unesterified cholesterol, esterified cholesterol, and triglycerides and colorimetrically for phospholipid and protein. The extracted HDL_3 lipids of one CTX patient (W.H.) and one normal control were also examined quantitatively by the other procedures described in Methods for unesterified cholesterol, cholesteryl esters, total triglycerides, and phospholipids. The extracted lipid moiety comprised a normal fraction, i.e., 45 to 48% by weight, of CTX HDL_3. The lipid compositions are given in Table V. The lipid data confirm the data of Table III, which shows a high ratio of protein to cholesterol in the HDL of all CTX subjects, and indicate a specific deficit in esterified cholesterol. The percentage of total phospholipids was essentially normal and the amount of triglycerides was about three times normal. Analysis by high-pressure liquid chromatography of the HDL_3 lipids confirmed the results on the content of free cholesterol and cholesteryl esters and showed that cholesteryl linoleate accounts for most of the deficit in cholesteryl esters (Table V). Results of our lipid analyses of normal HDL_3 are similar to values found in the literature (Table V). Since the size and density of CTX HDL are near normal, the deficit in cholesteryl esters must be compensated nearly quantitatively by triglycerides, as indicated by the data on percent composition of the lipid moiety. Since the molecular weights and partial

specific volumes (1.01 ml/g) of cholesteryl esters are somewhat lower than those of triglycerides ($\bar{v} = 1.09$ ml/g), the replacement probably is stoichiometrically <1 mol triglyceride for 1 mol cholesteryl ester.

Apolipoproteins of the HDL_2 and HDL_3. The apolipoproteins of six CTX patients were analyzed by SDS-polyacrylamide gel electrophoresis and polyacrylamide gel isoelectric focusing. During the period of study at least 20 normal HDL were examined. The SDS-gel (10% acrylamide) patterns of CTX apo-HDL_2 (Fig. 3) and apoHDL_3 showed little if any apoD and apoE, a higher than normal ratio of apoAI to apoAII, and generally low apoC. ApoD and apoE were concentrated mainly in the $d = 1.18$ g/ml subfraction, with lesser amounts in the adjacent fractions of $d = 1.15$ and 1.21 g/ml, of all subjects after the HDL of patients G.A., W.H., and E.S. and 12 normal subjects were separated by density gradient ultracentrifugation into 12 subfractions from $d = 1.067$ to 1.21 g/ml. There was no indication of abnormal amounts of apoD and apoE in the CTX HDL, but the possibility of preferential dissociation of apolipoproteins from the abnormal HDL during isolation from plasma was not investigated. For CTX HDL_3, the ratio of peak areas of apoAI to apoAII in SDS gels were in the range 4.5–6.1, which according to the standard apoAI and apoAII gels corresponded to weight ratios of 3.6–4.8. By comparison, the normal ratio was 1.9 to 2.9. Since the concentration of HDL is also low (Fig. 1 and Table IV), the plasma HDL reservoir of apoC available for transfer to

TABLE V

Composition of the Lipid Moieties of Normal and CTX HDL_3

Component	CTX	Normal	Literature
Phospholipid*	45.4±1.9	49.7±1.3	40.9–51.1 (29–31)
Triglyceride*	13.8±1.8	2.2±0.8	4.5–6.5 (29–31)
Cholesterol			
Free*	5.8±0.9	5.9±0.8	4.6, 5.6 (29)
Esterified*	15.9±1.1	22.0±1.2	18.7, 20.1 (29)
Cholesteryl esters			
Total*	26.9±1.8	37.2±1.5	
16:0‡	16.6	11.0	10.7–11.6 (31, 32)
18:1‡	25.0	17.0	18.9–23.6 (31, 32)
18:2‡	40.0	55.9	52.2–57.1 (31–33)
20:4‡	11.6	9.0	5.5–9.9 (31–33)
16:1‡			1.7–3.2 (31, 32)
18:0‡			0.9, 1.3 (32)
20:0‡			0.5, 0.8 (32)

* Percent by weight of total lipids. Values are the means ±SD for 4 CTX patients and 10 normal subjects.

‡ Percent by weight of total cholesteryl esters. Values are for CTX patient W.H. and a normal male. Literature values are for HDL.
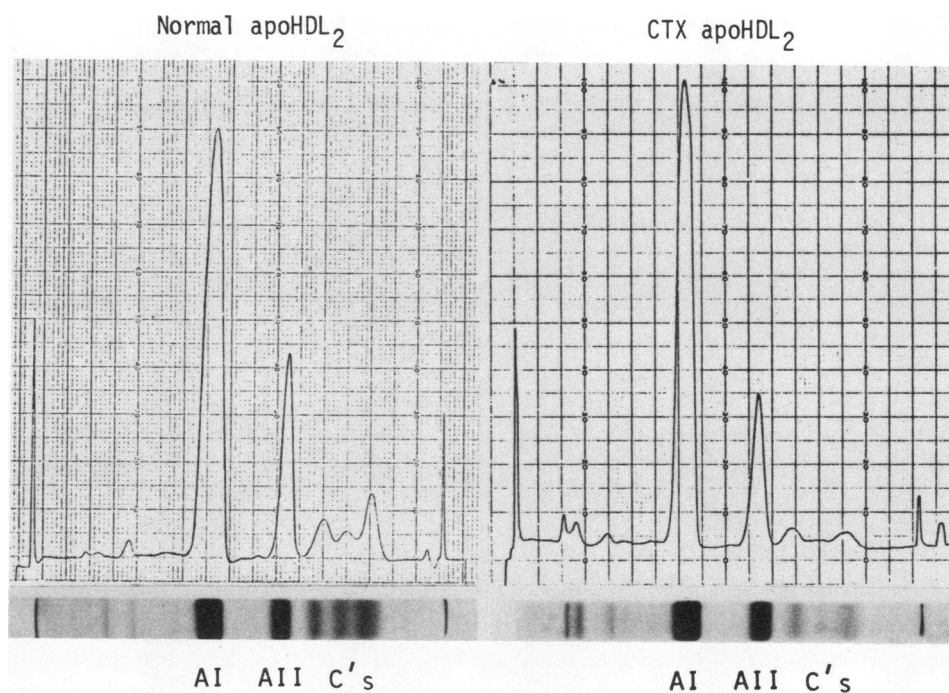
FIGURE 3   Densitometric scans of SDS-polyacrylamide gel electrophoretic patterns of apoHDL$_2$ of normal and CTX subjects.

nascent VLDL is low. However, the VLDL of CTX patients was not deficient in apoE or apoC as determined from the electrophoretic patterns (not shown). Electrophoresis of CTX apoHDL$_2$ in 4% SDS gels showed no detectable apoB (pattern not shown).

Isoelectric focusing patterns of CTX apoHDL$_2$ and apoHDL$_3$ confirmed the observations from SDS-gel electrophoresis and indicated that a minor form of apoAI (AI$_x$ in Fig. 4) was relatively enriched in all the patients' HDL as compared to normal, fasting HDL. ApoAI$_x$ accounted for ~14% of the total apoAI in CTX apoHDL$_3$ and 9% of the total apoHDL$_3$, according to the densitometric scans (Table VI). In normal HDL, apoAI$_x$ ranges from an undetectable amount to about 3% of the total protein. The densitometric scans of the isoelectric focusing gel patterns also indicated that the average ratio of total apoAI (AI$_x$ + AI) to apoAII in CTX apoHDL$_2$ and apoHDL$_3$, respectively, was 4.0 and 3.6. In samples from the control group in this study, the corresponding ratios of the peak areas for total apoAI and apoAII were 3.1 and 2.7, respectively.

ApoAI$_x$ was identified as a form of apoAI by the amino acid composition of the proteins eluted from the gels after isoelectric focusing of 300 μg apo HDL/gel (Table VII). These analyses also indicated that the bands containing the major forms of apoAI were not significantly contaminated with other apolipoproteins, e.g., apoE, since the composition corresponded closely to that expected for pure apoAI.

DISCUSSION

Results of this investigation indicate abnormal HDL composition to be a general feature of CTX and that abnormal plasma lipoprotein profiles with low concentrations of LDL and/or HDL are frequent. Particle size and density of the CTX HDL were normal. Thus the total number of HDL particles in CTX plasma, estimated from the analytical ultracentrifugation data (Table IV) are somewhat reduced (~30% on the average in males and less in females, in whom the HDL$_2$ are disproportionately low relative to HDL$_3$), but not to the extent suggested by the very low HDL-cholesterol (Table II). These data indicate that CTX HDL transport much less cholesterol per lipoprotein particle than normal.

The possible relationship of these abnormalities to the accumulation of cholesterol and cholestanol in extrahepatic tissues of CTX subjects is of some interest because lipoproteins are the transport system for sterol influx and efflux of cells. Cholestanol seems not to be distinguishable from cholesterol in transport, except in the CTX subject's brain, xanthomas, and bile, where cholestanol can account for 10–40% of total sterols (34, 35). In most other cases, red blood cells, and plasma, the proportions of cholestanol to cholesterol (about 1–3%) are very similar (34). Excess deposition (influx) and/or reduced capacity for removal (efflux) could cause the accumulation in tissue. Increased influx implies
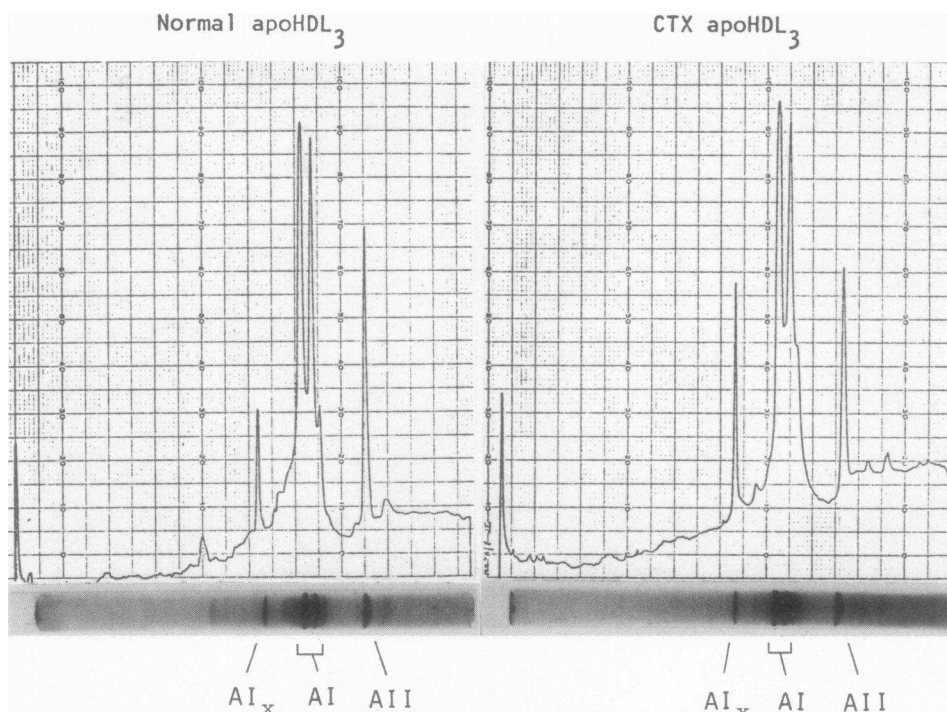
FIGURE 4 Densitometric scans of polyacrylamide gel isoelectric focusing patterns of apoHDL₃ of normal and CTX subjects. The pH range is 4 (at the extreme right of each pattern) to 8.

elevated plasma LDL (not low LDL as in CTX) and/or increased affinity for tissues. Decreased efflux and/or increased influx could be directly related to the HDL, if

TABLE VI
Apolipoprotein Composition of HDL₂ and HDL₃ from CTX Patients and Control Subjects*

Lipoprotein	Total apolipoprotein			
	AIₓ	AI	AII	Minor components‡
			%	
HDL₂				
Controls (3)	2±1	66±4	22±2	10±2
Patients (6)	7±1.5	64±5	18±2	10±2
HDL₃				
Controls (3)	3±1	65±5	25±2	7±1
Patients (6)	9±2	62±7	20±2	9±2

* After polyacrylamide-gel isoelectric focusing of HDL apolipoproteins, gels were scanned densitometrically and the area of each band was expressed as a percentage of total area of the apolipoproteins. Numbers are mean values±SD.
‡ Includes apoC-II, apoC-III, apoD, apoE, and unidentified bands.
Numbers in parentheses are numbers of subjects in the group. Only 30% of the control subjects had a measurable amount of an apolipoprotein with the same mobility as apoAIₓ on isoelectric focusing. These individuals (2 males and 1 female) were selected for this table. The patient group included 3 males and 3 females.

major functions of the HDL are the transport of cholesterol from peripheral tissues to the liver (10) and/or the control of LDL-sterol uptake by tissues by modulating LDL binding to membrane receptor sites and transendothelial transport (7–9).

The capacity of the HDL to accept sterols could be diminished by the abnormal composition of CTX HDL or, conversely, defective transfer of cholesterol across the cell membrane could result in abnormal HDL composition. The high ratio of cholesterol to cholesteryl esters, the low content of unesterified cholesterol, and the altered proportion of apolipoproteins AI and AII may diminish the capacity of the HDL of CTX patients to accept unesterified sterols. Esterification of cholesterol and/or the transfer of the lipids among lipoproteins also may be defective. Net transfer of unesterified cholesterol from erythrocytes (and very possibly other cells) to plasma depends on the free cholesterol to phospholipid ratios in cells and plasma lipoproteins and the transfer to plasma is driven by lecithin:cholesterol acyltransferase (36), which converts cholesterol of plasma HDL to cholesteryl esters. Furthermore, the phospholipid complexes of apoAI are less effective than the complexes of apoAII, apoCI, or apoCIII in releasing cellular sterol from Landschütz ascites cells (37). The abnormalities in composition of the HDL of CTX subjects, particularly the relative abundance of apoAI and free cholesterol, should favor the action of lecithin:cholesterol acyl-

TABLE VII
Amino Acid Composition of Apolipoproteins Isolated* from HDL of CTX Patients

	AI_x	AI_1	AI_2	AI_3	AII
			mol/1,000 mol		
Lys	78	79	78	80	120
His	19	19	19	18	0
Arg	62	63	64	58	0
Asp	87	92	93	90	40
Thr	38	39	40	39	78
Ser	64	62	59	63	80
Glu	189	192	190	188	208
Pro	38	40	40	41	54
Gly	44	39	41	43	43
Ala	75	76	78	77	68
½Cys	0	0	0	0	14
Val	50	48	51	52	75
Met	13	12	12	12	13
Ile	0	0	0	0	14
Leu	160	159	152	158	104
Tyr	26	25	27	27	47
Phe	23	21	24	24	48

* By preparative isolectric focusing.

transferase (38). However, the variant apoAI$_x$ may not be an effective cofactor for the reaction and it might even be inhibitory. ApoAI$_x$ (not characterized previously) seems to be a normal but minor form of apoAI that is considerably enriched in CTX HDL. The effect of increased cholestanol on the properties and metabolism of CTX HDL is not known. Perturbations are possible even though cholestanol accounts for only a small fraction of the total sterols.

Abnormalities in HDL concentration and apolipoprotein composition, particularly the relative abundance of apoAI$_x$ may derive in part from the known CTX abnormalities in synthesis and composition of bile acids (5) and morphology of the liver cells (39). Liver functions including synthesis and secretion of nascent lipoproteins may be coupled to the flux of the bile acid pool though the liver, which is deficient in CTX.

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