Isolation and characterization of an apoA-II-containing lipoprotein (LP-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia

P. Alaupovic,*† C. Knight-Gibson,* C-S. Wang,* D. Downs,* E. Koren,* H.B. Brewer, Jr., and R. E. Gregg[1,**]**

Lipoprotein and Atherosclerosis Research Program, Oklahoma Medical Research Foundation,* and Department of Biochemistry and Molecular Biology, Oklahoma University Health Sciences Center,† Oklahoma City, OK 73104, and Molecular Disease Branch, National Heart, Lung, and Blood Institute,** National Institutes of Health, Bethesda, MD 20892

Abstract Previous studies have shown that very low density lipoproteins (VLDL) from patients with Tangier disease are less effective as a substrate for human milk lipoprotein lipase (LPL) than VLDL from normal controls as assessed by measuring the first order rate constant (k_1) of triglyceride hydrolysis. Tangier VLDL also has a higher content of apolipoprotein (apo) A-II than normal VLDL. To explore the possible relationship between the relatively high concentration of apoA-II in VLDL and low k_1 values, Tangier VLDL were fractionated on an anti-apoA-II immunosorber. The retained fraction contained a newly identified triglyceride-rich lipoprotein characterized by the presence of apolipoproteins A-II, B, C-I, C-II, C-III, D, and E (LP-A-II:B:C:D:E or LP-A-II:B complex), whereas the unretained fraction consisted of previously identified triglyceride-rich apoB-containing lipoproteins free of apoA-II. In VLDL from patients with Tangier disease or type V hyperlipoproteinemia, the LP-A-II:B complex accounted for 70–90% and 25–70% of the total apoB content, respectively. The LP-A-II:B complexes had similar lipid and apolipoprotein composition; they were poor substrates for LPL as indicated by their low k_1 values (0.014–0.016 min^{-1}). In contrast, the apoA-II-free lipoproteins present in unretained fractions were effective substrates for LPL with k_1 values equal to or greater than 0.0313 min^{-1}. These results indicate that triglyceride-rich lipoproteins consist of several apoB-containing lipoproteins, including the LP-A-II:B complex, and that lipoprotein particles of similar size and density but distinct apolipoprotein composition also possess distinct metabolic properties. —**Alaupovic, P., C. Knight-Gibson, C-S. Wang, D. Downs, E. Koren, H. B. Brewer, Jr., and R. E. Gregg.** Isolation and characterization of an apoA-II-containing lipoprotein (LP-A-II:B complex) from plasma very low density lipoproteins of patients with Tangier disease and type V hyperlipoproteinemia. *J. Lipid Res.* 1991. **32:** 9–19.

Supplementary key words apoB-containing lipoproteins • apolipoproteins • neutral lipids • phospholipids • human milk lipoprotein lipase • immunoaffinity chromatography • monoclonal antibodies • electroimmunoassay

The characteristic biochemical feature of patients with Tangier disease is a severe deficiency of high density lipoproteins (HDL) and apolipoproteins A-I and A-II (1–3). In addition, patients with Tangier disease are characterized by hypocholesterolemia, mild to moderate hypertriglyceridemia, and reduced concentrations of apolipoproteins C-I, C-II, C-III, D, and E (4). Hypertriglyceridemia is manifested by the mild accumulation of chylomicrons and/or very low density lipoproteins (VLDL) and the marked triglyceride enrichment of low density lipoproteins (LDL) (5). Results of our previous study on the possible pathogenic mechanisms responsible for the impaired metabolism of triglyceride-rich lipoproteins in Tangier disease have shown that, in comparison with normal controls, Tangier patients had significantly decreased levels of plasma postheparin lipoprotein lipase (LPL) activity, increased levels of hepatic triglyceride lipase activity, and a lower reactivity of VLDL with human milk LPL (6). It has also been established that the protein composition of VLDL particles is characterized by a significant increase in the relative amount of apoA-II, suggesting a possible association between the abnormal apolipoprotein composition and an abnormally low reactivity of triglyceride-rich lipoproteins to LPL.

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LPL, lipoprotein lipase; EDTA, ethylenediamine tetraacetate; SDS, sodium dodecyl sulfate; CE, cholesteryl esters; FC, free cholesterol; TG, triglycerides; PL, phospholipids.

[1]Present address: Squibb Institute of Medical Research, P.O. 4000, Route 206, Princeton, NJ 08543-4000.

VLDL isolated from either normolipidemic or dyslipoproteinemic subjects are heterogeneous with respect to their physicochemical (7–12) and metabolic (13–17) properties. Studies from this (18–20) and other laboratories (12, 21–25) have indicated that VLDL may consist of several discrete lipoprotein particles rather than a single lipid-protein complex. We have suggested on the basis of these findings that apolipoprotein composition be used as the criterion for classifying and defining individual lipoprotein particles (18, 26, 27). Previous studies using immunoaffinity chromatography or a fractionation procedure based on sequential immunoprecipitation of VLDL with antibodies to apolipoproteins B, E, C-III, and C-II have disclosed (18–20) that apoB occurs in the form of at least four major lipoprotein particles identified on the basis of apolipoprotein composition as lipoprotein B (LP-B), lipoprotein B:C-I:C-II:C-III (LP-B:C), lipoprotein B:C-I:C-II:C-III:E (LP-B:C:E), and lipoprotein B:E (LP-B:E).

To explore the chemical nature of lipoprotein particles, including the presence of apoA-II-containing lipoproteins, Tangier VLDL were fractionated by immunoaffinity chromatography on an immunosorber with a monoclonal antibody to human plasma apoA-II (designated "pan" apoA-II antibody). Results of this study have revealed that Tangier VLDL contain a newly identified triglyceride-rich lipoprotein A-II:B:C-I:C-II:C-III:D:E (LP-A-II:B:C:D:E or further abbreviated as LP-A-II:B complex) characterized by a low pseudo first-order rate constant (k_1) of triglyceride hydrolysis; this lipoprotein accounts for 70–90% of the total apoB content of VLDL. In VLDL from patients with type V hyperlipoproteinemia, the LP-A-II:B complex accounted for 25–70% of the total apoB content. In contrast to the low lipolytic reactivity of the LP-A-II:B complex, the corresponding reactivity of the triglyceride-rich, apoA-II-free lipoprotein particles was normal. This study demonstrates that distinct triglyceride-rich lipoproteins characterized by specific apolipoprotein composition also have distinct substrate properties, and, possibly, specific roles in the transport and metabolism of triglycerides.

METHODS

Subjects

Four patients homozygous for Tangier disease and three patients with primary type V hyperlipoproteinemia were studied. The clinical features of two male patients and two female patients with Tangier disease have been previously reported (1, 4, 28). All four patients had classic biochemical characteristics of Tangier disease including concentrations of HDL < 2 mg/dl and apoA-I and apoA-II levels 1% and 5% of normal levels, respectively. The first male patient (No. 1, see Tables 2 and 3), age 32 years, had a plasma total cholesterol level of 52 mg/dl and triglyceride of 492 mg/dl; his plasma apolipoprotein B, C-I, C-II, C-III, D, and E concentrations were 58, 10.5, 2.4, 12.1, 4.2, and 7.4 mg/dl, respectively. The plasma total cholesterol and triglyceride levels of the second male patient (No. 2, see Tables 2 and 3), age 69 years, were 54 mg/dl and 112 mg/dl, respectively, wherease his apolipoprotein B, C-II, C-III, D, and E levels were 62.9, 0.9, 2.2, 3.9, and 5.5, respectively. This patient had a coronary bypass surgery 10 years ago. One of the female patients (No. 3, see Tables 2 and 3), age 33 years, had plasma total cholesterol and triglyceride levels of 90 and 243 mg/dl, respectively; her apolipoprotein B, C-II, C-III, D, and E levels were 94.3, 1.6. 9.4, 3.5, and 6.1 mg/dl, respectively. The other female patient (No. 4, see Tables 2 and 3) had plasma total cholesterol and triglyceride levels of 63 and 208 mg/dl, respectively, while her apolipoprotein B, C-I, C-II, C-III, D, and E levels were 91.2, 2.2, 2.1, 10.7, 1.6, and 2.8 mg/dl, respectively. Three hypertriglyceridemic male patients were selected from those attending the Lipid Clinic of the Oklahoma Medical Research Foundation. After excluding diseases predisposing to secondary hyperlipoproteinemias, patients were classified phenotypically as type V hyperlipoproteinemia on the basis of criteria outlined by the Lipid Research Clinics (29) of the National Institutes of Health, Bethesda, MD. None of the patients were on lipid-lowering drugs or diets expected to affect plasma lipids for at least 4 weeks prior to the blood collection for this study. The mean age of these three patients was 51 ± 3 years. The mean values of their plasma total cholesterol and triglyceride were 242 ± 89 and 1350 ± 544 mg/dl, respectively; the mean values for apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E were 121 ± 7, 56 ± 17, 117 ± 21, 15 ± 6, 9.6 ± 3, 34 ± 7, 16, and 21 ± 6 mg/dl, respectively. The concentrations of these apolipoproteins in normolipidemic, asymptomatic subjects have been reported previously (30).

Blood samples were obtained after an overnight fast (12 h) from donors in a sitting position. Blood was drawn by venipuncture into tubes that contained EDTA (1 mg/ml), and the plasma samples were collected by low-speed centrifugation. Blood from Tangier patients was drawn at the Molecular Disease Branch in Bethesda, MD, and the plasma samples were immediately shipped at 4°C and received in Oklahoma City, OK, the next morning. All analyses were performed immediately after the arrival of plasma samples in Oklahoma City. Plasma samples from patients with type V hyperlipoproteinemia had been kept for 24 h at 4°C before they were analyzed. All patients signed written informed consents. The protocols were approved by the Institutional Review Boards.

Production and characterization of monoclonal antibodies

Production and characterization of a "pan" monoclonal antibody to human plasma apoB has been previously described (31). Monoclonal antibodies to human plasma apoA-II were prepared according to a previously described procedure (32). Briefly, after immunization with purified apoA-II (33), mouse spleen cells were fused with mouse myeloma cells (Sp 2/0) using the polyethylene glycol method. Resulting hybridomas were cloned by the limiting dilution method. Clones were screened for the reactivity with chylomicrons, VLDL, LDL, HDL, and apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E using the enzyme-linked immunosorbent assay (ELISA) on microtiter plates (34). The antibody selected for this study (IgG_1, kappa) bound to delipidized apoA-II and all density classes with comparable affinity. By immunoblotting, the antibody was shown to bind to the apoA-II dimer, apoA-II monomer, and apoA-II:apoE complex. The microELISA assays revealed no reactivity with apolipoproteins other than apoA-II. When coupled to Affi-gel 10, the antibody to apoA-II allowed a complete removal of apoA-II-containing lipoproteins from normolipidemic as well as hyperlipidemic plasma. Based on these results, the antibody is considered to be a "pan" antibody (31) for apoA-II.

Isolation of very low density lipoproteins

Prior to the ultracentrifugal fractionation of triglyceride-rich lipoproteins, a preservative solution (35) (3.0 g penicillin-G, 0.5 g streptomycin sulfate, 13.1 g ϵ-amino caproic acid, and 10 g EDTA in 100 ml water, pH 7.0) was added to the plasma samples (0.1 ml/10 ml plasma) from the patients with Tangier disease and type V hyperlipoproteinemia. The ultracentrifugation was performed in a 50 Ti rotor using the Beckman L8-80 ultracentrifuge at 5°C according to a previously described procedure (7). To remove chylomicrons ($S_f < 400$), plasma samples were overlayered with an equal amount of a d 1.006 g/ml NaCl solution and centrifuged for 30 min at 17,500 rpm. The top layer was removed and the infranatant fraction was overlayered again with the d 1.006 g/ml NaCl solution; after ultracentrifugation for 22 h at 40,000 rpm, the top layer consisting of VLDL (S_f 20–400) was removed. The VLDL were washed once by recentrifugation at solution density 1.006 g/ml and, after exhaustive dialysis against 0.15 M NaCl, used for measurement of k_1 value and immunoaffinity chromatography.

Immunoaffinity chromatography

Monoclonal antibodies to human apolipoprotein B were coupled to Affi-gel 10 (Bio-Rad Laboratories, Richmond, CA) as previously described (34). The coupling to Affi-gel 10 of the antibody to human apoA-II was performed by the same procedure. The antibody to apoA-II was purified from ascites fluid by the use of protein A affinity column (Bio-Rad Laboratories). The Affi-gel 10 was washed with cold, double-distilled, deionized water and, after addition of the antibody solution to the gel slurry (10 mg protein/ml gel), the mixture was gently shaken for 2 h at 20°C. At the end of the incubation, the gel was allowed to settle and the supernatant liquid was carefully removed. To block the remaining active sites, the gel was incubated with 0.1 M Tris-HCl buffer containing 0.15 M NaCl, pH 7.4, for 18 h at 4°C. The gel was then washed with three volumes of 1.0 M NaCl and three volumes of 3 M NaSCN. This was followed by the equilibration with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 mg/ml EDTA. The binding capacity of the anti-apoA-II immunosorber was 0.05–0.1 mg of apoA-II per ml gel.

For analysis, 50-ml glass columns (1.2 × 49 cm, K-50 Pharmacia, Uppsala, Sweden) were packed under identical conditions as previously described (34, 36). Columns were first packed with 25 ml of Sephadex G-25 (medium) followed by 10 ml of antibody-coupled Affi-gel 10 and another protective layer of 5 ml of Sephadex G-25; the lower layer of Sephadex G-25 was used to minimize the exposure time between the lipoprotein and dissociating agent. The VLDL samples were applied to the column and incubated in the Affi-gel 10 layer for 12 h at room temperature. The unretained fraction was eluted with 0.05 M Tris-HCl buffer, pH 7.4, containing 0.15 M NaCl and 1.5 mg/ml EDTA. After the absorption at 280 nm returned to baseline, the column was washed with 150 ml of the Tris-HCl buffer and the retained fraction was eluted with 5 ml of 3 M NaSCN, pH 7.4. The bottom layer of Sephadex G-25 allowed an immediate separation of the retained lipoprotein fraction from the dissociating agent, which resulted in two distinct peaks at 280 nm, the first one consisting of lipoproteins and the second one of NaSCN. The structural integrity of apoB-containing lipoprotein particles isolated by immunoaffinity chromatography has been verified and documented in previous studies from this laboratory (34, 36, 37)

Determination of k_1 values for triglyceride-rich lipoproteins

The preparation of purified human milk LPL and the measurement of k_1 values were performed according to previously described procedures (6). The measurement of k_1 values was based on the use of 0.5 mM lipoprotein-triglyceride as substrate. The retained and unretained fraction eluted from the anti-apoA-II immunosorber were adjusted to this concentration of substrate by a procedure based on dialysis against sucrose placed outside the dialysis bags as previously described (37). Previous studies have established that this concentration procedure had no

effect on the lipoprotein composition or rate of lipolysis (34).

Immunoassays

Double diffusion analyses of lipoprotein particles were carried out in 1% agar (Special Agar Noble, Difco Laboratories, Detroit, MI) as previously described (38). Preparation of rabbit, goat, or sheep polyclonal antisera to human apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E was performed according to previously described procedures (38–41).

Apolipoproteins A-I, A-II, B, C-I, C-II, C-III, D, and E were quantified by previously described electroimmunoassays (39–44). All apolipoprotein assays were carried out in triplicate.

Lipid analyses

Plasma total cholesterol (CHOP/PAP, Boehringer, Mannheim, F.R.G.) and triglyceride (kits for total triglyceride and free glycerol, Abbott Laboratories, Irving, TX) levels were determined enzymatically on an Abbott VP-Super System analyzer. Both assays were standardized with serum calibrators and control samples supplied by the Centers for Disease Control, Atlanta, GA.

Neutral lipids (cholesteryl esters, free cholesterol, and triglycerides) of the lipoprotein particles were quantified by the gas-liquid chromatographic procedure described by Kuksis et al. (45). Phospholipid phosphorus content was determined by the method of Gerlach and Deuticke (46).

Polyacrylamide gel electrophoresis and immunoblotting

Basic (47) and acidic (48) polyacrylamide gel electrophoreses were carried out on 7% acrylamide gels containing 8 M urea.

Electrophoresis of lipoprotein particles was carried out in a 3.3% polyacrylamide slab gel containing SDS. The proteins were transferred to Immobilon transfer membrane (Millipore, Bedford, MA) by electroblotting and immunoblotted as described by Lee and Singh (49). A polyclonal antiserum to apoA-IV was provided by Dr. Walter J. McConathy, Oklahoma Medical Research Foundation.

RESULTS

We have reported that VLDL of patients with Tangier disease have a significantly higher concentration of apoA-II and a lower reactivity towards human milk LPL (k_1 values) than VLDL of normolipidemic, asymptomatic controls (6). To establish whether these abnormal features may also be characteristic of VLDL from patients with another dyslipoproteinemia, we selected patients with phenotype V hyperlipoproteinemia for comparison. There were marked differences in the concentrations but not in percent composition of VLDL lipid and protein constituents between patients with type V hyperlipoproteinemia and Tangier disease. The concentrations of apolipoproteins varied within and between patient groups to a greater extent than the corresponding percent composition (**Table 1**). In general, the relative content of apoA-II was higher and relative contents of apoC-III and apoE were lower in patients with Tangier disease than in patients with type V hyperlipoproteinemia. However, both the absolute and relative contents of apoA-II in VLDL from type V patients were still higher than those of VLDL from normolipidemic subjects (Table 1). To determine whether patients with Tangier disease differed from patients with type V hyperlipoproteinemia with respect to the substrate reactivity of their VLDL towards lipolysis and to gain further insight into the possible relationship between the substrate reactivity and the levels of apoA-II, we determined, in both groups of patients, the pseudo first-order rate constants (k_1) of VLDL triglyceride hydrolysis catalyzed by human milk LPL (6). The k_1 values of Tangier VLDL were lower (0.0168 ± 0.001 min^{-1}; mean ± SD) than those of VLDL from patients with type V hyperlipoproteinemia (0.0309 ± 0.009 min^{-1}).

TABLE 1. Concentration and composition of apolipoproteins in VLDL of patients with Tangier disease and type V hyperlipoproteinemia

Patients	Apolipoproteins						
	A-II	B	C-I	C-II	C-III	D	E
	mg/dl (%)						
Tangier disease (n = 4)	0.96 ± 0.81 (6.1 ± 1.3)	8.3 ± 6.9 (51.2 ± 3.8)	1.7 ± 1.5 (9.3 ± 1.4)	0.5 ± 0.1 (4.8 ± 2.9)	3.3 ± 3.1 (18.5 ± 1.7)	0.45 ± 0.4 (1.6 ± 0.9)	1.7 ± 1.6 (8.9 ± 4.5)
Hyperlipoproteinemia type V (n = 3)	2.1 ± 1.4 (1.4 ± 0.8)	68.3 ± 46.3 (43.5 ± 3.4)	10.6 ± 9.2 (6.0 ± 2.0)	7.5 ± 8.0 (4.4 ± 2.3)	38.2 ± 12.4 (28.2 ± 8.3)	2.8 ± 1.0 (2.8 ± 2.5)	24.3 ± 20.3 (14.0 ± 5.2)
Normal (n = 5)	0.03 ± 0.02 (0.7 ± 0.9)	3.5 ± 2.1 (37.0 ± 7.0)	0.5 ± 0.4 (6.3 ± 3.0)	0.4 ± 0.2 (6.6 ± 1.4)	1.2 ± 0.7 (21.1 ± 4.9)	Trace	0.6 ± 0.4 (8.3 ± 3.0)

Values are expressed as mean ± SD.

However, the k_1 values of VLDL from type V patients appeared to be lower than those of normolipidemic VLDL (0.036 ± 0.008 min^{-1}) (6). The apoA-II/triglyceride ratios of Tangier VLDL (0.0097 ± 0.0009) were higher than those of type V VLDL (0.0008 ± 0.0007) suggesting a possible inverse correlation between the apoA-II content of triglyceride-rich lipoproteins and their substrate reactivity towards LPL.

These findings have confirmed the abnormal apolipoprotein composition and substrate reactivity of Tangier VLDL (6) and indicated that the same abnormality may be present, but to a lesser degree, in VLDL of patients with primary hypertriglyceridemia. Furthermore, they have suggested that VLDL from patients with hypertriglyceridemia of different etiologies may contain varying concentrations of an apoA-II-containing triglyceride-rich lipoprotein which possesses a lower substrate reactivity towards human milk LPL than triglyceride-rich lipoproteins free of apoA-II.

Fractionation of VLDL by immunoaffinity chromatography on anti-apoA-II immunosorber

To isolate an apoA-II containing lipoprotein, we first applied Tangier VLDL to an immunosorber with a monoclonal "pan" apoA-II antibody. The fractionation of VLDL was monitored by determining apoA-II in the retained and unretained fractions by immunodiffusion and electroimmunoassay. The absence of apoA-II in the unretained fractions was used as the criterion for the complete separation of apoA-II-containing lipoprotein from lipoproteins free of apoA-II. The distribution of apoA-II-containing lipoproteins present in retained fractions and apoA-II-free lipoproteins present in unretained fractions was estimated by quantifying their contents of apoB. In the majority of patients, approximately 90% of Tangier VLDL was present in the retained and 10% in the unretained fraction (**Table 2**). Similar studies revealed that, in type V hyperlipoproteinemia, VLDL were also retained on the anti-apoA-II immunosorber, but to a lesser extent (25–70%) than Tangier VLDL. The mean k_1 value of anti-apoA-II retained fractions (0.0161 ± 0.002 min^{-1} (**Table 3**) from Tangier patients was only slightly lower than that of the corresponding VLDL (0.0168 ± 0.001 min^{-1}). In contrast, the mean k_1 value of anti-apoA-II retained fractions (0.0146 ± 0.001 min^{-1}, Table 3) from patients with type V hyperlipoproteinemia was not only substantially lower than the k_1 value of their corresponding VLDL (0.0309 ± 0.009 min^{-1}) but was also very similar to that of anti-apoA-II retained fractions isolated from Tangier plasma.

The lipid and apolipoprotein composition of the anti-apoA-II retained fraction isolated from VLDL of patients with Tangier disease differed very little from that of patients with type V hyperlipoproteinemia except for a slightly lower relative content of cholesteryl esters and slightly higher percentages of phospholipids and apoA-II (**Table 4**). The chemical composition of anti-apoA-II unretained fraction from VLDL of patients with type V hyperlipoproteinemia was similar to that of anti-apoA-II retained fractions except for the absence of apoA-II. In contrast to the compositional similarity between anti-apoA-II retained fractions from VLDL of Tangier and type V patients, the corresponding anti-apoA-II unretained fractions differed considerably in apolipoprotein composition (Table 4) suggesting that, apart from a lipoprotein characterized by the presence of apoA-II, VLDL of these two dyslipoproteinemic states may have qualitatively and/or quantitatively different apoB-containing lipoprotein particles such as LP-B, LP-B:C, LP-B:E, and LP-B:C:E (19, 20)

A comparative study on k_1 values of anti-apoA-II retained and unretained fractions could only be performed with VLDL from patients with type V hyperlipoproteinemia, because insufficient amounts of unretained fractions prevented a similar study with Tangier patients. As shown in Table 3, the k_1 values of retained fractions were significantly lower ($P < 0.0001$) than the k_1 values of unretained fractions isolated from the same patients. Despite potentially marked heterogeneity of apoB-containing lipoprotein particles in anti-apoA-II unretained fractions, their k_1 values were always higher than the k_1 values of the corresponding anti-apoA-II retained

TABLE 2. Percent distribution of anti-apoA-II retained and anti-apoA-II unretained fractions from VLDL of patients with Tangier disease and type V hyperlipoproteinemia

Patients	Fractions	Apolipoprotein B
		%
Tangier disease		
1	Anti-apoA-II-R	90.0
	Anti-apoA-II-U	10.0
2	Anti-apoA-II-R	70.0
	Anti-apoA-II-U	30.0
3	Anti-apoA-II-R	91.0
	Anti-apoA-II-U	9.0
4	Anti-apoA-II-R	91.2
	Anti-apoA-II-U	8.8
Hyperlipoproteinemia type V		
1	Anti-apoA-II-R	25.0
	Anti-apoA-II-U	75.0
2	Anti-apoA-II-R	61.0
	Anti-apoA-II-U	39.0
3	Anti-apoA-II-R	69.0
	Anti-apoA-II-U	31.0

Immunoaffinity chromatography was carried out on an immunosorber with "pan" monoclonal antibodies to human apoA-II as described in the Methods; anti-apoA-II-R, retained fraction; anti-apoA-II-U, unretained fraction. Distributions are expressed as percentages of apolipoprotein B present in retained and unretained fractions.

TABLE 3. The k_1 values of anti-apoA-II retained and anti-apoA-II unretained fractions from VLDL of patients with Tangier disease and type V hyperlipoproteinemia

Patients	k_1 Values	
	Anti-ApoA-II-Retained Fraction	Anti-ApoA-II-Unretained Fraction
	min^{-1}	
Tangier disease		
1	0.0138	ND
2	0.0154	ND
3	0.0185	ND
4	0.0168	ND
Mean ± SD	0.0161 ± 0.002	
Hyperlipoproteinemia type V		
1	0.0151	0.0315
2	0.0143	0.0313
3	0.0145	0.0313
Mean ± SD	0.0146 ± 0.001	0.0313 ± 0.0001
	$P < 0.0001$[a]	

Anti-apoA-II retained fraction is the VLDL fraction retained on immunosorber with "pan" monoclonal antibody to human apoA-II; anti-apoA-II unretained fraction is the VLDL fraction not retained on this immunosorber; ND, not determined. The quantities of anti-apoA-II unretained fraction from patients with Tangier disease were not sufficient to perform the measurement of k_1 values.

[a]Student's *t*-test.

fractions. On the other hand, there was no difference in the k_1 values between anti-apoA-II retained fractions isolated from VLDL of Tangier patients or patients with type V disease.

These studies have established that VLDL from patients with Tangier disease or type V hyperlipoproteinemia contain a distinct triglyceride-rich apoA-II-containing lipoprotein referred to as lipoprotein A-II:B complex (LP-A-II:B complex). Because of its low k_1 value, LP-A-II:B complex may be responsible for the relatively low substrate reactivity of VLDL towards lipolysis in Tangier disease and type V hyperlipoproteinemia.

Partial characterization of anti-apoA-II retained (LP-A-II:B complex) and unretained fractions of VLDL

Although present in different concentrations, the LP-A-II:B complexes isolated from VLDL of Tangier disease and type V disease had similar lipid and apolipoprotein composition (Table 4) and similar, if not identical, k_1 values (Table 3). The protein moiety of LP-A-II:B complex accounted for 9–11% of the total lipoprotein mass with triglycerides, phospholipids, cholesteryl esters, and free cholesterol constituting the remaining lipoprotein mass. A more detailed quantitative analysis revealed that LP-A-II:B complex contains apolipoproteins A-II, B, C-

TABLE 4. Percent lipid and apolipoprotein composition of anti-apoA-II-retained and anti-apoA-II-unretained fractions isolated from VLDL of patients with Tangier disease and type V hyperlipoproteinemia

Fractions	Lipids				Apolipoproteins			
	CE	FC	TG	PL	A-II	B	C-III	E
Tangier disease								
Anti-apoA-II-R (n = 4)	10.9 ± 1.5	5.2 ± 0.8	58.7 ± 1.7	24.9 ± 3.8	9.3 ± 3.1	49.4 ± 1.4	22.5 ± 7.8	18.7 ± 5.2
Anti-apoA-II-U (n = 1)	10.2	5.1	64.6	20.0	0.0	39.5	27.0	33.4
Hyperlipoproteinemia type V								
Anti-apoA-II-R (n = 3)	13.6 ± 2.9	6.1 ± 0.8	61.6 ± 8.9	18.6 ± 5.7	6.7 ± 3.1	52.1 ± 5.0	21.0 ± 1.8	19.9 ± 2.2
Anti-apoA-II-U (n = 3)	11.9 ± 3.8	5.6 ± 1.2	67.0 ± 4.1	15.4 ± 3.7	0.0	59.6 ± 2.6	22.1 ± 4.8	18.2 ± 6.1

Values are given as means ± SD. Abbreviations: CE, cholesteryl ester; FC, free cholesterol; TG, triglycerides; PL, phospholipids; anti-apoA-II-R, retained fraction; anti-apoA-II-U, unretained fraction.

TABLE 5. Apolipoprotein composition of anti-apoA-II retained (LP-A-II:B complex) and anti-apoA-II unretained fractions

Immunosorber	n	Apolipoproteins						
		A-II	B	C-I	C-II	C-III	D	E
		%						
Tangier disease								
Anti-apoA-II-R (LP-A-II:B complex)	3	4.7 ± 0.6	45.0 ± 4.7	7.8 ± 1.9	6.3 ± 1.1	20.3 ± 2.1	0.9 ± 0.3	14.4 ± 2.2
Anti-apoA-II-R:Anti-ApoB-R (LP-A-II:B complex)[a]	3	4.6 ± 0.9	48.6 ± 6.7	8.0 ± 2.5	6.4 ± 2.5	18.9 ± 5.4	0.7 ± 0.6	12.6 ± 1.5
Hyperlipoproteinemia type V								
Anti-apoA-II-R (LP-A-II:B complex)	3	5.5 ± 2.8	46.4 ± 7.1	6.0 ± 0.9	7.4 ± 1.7	16.6 ± 2.8	1.7 ± 0.9	16.0 ± 1.7
Anti-apoA-II-U[b]	3	0	48.8 ± 3.0	9.8 ± 2.5	7.9 ± 1.0	16.0 ± 3.5	0	17.2 ± 4.4

Values are given as mean ± SD; anti-apoA-II-R is the VLDL fraction retained on immunosorber with "pan" monoclonal antibody to human apoA-II; anti-apoA-II-U is the VLDL fraction not retained on this immunosorber.

[a]To test for the structural stability of LP-A-II:B complex, anti-apoA-II retained fractions were rechromatographed on an anti-apoB immunosorber; the retained fractions were characterized by determination of apolipoprotein composition. This test was only applied to anti-apoA-II retained fractions isolated from patients with Tangier disease.

[b]The amounts of anti-apoA-II unretained fractions from patients with Tangier disease were insufficient for a complete apolipoprotein characterization.

III, and E as the major and apolipoproteins C-I, C-II, and D as the minor protein constituents (**Table 5**). The presence of these apolipoproteins was confirmed qualitatively by basic and acidic polyacrylamide gel electrophoresis, immunodiffusion, and immunoblotting (patterns not shown). To ascertain the structural integrity of LP-A-II:B complex, the anti-apoA-II retained fractions isolated from VLDL of patients with Tangier disease were rechromatographed on an immunosorber with the "pan" monoclonal antibody to apoB. If apoB and other apolipoproteins identified in LP-A-II:B complex are true integral components of the complex, the percent lipid and apolipoprotein composition of fractions retained on anti-apoB immunosorber should not change appreciably from those of anti-apoA-II retained fraction. Results showed that the lipid composition of anti-apoB retained fraction was very similar to that of anti-apoA-II retained fraction (Table 4) with cholesteryl esters accounting for 8.6%, free cholesterol 4.8%, triglycerides 62.3%, and phospholipids 24.1% of total lipid. The apolipoprotein composition of anti-apoB retained fraction was also found to be very similar to that of anti-apoA-II retained fraction, indicating that apolipoproteins A-II, B, C-I, C-II, C-III, D, and E are, indeed, integral constitutents of a lipoprotein family of particles with neutral lipid and phospholipid composition typical of a very low density lipoprotein.

In some anti-apoA-II retained fractions, trace amounts of apoA-I could also be detected by electroimmunoassay. However, this retained apoA-I was not considered to be an integral protein component of the LP-A-II:B complex, because it was eliminated by subsequent chromatography on anti-apoB immunosorber. The presence of apoA-I in VLDL fractions seems to be due most probably to a nonspecific binding of lipid-poor LP-A-I or lipid-free apoA-I to the matrix of the immunosorber. Immunoblotting tests showed that, in the freshly isolated LP-A-II:B complex, apolipoproteins A-II and E did not occur as apo(E:A-II) complex but as separate apolipoprotein constituents. Immunoblotting also failed to detect the presence of apoA-IV in the LP-A-II:B complex. The only apoB form detected was apoB-100.

The lipid composition of the anti-apoA-II unretained fractions was very similar to that of retained fractions except for a slightly higher percentage of triglycerides and a lower percentage of phospholipids (Table 4). The main difference in the apolipoprotein composition was the absence in the anti-apoA-II unretained fraction of apoA-II and apoD as assessed by electro-immunoassay (Tables 4 and 5), basic polyacrylamide gel electrophoresis, and immunodiffusion analysis (not shown).

DISCUSSION

Apolipoproteins play an essential role in maintaining the structural stability and functional specificity of lipoprotein particles (1, 18, 26, 27, 50, 51). Furthermore, as unique constituents of lipoprotein particles, apolipoproteins are also recognized as ideal markers for identifying, characterizing, and classifying discrete lipoprotein particles irrespective of their sizes or hydrated densities (18, 21, 23, 26, 27, 52). We have suggested, on the basis of chemical and immunological evidence, that each of the major lipoprotein density classes consists of a variety of discrete, polydisperse lipoprotein particles defined by their unique chemical composition and possessing specific biological properties (27, 52). It has been demonstrated

that the major lipoprotein forms of apoB in very low and low density segments of the human plasma lipoprotein spectrum include LP-B, LP-B:C, LP-B:C:E (19, 20, 37, 53), and LP-B:E (54). Results of the present study show that VLDL from patients with type V hyperlipoproteinemia and Tangier disease contain another lipoprotein form of apoB identified by its apolipoprotein composition as LP-A-II:B:C-I:C-II:C-III:D:E (LP-A-II:B complex). Although the relative content of LP-A-II:B complex in Tangier VLDL was greater than that in VLDL of patients with phenotype V, the lipid and apolipoprotein composition and the k_1 values have shown a very close similarity between the LP-A-II:B complexes isolated from two different hypertriglyceridemic sources. The absence of apoA-II and apoD was the main characteristic of the apolipoprotein composition of triglyceride-rich fractions not retained by anti-apoA-II immunosorber. Because the anti-apoA-II unretained fractions consist of several discrete apoB-containing lipoproteins, their chemical composition may vary among individual patients to a greater extent than the chemical composition of anti-apoA-II retained fractions which only contain LP-A-II:B complex. However, the k_1 values of the unretained fractions were always significantly higher than those of correpsonding retained fractions or, in other words, lipoproteins present in anti-apoA-II unretained fractions were more effective as a substrate for LPL than the LP-A-II:B complex. This finding shows clearly that, within VLDL of type V and Tangier patients, there are two subpopulations of triglyceride-rich lipoprotein particles that differ both in their apolipoprotein composition and in one of their functional properties, i.e., the reactivity towards LPL.

The compositional and structural integrity of LP-A-II:B complex was assessed by sequential immunoaffinity chromatography using an anti-apoA-II immunosorber in the first step and an anti-apoB immunosorber in the second step. These experiments showed a minimal change in the lipid and apolipoprotein composition of LP-A-II:B complex present in the retained fractions from either of these two immunosorbers. This finding underscores not only the stability of such a complex lipoprotein particle but also the already documented suitability of immunoaffinity chromatography as a mild isolation procedure for lipoproteins defined by their apolipoprotein composition (34, 36, 37, 55–59). It should also be pointed out that the k_1 value of the LP-A-II:B complex recovered after its successive chromatographies on anti-apoA-II and anti-apoB immunosorbers was the same as the k_1 value of the LP-A-II:B complex present in the initial anti-apoA-II retained fraction, indicating no effect of an additional immunoaffinity chromatography step on this functional property of LP-A-II:B complex.

The site and mode of the LP-A-II:B complex formation is not known. Several in vitro studies have demonstrated the capacity of apoA-II to be incorporated into HDL by replacing apoA-I (60–62) and into VLDL by replacing apoE (63) without affecting the lipid composition of acceptor-lipoproteins. In plasma, apoA-II appears to be present mainly in the form of LP-A-I:A-II particles (56, 58). In view of its relatively high lipid binding capacity (62), apoA-II in LP-A-I:A-II particles would not seem to be readily available for the interaction with triglyceride-rich lipoprotein particles. However, it has been demonstrated that, in Tangier disease, apoA-II may occur without apoA-I as an LP-A-II particle (64) or, in another case of human plasma apoA deficiency as an LP-A-II:D particle (65). One or both of these particles may be the source of apoA-II or apoA-II and apoD in the LP-A-II:B complex. It was observed previously that, in Tangier patients 25 h after infusion of homologous HDL, apoA-II associated with β- and preβ-lipoproteins while apoA-I disappeared from HDL (66). Although it is not known whether LP-A-II particles occur in plasma of type V patients, it appears reasonable to speculate that they may serve as potential precursors of the LP-A-II:B complex by interacting, for example, with triglyceride-rich LP-B:C:E particles. Whether the LP-A-II:B complex is formed through such displacement reactions or some other mechanism remains to be tested in future experiments.

Kesaniemi and Grundy (67) have established that hypertriglyceridemia in patients with type V hyperlipoproteinemia is due to both overproduction and impaired degradation of VLDL triglycerides and suggested the possible presence of a structurally or compositionally defective triglyceride-rich lipoprotein as a major contributor to the catabolic defect. In view of the data presented in this report, the LP-A-II:B complex may be responsible for and considered as a marker for delayed clearance of VLDL triglycerides. This possibility remains to be tested and explored in future studies including the presence and distribution of LP-A-II:B complex in various hypertriglyceridemic states and its association with the clearance defect as one of the possible underlying causes.

Several recent studies have established a close relationship between the apolipoprotein composition and biological activity of lipoprotein particles of similar hydrated densities. For example, in VLDL, lipoprotein particles with and without apoE were shown to differ significantly with respect to their binding capacity to normal skin fibroblasts (68) or their conversion rates to particles of higher densities (69). Similarly in LDL, LP-B:E particles displayed higher binding affinity for HepG2 cell membranes than LP-B particles (54). In a study with cultured adipose cells, Barbaras et al. (70) observed that human plasma LP-A-I, but not LP-A-I:A-II particles, have the capacity to promote cholesterol efflux from the cells. The identification of LP-A-II:B complex as one of the VLDL lipoprotein particles with distinct apolipoprotein composition and biological properties provides further evidence that apolipoproteins are the essential constituents for the

structural integrity and functional specificity of plasma lipoproteins and that lipoprotein particles defined by their apolipoprotein composition may be considered as the fundamental chemical and metabolic entities of lipid transport.

We thank Dr. Walter J. McConathy for providing the antiserum to human apoA-IV. The technical assistance of Ms. Cindy SaeLim and Mr. Randall Whitmer is gratefully acknowledged. We also thank Ms. Margo French for her secretarial assistance and preparation of the manuscript. This study was supported, in part, by grant HL-23181 from the National Institutes of Health and by the resources of the Oklahoma Medical Research Foundation.

Manuscript received 18 September 1989 and in revised form 31 July 1990.

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