Properties of the Plasma Very Low and Low Density Lipoproteins in Tangier Disease

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ABSTRACT The absence of normal high density lipoproteins (HDL) in Tangier disease is well established, but the properties of very low density lipoproteins (VLDL) and low density lipoproteins (LDL) in this disorder have not been well defined. The profiles obtained by analytic ultracentrifugation and the chemical composition, morphology, and electrophoretic mobility of Tangier and normal VLDL and LDL were compared. Apolipoproteins were fractionated by gel chromatography and characterized by amino acid analysis, polyacrylamide-gel electrophoresis, and immunochemical reactivity.

Concentrations of low density lipoproteins of $S_f°$ 0–12 were reduced in three of six Tangier plasmas studied by analytic ultracentrifugation. Accumulation of intermediate density lipoproteins ($S_f°$ 12–20) was not observed. Two subjects with hypertriglyceridemia had normal VLDL ($S_f°$ 20–400) levels, suggesting that abnormalities of chylomicron metabolism probably account for the hypertriglyceridemia frequently observed in this disorder.

Tangier VLDL migrate more slowly than normal VLDL on paper electrophoresis, yet their morphology, gross chemical composition, and qualitative apolipoprotein content are similar. Quantitative abnormalities in C-apolipoproteins, however, were observed in Tangier VLDL. When patients were consuming unrestricted diets, C apoproteins accounted for 19–49% of the protein in lipoproteins of $d < 1.006$ g/ml. Ingestion of low-fat, high-carbohydrate diets reduced the VLDL-C-apoprotein content in all Tangier patients (mean = 17% of VLDL protein vs. 43% in controls). These findings suggested that a major proportion of the C apoproteins in Tangier plasma is associated with chylomicrons or their remnants, perhaps because the C-apoprotein reservoir normally provided by HDL is absent. This secondary mechanism for C-apoprotein conservation is lost when dietary fat is withdrawn.

LDL-2 ($1.035 < d < 1.063$) from Tangier and control plasma had identical electrophoretic mobilities. Tangier LDL-2 had slightly smaller median diameters (210–225 Å vs. 230–240 Å in controls) and a quite different composition than normal LDL-2. Triglyceride accounted for a mean of 29% of Tangier LDL-2 mass (control = 6%) and the cholesteryl ester content was reduced by about 50%. Thus, HDL may be required for the generation of chemically normal LDL. Alternatively, the fundamental defect in Tangier disease may involve all lipoprotein classes.

INTRODUCTION

Chylomicrons and very low density lipoproteins (VLDL)[1] transport triglycerides from the intestine and liver, respectively, to peripheral sites of utilization and storage. Low density lipoproteins (LDL) in man are believed to arise primarily or exclusively from VLDL catabolism. In contrast to the chylomicrons and the VLDL-LDL system, the high density lipoproteins (HDL) do not transport significant amounts of triglyceride. Indirect evidence, however, suggests that HDL may modulate the metabolism of the triglyceride-rich lipoproteins.

The major HDL apolipoproteins, apoA-I and

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[1] *Abbreviations used in this paper:* HDL, high density lipoproteins of $1.063 < d < 1.21$ g/ml; LCAT, lecithin:cholesterol acyltransferase; LDL, low density lipoproteins; LDL-1, low density lipoproteins of $1.006 < d < 1.035$ g/ml; LDL-2, low density lipoproteins of $1.035 < d < 1.063$ g/ml; VLDL, very low density lipoproteins of $d < 1.006$ g/ml; the prefix "apo-" designates the lipid-free protein(s) of a particular lipoprotein class.

apoA-II, have been identified in human thoracic duct chylomicrons (1, 2) and were found to account for about 15% of the total chylomicron protein (1). Presumably, these A-apoproteins arise in the gut, since the rat homologue of human apoA-I is present in rat intestinal lymph VLDL (3, 4) and much of it appears to be synthesized by the intestine (5). The intestine, however, may be incapable of C-apoprotein synthesis (5), and net transfer of C apoproteins from plasma HDL to chylomicrons has been demonstrated during alimentary lipemia in man (6). Much of the C-apoprotein complement of VLDL also appears to derive from circulating HDL, since the amount of newly synthesized C apoproteins in hepatic VLDL is relatively small (5). Hydrolysis of chylomicron and VLDL triglycerides likely requires adequate quantities of apoC-II, a potent activator of lipoprotein lipase (7–9); other apolipoproteins, at least in vitro, can influence lipolysis (10, 11).

HDL may additionally affect chylomicron and VLDL metabolism by accepting phospholipids removed during degradation of the triglyceride-rich lipoproteins (12) and by nonenzymatic exchange of cholesteryl esters for triglyceride (13). HDL are the preferred substrate for lecithin:cholesterol acyltransferase (LCAT) (14, 15), which catalyzes the production of most of the cholesteryl esters of human plasma lipoproteins (16), and apoA-I (17) and apoC-I (18) can specifically activate the LCAT enzyme.

The virtual absence of normal plasma HDL in Tangier disease (19) afforded a unique opportunity to examine the possible effects of HDL on the lower density classes of lipoproteins. Earlier observations suggested that abnormalities of chylomicron, VLDL, and LDL metabolism were present in this disorder. Delayed clearance of chylomicrons was observed more than a decade ago (20), and chylomicron remnants appear to accumulate abnormally in the plasma in Tangier disease (21). A distinct pre-beta band typical of VLDL has usually been absent on paper electrophoresis of whole Tangier plasma. Kocen and coworkers (22), moreover, found a low cholesterol: triglyceride ratio in their patient's LDL, an observation confirmed by Greten et al. (23) in another subject with Tangier disease. In the studies reported here, the lipid and apoprotein compositions of Tangier VLDL and LDL are compared with those of control subjects and striking deviations from the normal are defined. While it may be postulated that these abnormalities can be attributed to the HDL deficiency, direct proof of a causal relationship requires additional data.

METHODS

Study subjects. Seven patients homozygous for Tangier disease from five unrelated kindreds were studied. The clinical features of these subjects have been detailed in earlier reports from this laboratory (19, 20, 24–28). Plasma, anticoagulated with disodium EDTA (1 mg/ml), was obtained by venipuncture or by plasmapheresis when larger quantities were required. Samples were obtained after a 12- to 14-h fast. Diets consisted of solid food and were identical for patients and controls. All patients were studied on one or more occasions while consuming unrestricted diets in which 40–50% of calories were derived from fat. Four patients were additionally studied after eating a diet containing less than 5 g of fat and 500–700 g of carbohydrate for 7–10 days. Control subjects were volunteers, hospitalized at the National Institutes of Health Clinical Center, who had normal plasma lipid levels, took no medications, and were free of known disease.

Preparation of lipoproteins. The density of plasma was adjusted to 1.063 g/ml by adding solid KBr, and the samples were centrifuged for 16 h at 60,000 rpm in a Beckman 60 Ti rotor and L2-65B ultracentrifuge (Beckman Instruments, Inc., Fullerton, Calif.) at 5°C (1.66×10^8 *g*-min average). The supernatant fraction was recovered by tube slicing, adjusted to d 1.004 g/ml by dialysis, and centrifuged under identical conditions to recover the VLDL. The density of the infranatant fraction from the second ultracentrifugation was raised to 1.035 g/ml, and centrifugation was repeated to isolate the lipoprotein fraction here designated as LDL-1 ($1.006 < d < 1.035$ g/ml). The density of the infranatant fraction was finally adjusted to 1.063 g/ml and the LDL-2 ($1.035 < d < 1.063$ g/ml) recovered from the last ultracentrifugation. Lipoproteins were subjected to no further ultracentrifugal "washes," thus avoiding any additional structural and chemical changes known to attend repeated ultracentrifugation (29).

Chemical analysis. Protein concentrations were determined by the method of Lowry et al. (30). Turbidity in whole lipoprotein fractions was removed by extraction with diethyl ether or by the addition of 0.2 ml of 1 M sodium dodecylsulfate before absorbance determination. Phospholipid was assayed using the malachite green reagent (31, 32) and phospholipids estimated as the product of lipid phosphorus ×25. Plasma and lipoprotein cholesterol and triglyceride contents were measured with the Technicon AA-II AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.) (33, 34). The proportion of cholesterol esterified was estimated by the method of Zak et al. (35).

Amino acid analyses were performed on a Beckman model 121 amino acid analyzer (Beckman Instruments, Inc.) (36, 37). Proteins were hydrolyzed for 24 h at 110°C in 0.5 ml of constant boiling 6 N HCl containing 2-mercaptoethanol (1:2,000, vol/vol) (38).

Analytic ultracentrifugation and electron microscopy. Procedures used for the analytic ultracentrifugation of lipoproteins of $d < 1.063$ g/ml and the computer analysis of lipoprotein distribution have been described in detail (39). Lipoprotein fractions were prepared for electron microscopy by dialysis against 1% ammonium acetate buffer, pH 7.4, containing 0.1 mg/ml EDTA. Solutions were appropriately diluted and mixed with an equal volume of 2% sodium phosphotungstate. The final lipoprotein concentration was 0.1–0.25 mg/ml. Samples were examined on a Formvar/carbon-coated grid as reported elsewhere (40).

Characterization of apolipoproteins. Lipoprotein samples were concentrated in dialysis bags by dehydration with Aquacide C (Calbiochem, San Diego, Calif.) and dialyzed against 5 mM NH_4HCO_3 containing 0.1 mg/ml EDTA. The lipoprotein solutions (0.5–1.5 ml) were added dropwise to 24 ml of a methanol:diethylether (1:1) mixture with continuous vortex mixing. The 45-ml conical tube was

then filled with diethylether, thoroughly mixed, and left standing in wet ice for 10 min. Precipitated protein was recovered by low-speed centrifugation and was washed twice with the methanol:diethylether mixture and twice more with anhydrous diethylether. The protein was finally dried under a stream of nitrogen, and residual ether was removed by vacuum suction.

Apoproteins were solubilized in a buffer containing 0.1 M NH_4HCO_3 and 0.2 M sodium decylsulfate. Sodium decylsulfate was removed by dialysis against 5 mM NH_4HCO_3, and apoproteins were subsequently dialyzed into 7 M guanidine HCl, 0.1 M Tris-HCl, pH 8.0, in preparation for chromatography. Two dialysis procedures were necessary because apoVLDL is not completely soluble in 7 M guanidine and because sodium decylsulfate precipitates in guanidine solutions of high molarity. 15–20 mg of apoprotein were fractionated on 2.5 × 150 cm columns on Sephadex G-200 superfine packed as previously described (41) and eluted with 5 M guanidine HCl, 0.1 M Tris-HCl, pH 8.0. Verification of apoVLDL C-apoprotein content was also obtained by chromatography on columns of Biogel P-100 eluted with guanidine buffers. The estimates of the apoVLDL C-apoprotein content on the latter columns were within 5% of those estimated by Sephadex G-200 chromatography. A few preparations of Tangier apoVLDL were fractionated on 2.5 × 150 cm columns of Sepharose 6B-Cl eluted with 5 M guanidine HCl. Such columns permitted recovery of apoE (arginine-rich apoprotein) in more homogeneous form. Elution was monitored at 280 nm, and 3- to 4-ml fractions were collected. Appropriate fractions were pooled, dialyzed exhaustively against 5 mM NH_4HCO_3, and lyophilized. The void volume fraction was resolubilized in 0.1 M NH_4HCO_3 buffer containing 0.2 M sodium decylsulfate. The sodium decylsulfate was removed by dialysis before further analysis. The second column fraction was solubilized in 0.1 M NH_4OH and subsequent fractions in 0.1 M NH_4HCO_3. Recoveries of VLDL protein after delipidation, various dialyses, and chromatography ranged from 75% to 80%.

Apoprotein fractions were analyzed by polyacrylamide-gel electrophoresis in 7.5% acrylamide using the system of Reisfeld and Small (42), modified so that all solutions contained 8 M urea. Sodium dodecylsulfate polyacrylamide-gel electrophoresis was performed as described by Weber and Osborn (43). Gels were stained with 0.05% Coomassie Blue (44).

Ouchterlony double diffusion was performed in 1% agarose. All antisera were specific for single apolipoproteins.

RESULTS

Plasma lipid and lipoprotein concentrations. The mean plasma cholesterol concentration in the seven Tangier patients studied (Table I) was 69 mg/dl. The hypocholesterolemia was due in part to the near total absence of HDL, but the cholesterol carried in the LDL was significantly less than that usually found in age- and sex-matched controls (33). Four of the patients were hypertriglyceridemic, but the cholesterol transported in their VLDL fractions was within normal limits in every case, suggesting that chylomicrons or their remnants contributed to the mild hypertriglyceridemia. Chylomicrons, in addition, were frequently visualized as a faint creamy layer on the top of plasma left standing at 4°C.

Analytic ultracentrifugation was employed to quantitate the mass of VLDL and LDL in the plasma of all but patient Pa. Lo. (Fig. 1). Interpretation of the results is somewhat limited by the unavailability of comparable data from a population of age-matched controls (45). It is noteworthy, however, that C. No. and T. La, who were hypertriglyceridemic, had normal or only borderline elevations of VLDL concentrations. No accumulation of intermediate density lipoproteins ($S_f°$ 12–20) was observed (Fig. 1). The mass of total LDL ($S_f°$ 0–20) was reduced in patients E. La., T. La., and C. No., but normal in the other three subjects. These findings suggested that the measurement of LDL by cholesterol determination (33) does not satisfactorily reflect the mass of LDL in Tangier plasma and that the apparent "hypobetalipoproteinemia" is in part artifactual.

TABLE I
Plasma Lipid and Lipoprotein Cholesterol Concentrations in Tangier Patients and Controls

Patient	Sex	Age	Plasma		Lipoprotein cholesterol		
			Cholesterol	Triglyceride	VLDL	LDL	HDL
		yr		*mg/dl*			
E. La.	F	21	73	286	35	35	3
T. La.	M	19	31	211	4	24	3
C. No.	M	57	101	362	36	65	0
Pa. Lo.	F	23	48	165	7	36	5
Pe. Lo.	F	21	89	131	9	74	6
J. St.	M	9	57	110	5	50	2
R. Ja.	M	5	83	207	33	50	0
Controls							
1	F	34	167	38	5	104	58
2	M	22	143	119	13	106	24
3	M	22	150	113	12	94	44
4	M	34	162	135	18	101	43
5	F	21	151	72	10	78	63
6	F	61	187	63	7	111	63
7	F	52	202	80	16	151	35
8	M	25	113	82	19	67	27

Very low density lipoproteins. Chylomicrons and VLDL were not separated in this study. The persistence of chylomicrons was suggested, however, by the relative excess of triglyceride compared with cholesterol in the $d < 1.006$ g/ml lipoproteins of at least two of the patients (E. La. and T. La., Table II). The cholesteryl ester content of the $d < 1.006$ g/ml lipoproteins in Tangier plasma was also reduced, as was the proportion of free cholesterol (Table II). The phospholipid and protein contents were comparable to those in controls. These compositional differences were not apparent when patients and controls were maintained for 7–10 days on high-carbohydrate, low-fat diets of caloric content sufficient to maintain

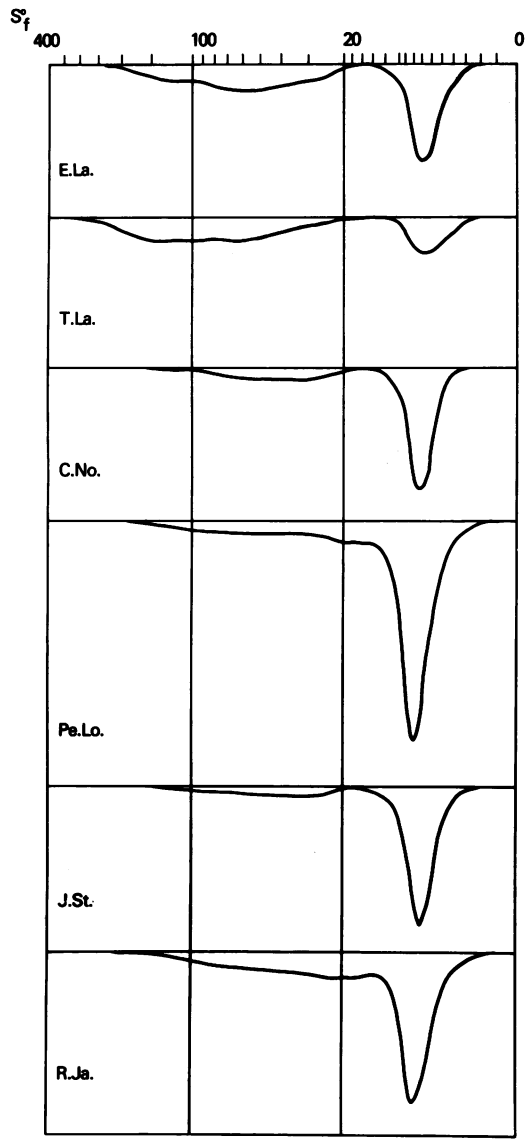

FIGURE 1 Analytical ultracentrifugal Schlieren patterns of $d < 1.063$ g/ml lipoproteins from the plasma of six patients with Tangier disease. Presentations are logarithmic and were traced from cathode ray tube computer plots.

body weight (Table III). One of the Tangier patients (E. La.), who was on oral contraceptives, had an exaggerated rise of plasma triglycerides (triglycerides = 639 mg/dl) with carbohydrate feeding, but the plasma triglyceride concentration fell in two of the patients (T. La. and C. No.) (Table III). The paradoxical response of the latter patients to high carbohydrate intake further supports the conclusion that the hypertriglyceridemia frequently observed in Tangier disease is often a reflection of impaired clearance of dietary fat (20).

A distinct pre-beta lipoprotein band is not visualized when Tangier plasma is examined by paper electrophoresis (46) and isolated Tangier VLDL has less than normal mobility (Fig. 2). This altered electrophoretic migration does not appear related to abnormalities of lipoprotein shape or size. Control and Tangier VLDL are spherical in shape, and the majority of particles in both cases fall between 300 and 500 Å (Fig. 2). The lack of gross compositional or morphological differences to account for the abnormal electrophoretic migration of Tangier VLDL prompted an investigation of the VLDL-apolipoprotein content.

Very low density apolipoproteins. The lipid-free protein (apoproteins) of Tangier and control VLDL was fractionated on columns of Sephadex G-200 eluted with a buffer containing 5 M guanidine HCl. Appropriate fractions were pooled (Fig. 3) and analyzed by polyacrylamide-gel electrophoresis, by Ouchterlony double diffusion against specific antisera, and by amino acid analysis.

The void volume peak (fraction 1, Fig. 3) contained large or highly aggregated proteins that did not enter 7.5% polyacrylamide gels (Fig. 4) but which produced a line of immunochemical identity with the B apoprotein from normal LDL (Fig. 5). The amino acid composition of the void volume peaks from Tangier and normal VLDL were nearly identical and similar to those reported by Gotto et al. (47) for the B apoprotein from normal VLDL and LDL.

Sephadex fraction 2 (Fig. 3) accounted for about 8% of the control and Tangier VLDL apoproteins. As previously reported (29, 41), this fraction is usually heterogeneous, often containing trace quantities of albumin and gammaglobulin. This fraction from both Tangier and control apoVLDL was immunochemically free of the A, B, and C apoproteins (Fig. 5). The so-called arginine-rich apoprotein (48), that has been designated R-X_2 (49) and apoE (50) by other laboratories, elutes in this fraction. An array of slowly migrating bands is usually visualized in alkaline polyacrylamide-gel electrophoresis (Fig. 4 *A*), but SDS-gel electrophoresis of Tangier and control apoVLDL demonstrated the protein of $\cong$ mol wt 35,000 that corresponds to apoE (Fig. 4 *B*). On unrestricted diets the arginine content of fraction 2 from Tangier apoVLDL ranged from 4.2 to 8.7 mol/100 mol of amino acid, and the mean of eight preparations was significantly less than that in control apoVLDL (Table IV). This low arginine content may reflect contamination of the $d < 1.006$ g/ml lipoproteins by chylomicron remnants in Tangier plasma (Fig. 4 *B*). This possibility is supported by two observations. When patient T. La. was on a high-carbohydrate, fat-free diet, the amino acid composition of his apoVLDL Sephadex fraction 2 approximated that of controls and published compositions for apoE (Table IV). A number of bands, however, were still apparent on SDS-gel electrophoresis. ApoE of greater homogeneity was recovered when the apoVLDL of patient E. La., ob-

TABLE II
*Composition of Density < 1.006 g/ml Lipoproteins of Tangier and Control Plasma**

Patient	UC‡	CE	Tg	PL	Pro
			% lipoprotein dry wt		
E. La.	3.1	12.0	68.9	11.2	4.8
T. La.	3.3	5.3	72.2	14.0	5.2
C. No.	3.5	13.8	60.0	15.7	7.0
Pe. Lo.	4.4	14.2	55.9	18.1	7.4
J. St.	5.0	12.1	58.1	17.6	7.2
R. Ja.	5.2	18.3	53.7	15.1	7.7
Mean (SD)	4.1 (0.9)	12.6 (4.3)	61.5 (7.4)	15.3 (2.5)	6.6 (1.2)
Controls					
1	7.6	18.5	50.6	17.0	6.3
2	7.2	28.1	39.3	17.9	7.5
3	5.2	20.5	48.5	19.5	6.3
4	5.2	16.2	55.5	16.3	6.8
8	4.1	16.6	60.0	14.2	5.3
Mean (SD)	5.9 (1.3)	20.0 (4.9)	50.8 (7.8)	17.0 (1.9)	6.4 (0.8)
P	<0.01	<0.05	<0.1	>0.2	>0.7

* All subjects on unrestricted diets.
‡ Abbreviations: UC, unesterified cholesterol; CE, cholesteryl esters; Tg, triglyceride; PL, phospholipid; Pro, protein.

tained while on a fat-free diet, was purified by chromatography on Sepharose 6B-C1. The amino acid composition of this preparation was almost identical with the apoE similarly prepared from the VLDL of a patient with type 3 hyperlipoproteinemia (Table IV), and SDS-gel electrophoresis (not shown) demonstrated a single major band with only trace contaminants. The apoE in Tangier VLDL, therefore, appears qualitatively identical with that in control VLDL.

The third Sephadex fraction (Fig. 3) contained less than 5% of the VLDL apoproteins; it generated several bands on polyacrylamide-gel electrophoresis (Fig. 4*A*). Both normal and Tangier apoVLDL frequently contained proteins with electrophoretic

TABLE III
Composition of Tangier and Control VLDL on High-Carbohydrate, Low-Fat Diets

Patient	UC*	CE	Tg	PL	Pro
			% lipoprotein dry wt		
E. La.	4.7	13.1	57.7	16.7	7.8
T. La.	6.4	20.4	50.1	15.3	7.8
C. No.	3.6	16.2	58.2	15.4	6.6
Pa. Lo.	7.4	25.4	39.0	18.9	9.3
Mean (SD)	5.5 (1.7)	18.8 (5.3)	51.2 (9.0)	16.6 (1.7)	7.9 (1.1)
Controls					
2	5.7	14.3	56.0	17.2	6.7
3	5.8	18.1	51.7	17.8	6.6
5	5.7	13.4	55.5	16.7	8.7
6	6.1	16.7	47.6	20.8	8.8
7	5.4	16.9	52.0	18.6	7.1
Mean (SD)	5.7 (0.3)	15.9 (2.0)	52.6 (3.4)	18.2 (1.6)	7.6 (1.1)
P	>0.8	>0.2	>0.9	>0.2	>0.8

* Abbreviations: UC, unesterified cholesterol; CE, cholesteryl esters; Tg, triglyceride; PL, phospholipid; Pro, protein.

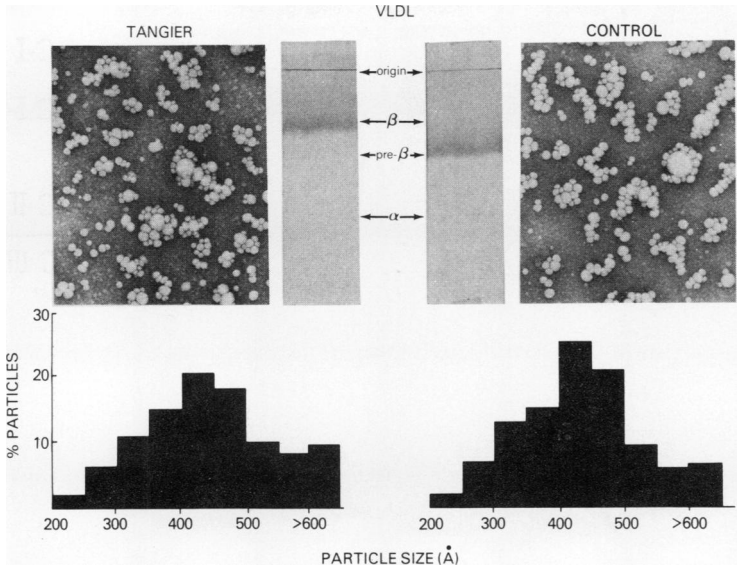

FIGURE 2 Very low density lipoproteins from the plasma of a normal subject and a patient with Tangier disease. Electron micrographs of negatively stained lipoproteins and lipoprotein paper electrophoresis patterns are in upper panels; particle size distributions are in the lower panels.

mobility similar to apoA-I and apoA-II, and their presence in this fraction was confirmed by Ouchterlony double diffusion (Fig. 5). Trace quantities of the C apoproteins were identified by immunochemical or electrophoretic techniques. The amino acid compositions of the fraction 3 obtained from both Tangier and control subjects were remarkably similar in spite of the obvious heterogeneity of proteins in this fraction.

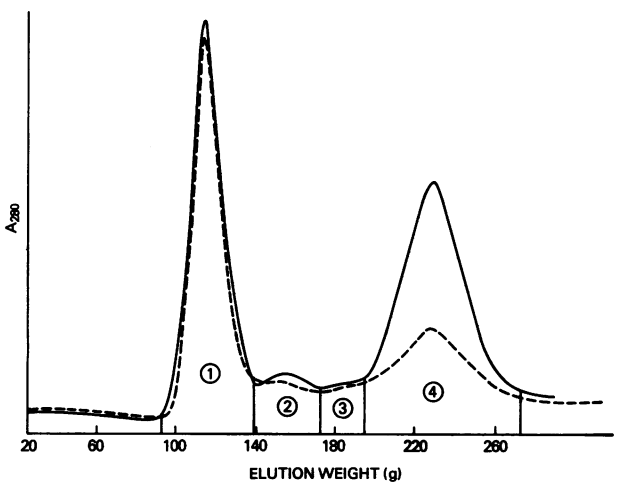

FIGURE 3 Sephadex G-200 SF chromatography of normal apoVLDL (solid line) and Tangier apoVLDL (broken line). A 2.5 × 150 cm column was eluted with buffer containing 5 M guanidine HCl, 0.1 M Tris-HCl, pH 8.0. Numbers and vertical lines define pooled fractions.

The C apoproteins (C-I, C-II, C-III-1, and C-III-2) accounted for most, if not all, of the protein in Sephadex fraction 4, as judged by electrophoretic (Fig. 4) and immunochemical (Fig. 5) criteria. The prominent protein band between apoC-I and apoC-II (C-I-1, Fig. 4) had an amino acid composition and immunochemical reactivity identical with apoC-I. This may be a polymorphic form of apoC-I or an artifact produced by the preparation procedures. Amino acid analysis revealed no homocitrulline, and the apoVLDL was not exposed to urea. Therefore, carbamylation of apoC-I does not account for the presence of this protein (41).

The apoVLDL obtained from T. La. and C. No. on unrestricted diets contained 48% and 49% C apoproteins, respectively (means of four determinations each). ApoVLDL from E. La. had a C-apoprotein content of 35% on one occasion and 19% on another. A single fractionation of apoVLDL from Pe. Lo. revealed C apoproteins to account for 17% of the total protein. The mean C-apoprotein content in six control preparations was 38% (range 36–41%). When the four Tangier patients were maintained on high-carbohydrate, low-fat diets, the C apoproteins comprised a mean of 17% of apoVLDL (range 10–28%). ApoVLDL isolated from control subjects maintained on an identical diet had a mean C-apoprotein content of 43% (range 38–46%).

LDL-1 ($1.006 < d < 1.035$ g/ml). LDL-1 are traditionally isolated in the density range 1.006–1.019 g/ml and are considered to represent short-lived intermediates in the conversion of VLDL to LDL. In

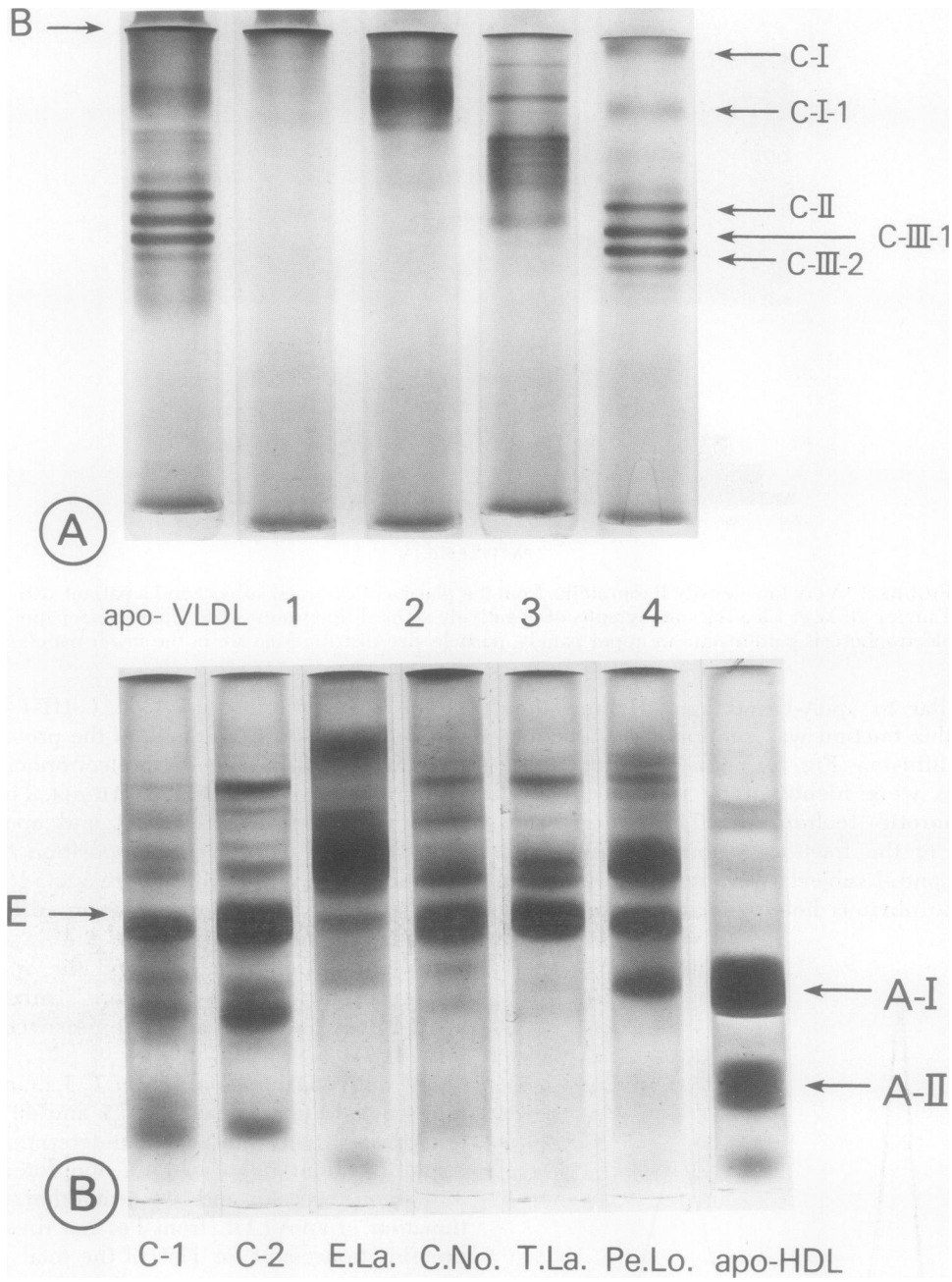

FIGURE 4 (A) Electrophoresis in 7.5% polyacrylamide gels containing 8 M urea at pH 9.4. The numbers refer to Sephadex G-200 SF fractions defined in Fig. 3. (B) Electrophoresis in 7.5% polyacrylamide gels containing sodium dodecylsulfate. The samples demonstrated are the second fractions from Sephadex G-200 SF chromatography (Fig. 3) of apoVLDL from two control subjects (C-1 and C-2) and four patients with Tangier disease. The pattern of apoHDL is provided for reference. Apolipoproteins A-I, A-II, and E are indicated.

the studies reported here, lipoproteins of d = 1.006–1.035 g/ml were included in the LDL-1 fraction in an effort to prepare a more homogeneous LDL-2 without contamination with intermediate density lipoproteins.

Such LDL-1 accounted for an average of 23% of the total LDL ($1.006 < d < 1.063$ g/ml) protein in Tangier plasma and for 16% in the controls. Their electrophoretic mobility and apoprotein content were

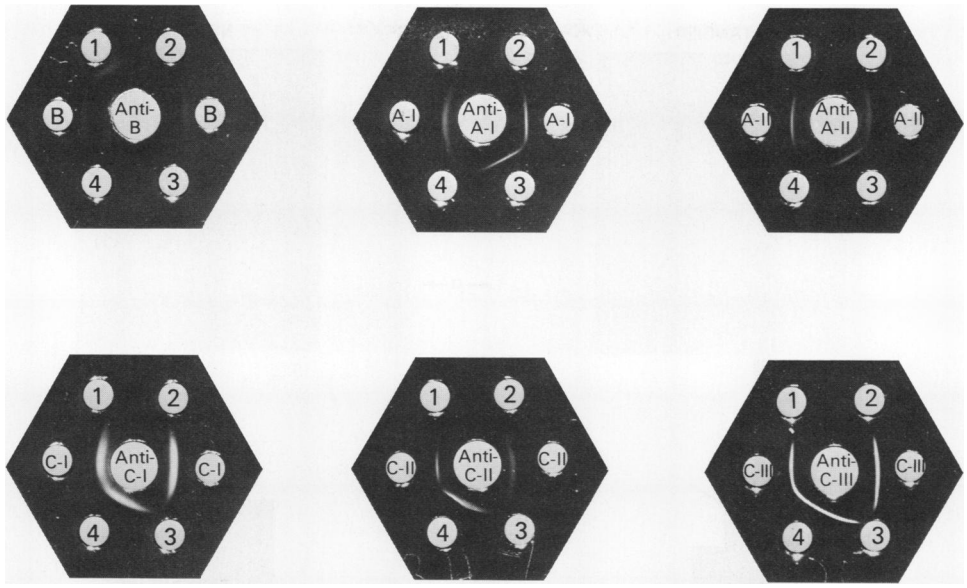

FIGURE 5 Immunoprecipitation reactions of the Sephadex G-200 SF fractions (Fig. 3) of the apoVLDL from a patient with Tangier disease. The antisera indicated were placed in the center wells and column fractions (1–4) and purified apolipoproteins in the outer walls.

TABLE IV

Amino Acid Composition of ApoE-Rich Fractions from Tangier and Control ApoVLDL

						ApoE		
	Tangier (8)*	Control (3)*	T. La.‡	E. La.§	S. Ja.§	(48)‖	(49)	(50)
				mol/100 mol amino acid				
Lysine	7.0 (1.0)	5.2 (0.3)	5.5	4.7	4.4	4.9	4.9	4.6
Histidine	1.7 (0.4)	1.0 (0.3)	1.1	1.0	0.6	0.8	1.3	1.2
Arginine	6.4 (1.8)	10.1 (1.0)	9.1	10.3	10.6	9.1	10.9	9.2
Aspartic acid and asparagine	8.5 (1.4)	5.6 (0.2)	6.6	5.5	5.1	6.8	4.9	5.7
Threonine	5.1 (0.7)	4.1 (0.2)	4.4	4.1	4.0	4.5	3.9	5.0
Serine	6.6 (1.1)	5.4 (0.8)	5.3	5.7	5.9	6.0	5.6	7.6
Glutamic acid and glutamine	17.8 (3.1)	21.6 (0.5)	20.6	22.2	22.7	17.8	23.9	23.7
Proline	4.2 (0.7)	3.2 (0.2)	3.5	3.2	3.0	3.8	2.8	3.7
Glycine	6.0 (0.5)	6.5 (0.2)	6.4	6.4	6.1	6.7	6.0	5.9
Alanine	9.0 (1.2)	11.1 (0.6)	10.6	11.5	11.8	10.4	11.1	9.2
Valine	6.2 (0.5)	7.1 (0.3)	7.0	7.1	7.0	7.4	7.0	6.0
Methionine	1.3 (0.4)	2.2 (0.2)	2.2	1.8	1.9	0.7	2.5	1.1
Isoleucine	2.8 (1.0)	1.4 (0.2)	2.2	1.1	0.9	1.8	1.3	1.1
Leucine	11.5 (0.7)	12.0 (0.4)	12.1	12.0	11.9	12.0	11.2	10.5
Tyrosine	2.5 (0.6)	1.5 (0.5)	1.6	1.7	2.2	1.7	1.4	1.7
Phenylalanine	3.3 (1.1)	1.6 (0.5)	1.7	1.9	2.0	2.1	1.4	1.9

* Fraction 2 from Sephadex G-200 columns (Fig. 3). Number of preparations is in parentheses.

‡ Fraction 2 from apoVLDL of Tangier patient T. La. while on a low-fat, high-carbohydrate diet.

§ ApoE-rich fraction from Tangier patient E. La. and subject S. Ja. (type 3 hyperlipoproteinemia). ApoVLDL chromatographed on columns of Sepharose CL-6B.

‖ Literature data source indicated in parentheses.

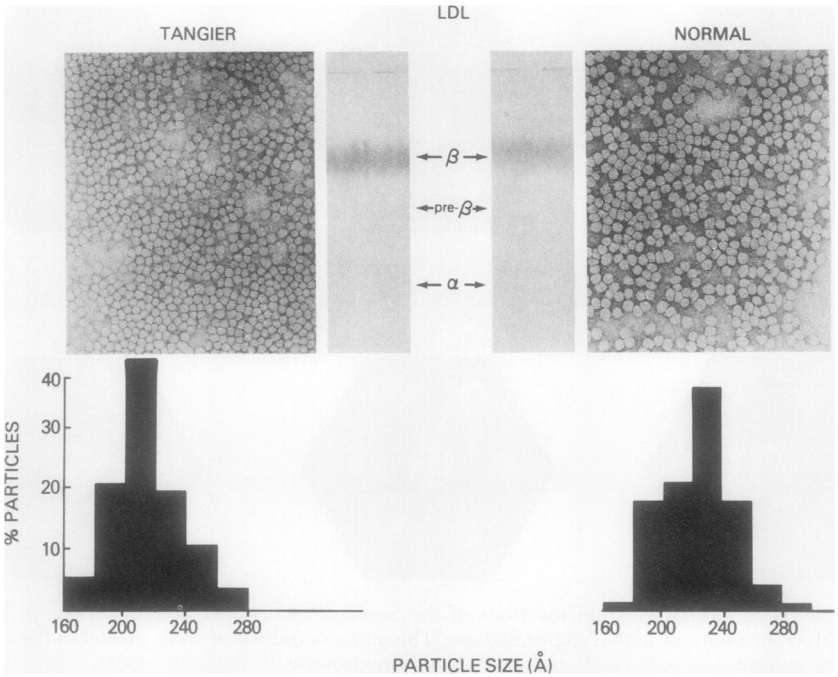

FIGURE 6 Low density lipoproteins of $1.035 < d < 1.063$ g/ml from the plasma of a normal subject and a patient with Tangier disease. Electron micrographs of negatively stained lipoproteins and lipoprotein paper electrophoresis patterns are in upper panels. Particle size distributions are in lower panels.

indistinguishable from that in normal LDL-1. Three preparations of Tangier LDL-1, examined by electron microscopy, had median diameters of 240, 240, and 250 Å. LDL-1 in control preparations were larger, with median diameters of 250, 290, and 295 Å.

There were striking differences between the chemical compositions of normal and Tangier LDL-1. The cholesteryl ester content of Tangier LDL-1 ranged from 13% to 29% (38% in controls), and triglycerides accounted for 27–54% of the mass (18% in controls). The different experimental diets did not affect the composition of Tangier or control LDL-1.

LDL-2 ($1.035 < d < 1.063$ g/ml). Tangier and control LDL-2 proved to be spherical particles of 160–280 Å diameter. The median particle diameters of LDL-2 from three Tangier patients were slightly smaller than in three controls (210, 215, 220 vs. 220, 235, and 240 Å). Tangier and control LDL-2 had identical mobilities on paper electrophoresis (Fig. 6).

As was true for LDL-1, Tangier LDL-2 contained much less cholesteryl esters and proportionately more protein and triglyceride than control LDL-2 (Table V). Triglycerides, rather than cholesteryl esters, were the major lipid in Tangier LDL-2, comprising 23–41% of the lipoprotein dry weight (6% in controls). The percentage of total cholesterol that was esterified was similar in Tangier (76%) and control (78%) LDL-2. The composition of Tangier LDL-2 did not vary with diet.

Identical chromatograms were obtained when the apoproteins from Tangier and normal LDL-2 were fractionated by gel chromatography. Over 90% of the protein eluted in the void volume peak which contained apoB, and C-apoproteins comprised less than 5% of the total apoLDL. Therefore, triglyceride enrichment of Tangier LDL does not appear to enhance the association of C-apoproteins with this lipoprotein fraction.

DISCUSSION

The paucity of C apoproteins in Tangier VLDL may be related to the absence of normal HDL from Tangier plasma. It can be calculated that in normal, post-absorptive state subjects, VLDL and HDL each contain about half of the total plasma C apoproteins. Studies with radiolabeled VLDL have revealed rapid in vitro and in vivo exchange of C apoproteins between VLDL and HDL (51), and LaRosa et al. (52) have provided evidence that heparin-induced lipolysis could induce net transfer of C apoproteins from VLDL to HDL. The small amount of newly synthesized C

TABLE V
Chemical Composition of Tangier and Control LDL-2 (1.035 < d < 1.063)

Tangier	UC*	CE	Tg	PL	Pro
			% lipoprotein dry wt		
E. La.	2.4	16.2	35.6	18.5	27.3
T. La.	4.1	10.8	41.0	17.9	26.3
C. No.	4.6	30.2	24.5	18.9	21.8
Pe. Lo.	5.4	30.2	23.3	20.1	21.0
J. St.	5.4	27.6	24.2	21.2	21.6
R. Ja.	3.9	21.7	27.1	24.1	23.2
Mean (SD)	4.3 (1.1)	22.8 (8.0)	29.3 (7.3)	20.1 (2.3)	23.5 (2.6)
Controls					
1	8.0	47.3	2.8	24.5	17.4
2	8.0	45.1	6.8	21.9	18.2
3	5.6	46.9	8.3	20.5	18.7
4	8.2	49.7	6.9	16.3	18.9
8	8.1	48.5	4.1	21.1	18.2
Mean (SD)	7.6 (1.1)	47.4 (2.7)	5.8 (2.3)	20.9 (3.0)	18.3 (0.6)
P	<0.005	<0.001	<0.001	>0.6	<0.005

* Abbreviations: UC, unesterified cholesterol; CE, cholesteryl esters; Tg, triglyceride; PL, phospholipid; Pro, protein.

apoproteins in freshly secreted hepatic VLDL (5) and their transfer to and from chylomicrons during alimentary lipemia (6) have suggested that HDL conserve the C apoproteins in plasma for cyclic reincorporation into chylomicrons and VLDL. In Tangier disease, however, only trace quantities of C apoproteins are found in the HDL density range, and heparin-induced lipolysis does not increase the C-apoprotein content of Tangier HDL (unpublished observation). When the C apoproteins in VLDL are labeled with ^{125}I and incubated with Tangier plasma, a significant amount of radioactivity is not recovered in the 1.063–1.21 g/ml fraction of plasma (53). These observations suggest that the C apoproteins do not form independent lipoproteins of density 1.063–1.21 g/ml in vivo, and that C-apoprotein conversation in this density class depends on the availability of normal HDL to complex with the C apoproteins.

When two of the Tangier patients were consuming unrestricted diets, a normal C-apoprotein content was found in the lipoproteins of $d < 1.006$ g/ml. However, no lipoproteins with pre-beta mobility were observed on paper electrophoresis. If the usual pre-beta mobility of VLDL requires a normal complement of C apoproteins, then the beta mobility of Tangier VLDL suggests that much of the C-apoprotein content of Tangier lipoproteins of $d < 1.006$ g/ml is associated with chylomicrons or their remnants. The uniformly low C-apoprotein content of the VLDL from Tangier patients on high-carbohydrate, fat-free diets is consistent with this hypothesis. The feeding of such diets to normals with an intact HDL C-apoprotein reservoir does not lead to C-apoprotein depletion in their VLDL. We have also shown that infusion of large quantities of normal HDL produce dramatic and immediate changes in the electrophoretic properties of Tangier VLDL (54). This effect has yet to be directly correlated with an increase in content of C apoproteins. It is still possible that quantitative changes in some of the minor VLDL apoproteins may also affect the mobility of Tangier VLDL on paper electrophoresis. The VLDL of all Tangier plasmas examined appeared to contain apoE (Fig. 4 *B*), but precise quantitation was not possible.

The normal cholesteryl ester content of Tangier VLDL (low-fat, high-carbohydrate diets, Table III) is of theoretical interest since the origins of the cholesteryl esters in VLDL are disputed. Man lacks the hepatic acyl-CoA:cholesterol acyltransferase (55) which is thought to contribute to the VLDL cholesteryl esters of lower animals (56–58). Cholesteryl esters in human plasma lipoproteins are believed to derive primarily from the lecithin:cholesterol acyltransferase (LCAT) reaction in the plasma itself, with a minor contribution from cholesteryl esters in lipoproteins of intestinal origin (59). Human VLDL do not serve as substrates for the LCAT enzyme (60). Since net transfer of cholesteryl esters from HDL to VLDL has been demonstrated (60, 61), the cholesteryl esters of VLDL are widely believed to be derived from HDL (62).

Barter (63) has presented evidence, however, that VLDL cholesteryl esters are not derived exclusively

from HDL, but his experiments could not distinguish between hepatic and intraplasmic origins of the VLDL cholesteryl esters. The cholesteryl ester content of the $d < 1.006$ g/ml lipoproteins from Tangier patients on unrestricted diets was significantly less than in control VLDL (Table II). This was probably related to the presence of chylomicrons or their remnants. When the donors ingested fat-free diets, the amount of cholesteryl esters in Tangier VLDL was comparable to controls (Table III). Since Tangier plasma contains virtually no HDL, it appears likely that VLDL cholesteryl esters are derived either directly from the intraplasmic action of LCAT or from the liver. The liver is a more likely source, in view of the fact that the VLDL in patients with familial LCAT deficiency are richer in cholesteryl esters than are their LDL or HDL (64). In LCAT deficiency, as in Tangier disease, isocaloric substitution of carbohydrate for fat eliminates circulating chylomicron remnants and increases the cholesteryl ester content of the VLDL (65).

The LDL in Tangier plasma had electrophoretic mobility on paper electrophoresis indistinguishable from controls, but both the LDL-1 and LDL-2 subfractions were slightly smaller than their normal counterparts. Utermann et al. (66) have reported that Tangier LDL migrates abnormally fast in 3.75% polyacrylamide gels, and we have observed that Tangier LDL has slightly greater than normal mobility on agarose-gel electrophoresis. It is possible that the smaller size of Tangier LDL-2 results in abnormally rapid mobility in electrophoretic media capable of molecular sieving. Utermann et al. (66) attributed the differences in mobility to an increased content of apoD in Tangier LDL. The apoD content of Tangier LDL was not specifically investigated in the studies reported here, but the fractionation of apoLDL by column chromatography indicated that the B and C apoproteins accounted for more than 95% of the total protein in both normal and Tangier LDL.

The most striking abnormality in Tangier LDL was the relative enrichment in triglyceride and impoverishment of cholesteryl ester content (Table V). While these findings may suggest that HDL participate in the production of chemically normal LDL, such a role must still be considered speculative. Most of the protein and triglycerides of LDL appear to derive from VLDL catabolism (67–70), although direct secretion of LDL by the liver has not been excluded. The origins of the cholesteryl esters in LDL are less clear. The cholesteryl ester content of a VLDL macromolecule is more than sufficient to account for that in a typical LDL particle. If VLDL catabolism proceeds normally in Tangier disease, the paucity of cholesteryl esters in Tangier LDL suggests that cholesteryl esters may be lost as VLDL are converted to LDL. The normally high cholesteryl ester content of LDL could be produced by net transfer of cholesteryl esters from HDL to LDL, or from exchange of LDL-triglyceride for HDL cholesteryl esters. When HDL are absent from the plasma, triglyceride appears to be preserved as the major neutral lipid in LDL.

We have attempted to interpret the abnormalities observed in Tangier VLDL and LDL as an effect of the profound HDL deficiency in this disease. If this presumption is valid, the results are of great interest because a complex involvement of HDL in the metabolism of the chylomicron-VLDL-LDL system may be inferred. The possibility cannot be dismissed, however, that some or all of the lipoprotein abnormalities documented are related to an unrecognized but fundamental defect affecting all lipoprotein classes in Tangier disease.

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REFERENCES

1. Kostner, G., and A. Holasek. 1972. Characterization and quantitation of the apolipoproteins from human chyle chylomicrons. *Biochemistry.* **11:** 1217–1223.
2. Glickman, R. M., and K. Kirsch. 1974. The apoproteins of various size classes of human chylous fluid lipoproteins. *Biochim. Biophys. Acta.* **371:** 255–266.
3. Ockner, R. K., K. J. Bloch, and K. J. Isselbacher. 1968. Very-low-density lipoprotein in intestinal lymph: evidence for presence of the A protein. *Science (Wash. D. C.).* **162:** 1285–1286.
4. Mjøs, O. D., O. Faergman, R. H. Hamilton, and R. J. Havel. 1975. Characterization of remnants produced during the metabolism of triglyceride-rich lipoproteins of blood plasma and intestinal lymph in the rat. *J. Clin. Invest.* **56:** 603–615.
5. Windmueller, H. B., P. N. Herbert, and R. I. Levy. 1973. Biosynthesis of lymph and plasma lipoprotein apoproteins by isolated perfused rat liver and intestine. *J. Lipid Res.* **14:** 215–223.
6. Havel, R. J., J. P. Kane, and M. L. Kashyab. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32–38.
7. Bier, D. M., and R. J. Havel. 1970. Activation of lipoprotein lipase by fractions of human serum. *J. Lipid Res.* **11:** 565–570.
8. Havel, R. J., V. G. Shore, B. Shore, and D. M. Bier. 1970. Role of specific glycopeptides of human serum lipoproteins in the activation of lipoprotein lipase. *Circ. Res.* **27:** 595–600.
9. LaRosa, J. C., R. I. Levy, P. N. Herbert, S. E. Lux, and D. S. Fredrickson. 1970. Specific apoprotein activator for lipoprotein lipase. *Biochem. Biophys. Res. Commun.* **41:** 57–62.
10. Brown, W. V., and M. L. Baginsky. 1972. Inhibition of lipoprotein lipase by an apoprotein of human very low density lipoprotein. *Biochem. Biophys. Res. Commun.* **46:** 375–382.
11. Krauss, R. M., P. N. Herbert, R. I. Levy, and D. S. Fredrickson. 1973. Further observations on the activation

and inhibition of lipoprotein lipase by apolipoproteins. *Circ. Res.* **33:** 403–411.
12. Eisenberg, S., and D. Schurr. 1976. Phospholipid removal during degradation of rat plasma very low density lipoprotein *in vitro*. *J. Lipid Res.* **17:** 578–587.
13. Nichols, A. V., and L. Smith. 1965. Effect of very low-density lipoproteins on lipid transfer in incubated serum. *J. Lipid Res.* **6:** 206–210.
14. Glomset, J. A., E. T. Janssen, R. Kennedy, and J. Dobbins. 1966. Role of plasma lecithin:cholesterol acyltransferase in the metabolism of high density lipoproteins. *J. Lipid Res.* **7:** 639–648.
15. Fielding, C. J., and P. E. Fielding. 1971. Purification and substrate specificity of lecithin:cholesterol acyltransferase from human plasma. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **15:** 355–358.
16. Norum, K. R., J. A. Glomset, and E. Gjone. 1972. Familial lecithin:cholesterol acyltransferase deficiency. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 3rd edition. 531–544.
17. Fielding, C. J., V. G. Shore, and P. E. Fielding. 1972. A protein cofactor of lecithin:cholesterol acyltransferase. *Biochem. Biophys. Res. Commun.* **46:** 1493–1498.
18. Soutar, A., C. Garner, H. N. Baker, J. T. Sparrow, R. L. Jackson, A. M. Gotto, and L. C. Smith. 1975. The effects of plasma apolipoproteins on lecithin:cholesterol acyltransferase. *Biochemistry.* **14:** 3057–3064.
19. Fredrickson, D. S., A. M. Gotto, and R. I. Levy. 1972. Familial lipoprotein deficiency. Abetalipoproteinemia, hypobetalipoproteinemia, and Tangier disease. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredickson, editors. McGraw-Hill Book Company, New York. 3rd edition. 493–530.
20. Fredrickson, D. S. 1966. Familial high density lipoprotein deficiency: Tangier disease. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 2nd edition. 486–508.
21. Herbert, P., D. Fredrickson, R. Heinen, T. Forte, and G. Assmann. 1974. Tangier disease: a pathogenetic mechanism. *Circulation.* **50:** III-19.
22. Kocen, R. S., J. K. Lloyd, P. T. Lascelles, A. S. Fosbrooke, and D. Williams. 1967. Familial α-lipoprotein deficiency (Tangier disease) with neurological abnormalities. *Lancet.* **I:** 1341–1345.
23. Greten, H., T. Hanneman, W. Gusek, and O. Vivell. 1974. Lipoprotein and lipolytic enzymes in a case of Tangier disease. *N. Engl. J. Med.* **291:** 548–552.
24. Fredrickson, D. S., P. H. Altrocchi, L. V. Avioli, D. S. Goodman, and H. C. Goodman. 1961. Tangier disease. *Ann. Intern. Med.* **55:** 1016–1031.
25. Fredrickson, D. S. 1964. The inheritance of high density lipoprotein deficiency (Tangier disease). *J. Clin. Invest.* **43:** 228–234.
26. Hoffman, H. N., and D. S. Fredrickson. 1965. Tangier disease (familial high density lipoprotein deficiency). Clinical and genetic features in two adults. *Am. J. Med.* **39:** 582–593.
27. Engel, W. K., J. D. Dorman, R. I. Levy, and D. S. Fredrickson. 1967. Neuropathy in Tangier disease: α-lipoprotein deficiency manifesting as familial recurrent neuropathy and intestinal lipid storage. *Arch. Neurol.* **17:** 1–9.
28. Ferrans, V. J., and D. S. Fredrickson. 1975. The pathology of Tangier disease: a light and electron microscopic study. *Am. J. Pathol.* **78:** 101–136.
29. Herbert, P. N., T. M. Forte, R. S. Shulman, M. J. LaPiana, E. L. Gong, R. I. Levy, D. S. Fredrickson, and A. V. Nichols. 1975. Structural and compositional changes attending the ultracentrifugation of very low density lipoproteins. *Prep. Biochem.* **5:** 93–129.
30. Lowry, O. H., N. O. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
31. Itaya, K., and M. Ui. 1966. A new micromethod for the colorimetric determination of inorganic phosphate. *Clin. Chim. Acta.* **14:** 361–366.
32. Chalvardjian, A., and E. Rudnicki. 1970. Determination of lipid phosphorus in the nanomolar range. *Anal. Biochem.* **36:** 225–226.
33. Fredrickson, D. S., R. I. Levy, and R. S. Lees. 1967. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. *N. Engl. J. Med.* **276:** 94–103.
34. Lipid and lipoprotein analysis. 1974. *In* Manual of Laboratory Operations, Lipid Research Clinics Program, Vol. 1. Department of Health, Education, and Welfare, Pub. No. (NIH) 75–628.
35. Zak, B., R. C. Dickenman, E. G. White, H. Burnett, and P. J. Chemey. 1954. Rapid estimation of free and total cholesterol. *Am. J. Clin. Pathol.* **24:** 1307–1315.
36. Moore, F., D. H. Spackman, and W. H. Stein. 1958. Chromatography of amino acids on sulfonated polystyrene resins. An improved system. *Anal. Chem.* **30:** 1185–1190.
37. Hubbard, R. W. 1965. Studies in accelerated amino acid analysis. *Biochem. Biophys. Res. Commun.* **19:** 679–685.
38. Keutman, H. T., and J. T. Potts, Jr. 1969. Improved recovery of methionine after acid hydrolysis using mercaptoethanol. *Anal. Biochem.* **29:** 175–185.
39. Lindgren, F. T., and L. C. Jensen. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. Wiley-Interscience, New York. 181–274.
40. 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 lipoprotein as revealed by electron microscopy. *J. Clin. Invest.* **50:** 1141–1148.
41. Herbert, P. N., R. S. Shulman, R. I. Levy, and D. S. Fredrickson. 1973. Fractionation of the C-apoproteins from human plasma very low density lipoproteins: artifactual polymorphism from carbamylation in urea containing solutions. *J. Biol. Chem.* **248:** 4941–4946.
42. Reisfeld, R. A., and P. A. Small, Jr. 1966. Electrophoretic heterogeneity of polypeptide chains of specific antibodies. *Science (Wash. D.C.).* **152:** 1253–1255.
43. Weber, K., and M. Osborn. 1969. The reliability of molecular weight determinations by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
44. Chrambach, A., R. A. Reisfeld, M. Wyckoff, and J. Zaccari. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. *Anal. Biochem.* **20:** 150–154.
45. Lindgren, F. T., G. L. Adamson, L. C. Jenson, and P. D. Wood. 1975. Lipid and lipoprotein measurements in a normal adult American population. *Lipids.* **10:** 750–756.
46. Fredrickson, D. S., R. I. Levy, and F. T. Lindgren. 1968. A comparison of heritable abnormal lipoprotein patterns as defined by two different techniques. *J. Clin. Invest.* **47:** 2446–2457.
47. Gotto, A. M., W. V. Brown, R. I. Levy, M. E. Birnbaumer, and D. S. Fredrickson. 1972. Evidence for the identity of the major apoprotein in low density and very low density lipoproteins in normal subjects and patients with familial hyperlipoproteinemia. *J. Clin. Invest.* **51:** 1486–1494.

48. Shelburne, F. A., and S. H. Quarfordt. 1974. A new apoprotein of human plasma very low density lipoproteins. *J. Biol. Chem.* **249**: 1428–1433.
49. Shore, V. G., and B. Shore. 1972. The apolipoproteins: their structure and functional roles in human serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. Wiley-Interscience, New York. 789–824.
50. Utermann, G. 1975. Isolation and partial characterization of an arginine-rich apolipoprotein from human plasma very low density lipoproteins: apolipoprotein E. *Hoppe-Seyler's Z. Physiol. Chem.* **356**: 1113–1121.
51. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoprotein proteins. I. Preliminary *in vitro* and *in vivo* observations. *Biochim. Biophys. Acta.* **260**: 212–221.
52. LaRosa, J. C., R. I. Levy, W. V. Brown, and D. S. Fredrickson. 1971. Changes in high density lipoprotein protein composition after heparin-induced lipolysis. *Am. J. Physiol.* **220**: 785–791.
53. Assmann, G., P. N. Herbert, D. S. Fredrickson, and T. Forte. 1977. Isolation and characterization of an abnormal high density lipoprotein in Tangier disease. *J. Clin. Invest.* **60**: 242–252.
54. Herbert, P. N., L. O. Henderson, N. M. Papadopoulos, E. C. Church, and D. S. Fredrickson. 1977. Effects of high density lipoprotein infusion in Tangier disease. *In* XXV Annual Colloquium, Protides of the Biological Fluids. H. Peeters, editor. Pergamon Press, Oxford. In press.
55. Stokke, K. T. 1972. The existence of an acid cholesterol esterase in human liver. *Biochim. Biophys. Acta.* **270**: 156–166.
56. Gidez, L. I., P. S. Roheim, and H. A. Eder. 1967. Turnover of cholesteryl esters of plasma lipoproteins in the rat. *J. Lipid Res.* **8**: 7–15.
57. Rose, H. G. 1972. Origin of cholesteryl esters in the blood of cholesterol-fed rabbits: relative contributions of serum lecithin:cholesteryl acyltransferase and hepatic ester synthesis. *Biochim. Biophys. Acta.* **260**: 312–326.
58. Stokke, K. T. 1974. Cholesteryl ester metabolism in liver and blood plasma of various animal species. *Atherosclerosis.* **19**: 393–406.
59. Glomset, J. A. 1970. Physiological role of lecithin:cholesterol acyltransferase. *Am. J. Clin. Nutr.* **23**: 1129–1136.
60. Akanuma, Y., and J. Glomset. 1968. *In vitro* incorporation of cholesterol-^{14}C into very low density lipoprotein cholesteryl esters. *J. Lipid Res.* **9**: 620–626.
61. Nichols, A. V., and L. Smith. 1965. Effect of very low density lipoproteins on lipid transfer in incubated serum. *J. Lipid Res.* **6**: 206–210.
62. Norum, K. R., J. A. Glomset, and E. Gjone. 1972. Familial lecithin:cholesterol acyl transferase deficiency. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill Book Company, New York. 3rd edition. 531–544.
63. Barter, P. J. 1974. Origin of esterified cholesterol transported in the very low density lipoproteins of human plasma. *J. Lipid Res.* **15**: 11–19.
64. Glomset, J. A., K. R. Norum, and W. King. 1970. Plasma lipoproteins in familial lecithin:cholesterol acyltransferase deficiency: lipid composition and reactivity in vitro. *J. Clin. Invest.* **49**: 1827–1837.
65. Glomset, J. A. 1973. The metabolic role of lecithin:cholesterol acyltransferase: perspectives from pathology. *Adv. Lipid Res.* **11**: 1–64.
66. Utermann, G., H. J. Menzel, and W. Schoenborn. 1975. Plasma lipoprotein abnormalities in a case of primary high-density-lipoprotein (HDL) deficiency. *Clin. Genet.* **8**: 258–268.
67. Gitlin, D., D. G. Cornwell, D. Nakasato, J. L. Oncley, W. L. Hughes, Jr., and C. A. Janeway. 1958. Studies on the metabolism of plasma proteins in the nephrotic syndrome. II. The lipoproteins. *J. Clin. Invest.* **37**: 172–184.
68. Havel, R. J., J. M. Felts, and C. M. Van Duyne. 1962. Formation and fate of endogenous triglycerides in blood plasma of rabbits. *J. Lipid Res.* **3**: 297–308.
69. Quarfordt, S. H., A. Frank, D. M. Shames, M. Berman, and D. Steinberg. 1970. Very low density lipoprotein triglyceride transport in type IV hyperlipoproteinemia and the effects of carbohydrate-rich diets. *J. Clin. Invest.* **49**: 2281–2297.
70. Eisenberg, S., D. W. Bilheimer, R. I. Levy, and F. T. Lindgren. 1973. On the metabolic conversion of human plasma very low density lipoprotein to low density lipoprotein. *Biochem. Biophys. Acta.* **323**: 361–377.