Characterization of Apolipoprotein A-IV Complexes and A-IV Isoforms in Human Lymph and Plasma Lipoproteins

(Received for publication, January 3, 1984)

Takao Ohta†, Noel H. Fidge‡, and Paul J. Nestel

From the Baker Medical Research Institute, Commercial Road, Prahran, Victoria 3181, Australia

We have isolated and characterized A-IV apolipoprotein (apo-A-IV) from human lymph and plasma by immunoadsorbance chromatography and two-dimensional electrophoresis. Two different apo-A-IV-containing lipoproteins were isolated from four different sources, human lymph triglyceride-rich fraction (TRL), lymph lipoprotein-deficient fraction (LDF), plasma high-density lipoprotein (HDL), and plasma lipoprotein-deficient fraction (LDF). The lipoprotein complexes obtained from lymph TRL and plasma HDL were similar and contained apo-A-IV, apo-A-I, and small molecular weight peptides (apo-C or -A-II). The second lipoprotein complex was isolated from lymph LDF and plasma LDF, and contained apo-A-IV, apo-A-I, and a peptide of Mr = 59,000. The lipid composition of the lipoprotein complexes varied according to the source: triglyceride predominating in lymph TRL and phospholipid and cholesterol ester from the other sources. Free cholesterol was conspicuously present in very small amounts.

Using two-dimensional electrophoresis and immunoblotting techniques, eleven isoproteins of apo-A-IV were identified (pI=4.98, 5.06, 5.10, 5.15, 5.20, 5.22, 5.25, 5.30, 5.34, 5.42, and 5.48). The isoprotein pattern of lymph TRL and plasma HDL was similar, but that of lymph and plasma LDF were different patterns. These results suggest that apo-A-IV associated with d < 1.21 lipoproteins and apo-A-IV present in LDF may be in metabolically separate lipoproteins and may have different physiological roles.

A recently described plasma protein, A-IV apolipoprotein, is a major component of newly synthesized chylomicrons obtained from human rat, and canine lymph (1–3). It is immunologically distinct from other apolipoproteins and has a Mr = 46,000. In human plasma, apo-A-IV is mainly present in the lipoprotein-deficient fraction and in minor amounts within HDL (2–4). The distribution within plasma varies among individuals, with a slight increase in the proportion associated with triglyceride-rich lipoproteins in lipemic plasma. It is found in human intestinal epithelial cells (4) and its concentration in plasma rises modestly after fat ingestion (4); in the rat apo-A-IV is synthesized mainly in the intestine and liver (5). While the physiological function of apo-A-IV appears to be linked to the transport of absorbed triglyceride, the unusual distribution of this apolipoprotein, with the bulk unassociated with lipoproteins, is puzzling. Furthermore, since it has been established that other apolipoproteins, such as A-I, exist as isoforms some of which are pro-proteins (6–9) it seemed possible that the distribution of apo-A-IV in plasma might reflect a variable distribution of individual isoforms. We have therefore carried out a detailed investigation of the structure of apo-A-IV using an immunoadsorbance technique which has enabled us to compare the native states of A-IV apolipoprotein in plasma and lymph chylomicrons and to identify the existence of complexes of apo-A-IV with other apolipoproteins.

MATERIALS AND METHODS

Isolation of Apolipoprotein A-IV from LDF of Plasma—Plasma was obtained from the Red Cross Bloodbank (Melbourne) on the same day of blood collection. The blood contained citrate/phosphate/dextrose as anticoagulant and lipoprotein fractionation was started immediately on arrival. Solid KBr was added to give a non-protein solvent density of 1.25 g/ml and lipoproteins were separated by centrifugation in a Ti 45 rotor at 43,000 rpm for 48 h. The floating lipoproteins were removed by aspiration and the infranatant protein solution was dialyzed exhaustively against 0.15 M NaCl, containing 1 mM EDTA buffered to pH 7.4 with 2-amin-2-hydroxymethyl-1,3-propanediol base (buffer A).

The dialyzed 1.25 density infranatant fraction (approximately 600 ml) was mixed with 0.3 volumes of Neutralipid (artificial fat emulsion, Pharmacia) and incubated at 37 °C for 2 h. After centrifugation in a Beckman SW 41 rotor for 30 min at 4 °C, the supernatant lipid layer was dispersed in buffer A to yield the original volume of Neutralipid and centrifuged twice more to remove unbound plasma protein. Finally, the lipid layer was resuspended in 30 ml of buffer A and delipidated with chloroform/methanol/ether as described previously (10).

The delipidated protein was immediately dissolved in 10 ml of 0.1 M Tris-HCl, pH 7.5, containing 8 M urea and subjected to chromatography on a column (1.5 × 30 cm) of QAé (quaternary aminomethyl)-Sephadex A-50 (Pharmacia). The column was eluted using a linear gradient of NaCl which was established between 0 and 0.3 M NaCl in 6 M urea, 0.1 M Tris-HCl, pH 7.5 (300 ml each). Fractions of 4 ml were collected at 4 °C at a flow rate of 15 ml/h. Final purification of apolipoprotein A-IV was carried out using preparative gel electrophoresis on 10% polyacrylamide gels (in 0.1% SDS) in the presence of β-mercaptoethanol (11). Protein bands were visualized using 4 M sodium acetate and the proteins eluted from crushed gel slices into 20 volumes of 0.1% SDS in 0.05 M NH₄HCO₃, pH 8.0 (eluting buffer), containing 0.01% sodium azide by incubating overnight at 37 °C on a shaking waterbath. The gel pieces were sedimented by centrifugation (400 × g), the supernatant filtered, and the gel slices washed once with elution buffer. The filtrates were pooled and the SDS removed by dialysis for 1 week (with changes of dialysate twice daily) against 5 mM NH₄HCO₃, pH 8.0, containing sodium azide and thimerosal. Apolipoproteins were then lyophilized (12). Recovery of apo-A-IV from SDS gels was approximately 65%.

Preparation of Antiserum—New Zealand White rabbits (2.5–3 kg)
were immunized with 100 μg of purified apolipoprotein A-IV from plasma LDF or lymph TRL. The protein solution was emulsified with an equal volume of Freund's complete adjuvant and injected intradermally into multiple sites on the back of the rabbits. The injection was repeated three times at intervals of 2-3 weeks and animals were bled 8 days after the last injection. The antiserum did not react with purified A-I, E, B, C-III, C-IV, or C apolipoproteins or albumin. Anti-human β2-glycoprotein I serum was purchased from Calbiochem-Behring Australia Pty. Ltd.

Apolipoprotein A-IV-Sepharose 4B Affinity Column—5 mg of purified apo-A-IV from plasma LDF were extensively dialyzed against 0.1 M NaHCO₃, containing 0.5 M NaCl, pH 8.0, and coupled to CNBr-activated Sepharose 4B (Pharmacia) at pH 8.0 as described by Cuatrecasas (13). A ratio of 1 mg of apo-A-IV/ml of activated Sepharose 4B was utilized. Finally, apo-A-IV-Sepharose 4B was equilibrated with 0.1 M borate containing 1.0 M NaCl and 0.1% Tween 20, pH 8.0, and transferred to chromatography columns making a gel bed of 1.0 X 7.0 cm.

100 ml of antiserum to apo-A-IV was used for the isolation of apo-A-IV specific IgG. Antiserum was dialyzed against equilibrating buffer and applied to the apo-A-IV-Sepharose 4B affinity column. Material bound to the column was eluted with 0.2 M glycine/HCl, pH 2.2, and dialyzed against buffer A. The eluate was monitored for reaction with apo-A-IV. 80 mg of apo-A-IV specific IgG were obtained from 100 ml of apo-A-IV antiserum.

Anti-apo-A-IV-Sepharose 4B Column (A-IV Immunoblotting Column)—2 mg of apo-A-IV specific IgG were coupled to CNBr-activated Sepharose 4B as described by Cuatrecasas (13). A ratio of 2 mg of IgG/ml of activated Sepharose 4B was utilized. Chromatography was performed on a column of 2.5 X 30 cm. Anti- apo-A-IV-Sepharose 4B was packed on twice the bed volume of Sephadex G-25 (Medium, Pharmacia) in 0.05 M Tris containing 0.5 M NaCl, pH 7.5, to avoid prolonged interaction of the desorbing reagent with protein eluted from the column. The column was equilibrated with 0.05 M Tns containing 0.5 M NaCl and 0.01% NaN₃ at pH 7.5.

Isolation of Apolipoprotein-A-IV from Human Lymph Tryglyceride-rich Lipoprotein—Tryglyceride-rich lipoprotein was isolated from lymph obtained from chylous pleural effusion of a patient with tuberos sclerosis and lymphangiomatosus of the thoracic duct by continuous low-pressure suction. Antibacterial agent, sodium azide (0.01%), and thimerosal (0.005%), were added to the sample which was ultracentrifuged for 5 h at 4 °C within 24 h of collection. Omission of azide and thimerosal in preliminary experiments made no difference to the pattern of apo-A-IV isoforms. The top fraction was recentrifuged for 8 h at 50,000 rpm in a Beckman 50 Ti rotor. A portion of the lipoprotein was delipidated with chloroform/methanol/ether as described above. The apolipoprotein moiety was dissolved in 0.1 M K₂HPO₄, containing 0.5 M NaCl, pH 6.5, and coupled to CNBr-activated Sepharose 4B as described by Cuatrecasas (13). A ratio of 2 mg of IgG/ml of activated Sepharose 4B was utilized. Chromatography was performed on a column of 2.5 X 30 cm. Anti-apo-A-IV-Sepharose 4B was packed on twice the bed volume of Sephadex G-25 (Medium, Pharmacia) in 0.05 M Tris containing 0.5 M NaCl, pH 7.5, to avoid prolonged interaction of the desorbing reagent with protein eluted from the column. The column was equilibrated with 0.05 M Tns containing 0.5 M NaCl and 0.01% NaN₃ at pH 7.5.

Amino acid analysis was performed on a Beckman 6300 analyzer. Samples were dialyzed against distilled water, lyophilized, and hydrolyzed in 6 N HCl at 115 °C for 24 h in evacuated sealed tubes.

Isoelectric Focusing and Two-dimensional Gel Electrophoresis—In order to determine the pl values of A-IV isoforms, isoelectric focusing was performed in polyacrylamide disc gels containing 8% urea, and ampholytes (4-6 range) essentially as described by Gidez et al. (15). pl values were determined by comparison with standards (isoelectric focusing kit, Pharmacia) focused in the same run as unknown samples.

Two-dimensional gel electrophoresis was performed first in isoelectric focusing gels (2 X 150 mm) using ampholytes in the pH 4-6 range, as described above, for 18 h at 400 V. The gels were then equilibrated in sample buffer for 20 min and placed above SDS slab gels (15% polyacrylamide containing 0.1% SDS), using 1% agarose in the imbedding gel (16). A standard protein mixture was applied to a sample lane in all slab gels in order to compare unknown proteins with purified A-I, E, A-IV, C, and A-II apolipoprotein. After electrophoresis, gels were fixed and stained with Coomassie Blue (17).

Electrophoretic transfer was accomplished at 6 V/cm (with respect to electrode separation) for 3.5 h using the Bio-Rad model 160/18 power supply.

Iodination of Apo-A-IV—Apo-A-IV (0.1 mg dissolved in 0.2 ml of 4 M borate buffer, pH 8.0) was labeled with 125I by the iodine monochloride technique (12). Efficiency of labeling was approximately 25% and the iodine/protein ratio was less than 1 g/atom of iodine/mg of protein. Removal of free 125I was accomplished by gel filtration through Sephadex G-50 columns (0.7 X 20 cm), equilibrated with buffer A followed by dialysis against the same buffer.

Lipid Analysis—Fractions obtained from immunoabsorbance columns were extracted with chloroform/methanol according to Folch et al. (19). Lipids other than phospholipid were analyzed by thin-layer chromatography on silica gel rods using the Iatroscan Th-10 (Iatron Lab) (20). Phospholipid was analyzed by the method of Bartlett (21).

Protein content of each fraction from immunoabsorbance columns was measured by the method of Lowry et al. (22).

RESULTS

Isolation of Apo-A-IV from Plasma LDF—As shown in Fig. 1, the neutral lipid-bound protein consisted of apolipoproteins and several plasma proteins. 30% of apo-A-IV in LDF was bound to Neutral lipid as assessed by electroimmunoassay. When the bound proteins were subjected to chromatography on QAE-Sephadex A-50, 80-85% was recovered in two major and three minor fractions (A, B, C, D, and E). SDS-polyacrylamide gel patterns of each fraction are also shown in Fig. 1. Fractions A and B reacted against anti-apo-A-1 while fractions D and E gave precipitin lines against anti-albumin after immunodiffusion. Fraction C consisted of three major proteins and also reacted with anti-apo A-IV, anti-apo A-I, and anti-albumin. Fraction C was therefore subjected to preparative SDS-disc gel electrophoresis in order to purify A-IV apolipoprotein. The purified protein had a similar amino acid composition to that previously reported for A-IV (Table I) and the molecular weight, determined by SDS-gel electrophoresis, was approximately 46,000. Electrophoresis had been performed in the presence of mercaptoethanol to dissociate the apo-E-A-II complex which otherwise migrates in a position similar to that of apo-A-IV (23, 24).

Isolation of Apo-A-IV from Delipidated Lymph TRL Using Immunoabsorbance—We attempted desorption with several reagents including Tris-glycine, pH 2.5, and 2 M NaSCN, pH 7.5. These reagents resulted in only 10-20% of the expected recovery of apo-A-IV when lymph triglyceride-rich apolipoprotein was applied to the column. The use of 6 M guanidine HCl resulted in an 85-90% recovery of apo-A-IV as calculated from co-chromatography of 125I-labeled apo-A-IV.

Fig. 2 shows the result obtained when delipidated lymph TRL (10 mg) was chromatographed through an anti-apo-A-IV immunobeadsosorbance column, which resulted in a clear separation of apo-A-IV from other lymph triglyceride-rich apolipoproteins. The purified protein had a similar amino acid composition to that separated from plasma LDF (Table I) and the molecular weight determined, as described before,
Characterization of Apolipoprotein A-IV Complexes and Isoforms

Amino acid composition of human apo-A-IV

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Source of apo-A-IV</th>
<th>LDF^a</th>
<th>Lymph^b</th>
<th>LDF^a</th>
<th>lymph^c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>mol %</td>
<td></td>
<td>mol %</td>
<td></td>
</tr>
<tr>
<td>Asp</td>
<td>9.4</td>
<td>10.0</td>
<td>9.1</td>
<td>10.0</td>
<td></td>
</tr>
<tr>
<td>Thr</td>
<td>3.9</td>
<td>4.2</td>
<td>4.0</td>
<td>3.6</td>
<td></td>
</tr>
<tr>
<td>Ser</td>
<td>4.6</td>
<td>4.0</td>
<td>5.0</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Glu</td>
<td>25.0</td>
<td>25.7</td>
<td>22.9</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>Pro</td>
<td>3.4</td>
<td>3.9</td>
<td>3.0</td>
<td>3.7</td>
<td></td>
</tr>
<tr>
<td>Gly</td>
<td>5.1</td>
<td>4.2</td>
<td>5.2</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Ala</td>
<td>7.7</td>
<td>6.9</td>
<td>8.2</td>
<td>8.2</td>
<td></td>
</tr>
<tr>
<td>Cys</td>
<td>—</td>
<td>—</td>
<td>0.5</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td>Val</td>
<td>5.1</td>
<td>5.4</td>
<td>5.2</td>
<td>4.6</td>
<td></td>
</tr>
<tr>
<td>Met</td>
<td>0.8</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Ile</td>
<td>1.5</td>
<td>1.8</td>
<td>1.6</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>Leu</td>
<td>12.8</td>
<td>13.1</td>
<td>13.1</td>
<td>13.6</td>
<td></td>
</tr>
<tr>
<td>Tyr</td>
<td>2.0</td>
<td>2.3</td>
<td>1.9</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Phe</td>
<td>2.9</td>
<td>3.0</td>
<td>2.6</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>6.7</td>
<td>6.8</td>
<td>6.9</td>
<td>6.8</td>
<td></td>
</tr>
<tr>
<td>His</td>
<td>1.8</td>
<td>1.5</td>
<td>2.0</td>
<td>2.0</td>
<td></td>
</tr>
<tr>
<td>Arg</td>
<td>7.3</td>
<td>6.3</td>
<td>6.4</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>Trp</td>
<td>—</td>
<td>—</td>
<td>1.7</td>
<td>—</td>
<td></td>
</tr>
</tbody>
</table>

^a Our data, mean of duplicate analysis.
^b Weinberg and Scapu (31).
^c Weinsohler et al. (2).
^d —, not detected.

The lipid composition of both 2nd peaks are shown in Table II.
Characterization of Apolipoprotein A-IV Complexes and Isoforms

**FIG. 3.** SDS-polyacrylamide gel electrophoresis of human lymph TRL (A), bound fractions of lymph TRL (B), plasma HDL (C), plasma LDF (D), and lymph LDF (E) from the anti-apo-A-IV-IgG immunoabsorbance column. Positions labeled are: I, high-molecular weight protein (presently unidentified); 2, β-glycoprotein I; 3, apolipoprotein A-IV; 4, apolipoprotein E; 5, apolipoprotein A-I; 6 and 7, small M, peptides, comprising C and A-II apoproteins.

**TABLE II**

Per cent distribution of apolipoproteins in lymph TRL and the fractions which bound to anti-A-IV immunoabsorbance columns after chromatography of lymph TRL, lymph LDF, plasma HDL, and plasma LDF.

<table>
<thead>
<tr>
<th>Identity</th>
<th>Native lymph TRL</th>
<th>Immunoabsorbed fractions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lymph TRL</td>
<td>Plasma HDL</td>
</tr>
<tr>
<td>High M, proteins</td>
<td>6.8*</td>
<td>—</td>
</tr>
<tr>
<td>M, = 59,000 peptide</td>
<td>8.2</td>
<td>1.2</td>
</tr>
<tr>
<td>A-IV</td>
<td>26.2</td>
<td>39.8</td>
</tr>
<tr>
<td>E</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>A-I</td>
<td>23.1</td>
<td>27.0</td>
</tr>
<tr>
<td>Small M, peptides (C + A-II)</td>
<td>32.4</td>
<td>31.3</td>
</tr>
</tbody>
</table>

*Native lymph TRL (before immunoabsorbance).

* Due to slight differences in chromatogenicity of apolipoprotein, quantitation by this gel technique should be considered semiquantitative.

**TABLE III**

Per cent composition of the lipids in the fractions which bound to anti-A-IV immunoabsorbance column after chromatography of lymph TRL, plasma HDL, plasma LDF, and lymph LDF.

<table>
<thead>
<tr>
<th></th>
<th>Lymph TRL</th>
<th>Plasma HDL</th>
<th>Plasma LDF</th>
<th>Lymph LDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>3.6*</td>
<td>54.5</td>
<td>60.2</td>
<td>59.8</td>
</tr>
<tr>
<td>Phospholipid</td>
<td>7.3</td>
<td>21.8</td>
<td>31.5</td>
<td>33.7</td>
</tr>
<tr>
<td>Triglyceride</td>
<td>86.2</td>
<td>9.8</td>
<td>2.7</td>
<td>ND†</td>
</tr>
<tr>
<td>Cholesterol†</td>
<td>3.0</td>
<td>14.1</td>
<td>6.0</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Mean of duplicate analysis.

† ND, not determined.

**FIG. 4.** Analytical isoelectric focusing on urea-polyacrylamide gel in a pH gradient from 4 (bottom) to 6 (top) of isolated apolipoprotein A-IV from lymph TRL. The pI value of each bands are: 1, 5.48; 2, 5.42; 3, 5.34; 4, 5.30; 5, 5.25; 6, 5.22; 7, 5.20; 8, 5.15; 9, 5.10; 10, 5.06; 11, 4.98.

**FIG. 5.** Two-dimensional polyacrylamide gel electrophoresis of the bound fraction of lymph TRL obtained from the anti-apo-A-IV-IgG immunoabsorbance column. Rat HDL was used as a molecular weight marker (left lane) and as a standard for identifying unknown peptides.

**III.** The bound fraction of plasma LDF and lymph LDF contained all major plasma lipids. Following rechromatography of each second peak, the protein profile and lipid composition was identical to that in the original material. Presumably the short exposure of the apo-A-IV to 6 M guanidine HCl in our study did not disrupt the particles isolated by immunoabsorption.

Isolation of Apo-A-IV from Plasma HDL and Lymph TRL—The composition of the apolipoproteins in the fractions which bound to anti-A-IV immunoabsorbance columns after chromatography of plasma HDL and lymph TRL is also shown in Fig. 3. The complexes in these fractions comprised predominantly apo-A-IV, apo-A-I, and small M, peptides (apo-C or apo-A-II). The proportion of these proteins was also determined after scanning the gels and are shown in Table II.

Lipid compositions of apo-A-IV containing lymph TRL and plasma HDL are shown in Table III. Apo-A-IV containing lymph TRL consisted of all major plasma lipids with triglyceride as a predominant lipid component. Apo-A-IV containing plasma HDL contained all major plasma lipids.

**Isoforms of Apo-A-IV**—Isoelectric focusing of purified apo-A-IV (obtained from lymph triglyceride-rich lipoprotein) showed the presence of 3 major and 8 minor isoforms (Fig. 4).
Characterization of Apolipoprotein A-IV Complexes and Isoforms

Their pl values ranged from 4.98 to 5.48. Isoforms of apo-A-IV partially purified from four different sources (lymph TRL, lymph LDF, plasma HDL, and plasma LDF) were analyzed by two-dimensional gel electrophoresis (Figs. 5 and 6). Confirmation of their identity as apo-A-IV isoforms was obtained by comparison with purified apoprotein standards and by immunoblotting using monospecific anti-A-IV IgG (Fig. 7). All isoforms migrated to the same position as the apo-A-IV standard (Fig. 5). As shown in Fig. 6, 11 isoforms were identified in lymph LDF and 9 isoforms in lymph TRL, plasma HDL, and plasma LDF. These comparisons are based on the pattern obtained for purified apo-A-IV from lymph TRL shown in Fig. 4. After two-dimensional gel electrophoresis, isoforms 6 and 11 were not detectable, presumably due to sensitivity of this staining method.

Apo-A-IV from all sources except lymph LDF showed a similar pattern for the major isoproteins 7, 8, and 9. Isoprotein 7 was predominant in lymph LDF. The proportions of the minor isoforms of apo-A-IV from lymph TRL and plasma HDL were similar but differed from those obtained from lymph and plasma LDF. For example, as shown in Fig. 6, a total of 7 minor isoforms (1-6 and 11) were identified in lymph LDF but isoform 6 was not detected in lymph TRL, plasma HDL, and plasma LDF. Also, 3 isoforms (3, 4, and 5) were more predominant in plasma LDF than in the other samples.

Using the immunoblotting technique we confirmed the identification of the major and minor isoforms of apo-A-IV from each source as shown in Fig. 7 for plasma LDF and lymph LDF. Although the positions superimpose, the intensity of staining of the various isoforms varies between Coo-massie Blue stained gels and transblots, which is due to interference of SDS in quantitative transfer of proteins, also observed with other protein systems (18).

DISCUSSION

This paper describes the isolation and purification of A-IV apolipoprotein by two techniques, first, using conventional protein purification procedures and second, immunochemical absorption. Following absorption to neutral lipid, absorbed A-IV apolipoprotein could be partially purified by initial ion-exchange chromatography and required further preparative SDS-gel electrophoresis for final purification. The properties of apo-A-IV thus prepared were similar to those reported previously (3, 4, 27). The characterization of this unique apolipoprotein required its isolation by procedures which would minimize structural changes to maintain it as closely as possible to its native form. Immunoabsorbance columns using purified anti-A-IV IgG were therefore used.

The results showed that when delipidated chylomycin apolipoprotein was applied to immunoabsorbance columns, apo-A-IV was obtained in pure form. However, when nonde-lipidated lymph TRL and plasma HDL were applied, apo-A-IV could be recovered only in association with other major apolipoproteins (apo-A-I) and small M, peptides (apo-C or A-II). These complexes also contained lipid which varied in composition. Lymph TRL A-IV complex contained mainly triglyceride and smaller amounts of cholesterol (Table III), whereas A-IV complex from plasma HDL contained phospholipids, triglyceride, and cholesterol. This suggests that apo-A-IV exists in associated forms, comprising other apolipoproteins and lipid, and might thus be designated as lipoprotein A-IV complexes. Separation of lymph and plasma lipoprotein-deficient fractions showed the presence of an additional higher molecular weight apolipoprotein, which did not react against antisera to β₂-glycoprotein I (25, 26) or albumin.

The findings of similar lipoprotein A-IV complexes in triglyceride-rich lipoproteins and in HDL, but of a different complex in lipoprotein-deficient plasma is consistent with the metabolism of apo-A-IV that we had reported previously (28). When 125I-labeled apo-A-IV was injected into humans within either triglyceride-rich lipoprotein, HDL, or lipoprotein-deficient plasma, isotopic equilibrium occurred rapidly between apo-A-IV in triglyceride-rich lipoprotein and in HDL. By contrast, the equilibrating specific radioactivity of apo-A-IV in lipoprotein-deficient plasma was always about one-tenth of that in the lipoproteins even after 48 h. This phenomenon was also observed in vitro. When 125I-labeled apo-A-IV was incubated with both human lymph and human plasma, specific radioactivity of lymph chylomicrons and plasma HDL were 15 times higher than those of lymph lipoprotein-deficient
fractions and plasma lipoprotein-deficient fractions. This suggests that lipoprotein A-IV complexes in triglyceride-rich lipoprotein and in HDL have a similar origin and have evolved through similar metabolic processes that are not immediately related to the apo-A-IV complexes in lipoprotein-deficient plasma or lymph. This may also be compatible with the triglyceride-rich lipoproteins and in HDL, but unlike those analyzed from the lipoprotein-deficient fraction, although further investigation is required to confirm this suggestion.

It is of interest therefore that most of the circulating apo-A-IV in human plasma is nevertheless present in lipoprotein-deficient plasma. It is conceivable that this reflects two separate functions for apo-A-IV, one related to the transport of absorbed triglyceride and the other to the transport of cholesterol or phospholipids as suggested by Delamatre and Roheim (29). The low proportion of free versus esterified cholesterol in all lipoprotein A-IV complexes found in this study may also suggest that these particles are relatively aged and represent the end products of lecithin cholesterol acyltransferase activation, in which A-IV apoprotein may also act as a cofactor protein (30).

The amino acid composition of the apo-A-IV obtained from all sources was similar. Apo-A-IV from lymph chylomicrons and from plasma LDF reacted with lines of identity against anti-plasma LDF apo-A-IV serum. Further studies are in process to investigate the physiological relevance of the microheterogeneity of apo-A-IV, demonstrable in the differing isoform patterns within the lipoprotein A-IV complexes in plasma LDF and in the other sources of apo-A-IV.

REFERENCES