Plasma metabolism of apolipoprotein A-IV in humans[1]

Giancarlo Ghiselli, Satya Krishnan, Yitzhak Beigel, and Antonio M. Gotto, Jr.

Baylor College of Medicine and The Methodist Hospital, Department of Medicine, Houston, TX 77030

Abstract As assessed by molecular sieve chromatography and quantitation by a specific radioimmunoassay, apoA-IV is associated in plasma with the triglyceride-rich lipoproteins, to a high density lipoprotein (HDL) subfraction of smaller size than HDL_3, and to the plasma lipoprotein-free fraction (LFF). In this study, the turnover of apoA-IV associated to the triglyceride-rich lipoproteins, HDL and LFF was investigated in vivo in normal volunteers. Human apoA-IV isolated from the thoracic duct lymph chylomicrons was radioiodinated and incubated with plasma withdrawn from normal volunteers after a fatty meal. Radioiodinated apoA-IV-labeled triglyceride-rich lipoproteins, HDL, and LFF were then isolated by chromatography on an AcA 34 column. Shortly after the injection of the radioiodinated apoA-IV-labeled triglyceride-rich lipoproteins, most of the radioactivity could be recovered in the HDL and LFF column fractions. On the other hand, when radioiodinated apoA-IV-labeled HDL or LFF were injected, the radioactivity remained with the originally injected fractions at all times. The residence time in plasma of ^{125}I-labeled apoA-IV, when injected in association with HDL or LFF, was 1.61 and 0.55 days, respectively. When ^{125}I-labeled apoA-IV was injected as a free protein, the radioactivity distributed rapidly among the three plasma pools in proportion to their mass. The overall fractional catabolic rate of apoA-IV in plasma was measured in the three normal subjects and averaged 1.56 pools per day. The mean degradation rate of apoA-IV was 8.69 mg/kg · day. The results are consistent with the conclusions that: *1*) apoA-IV is present in human plasma in three distinct metabolic pools; *2*) apoA-IV associated with the triglyceride-rich lipoproteins is a precursor to the apoA-IV HDL and LFF pools; *3*) apoA-IV in LFF is not a free protein and its turnover rate is faster than that of apoA-IV in HDL; *4*) since no transfer of apoA-IV from the HDL or the LFF occurs, these pools may represent a terminal pathway for the catabolism of apoA-IV; and *5*) the catabolism of apoA-IV in HDL is dissociated from that of apoA-I although both apoproteins may reside on the same lipoprotein particles. — **Ghiselli, G., S. Krishnan, Y. Beigel, and A. M. Gotto, Jr.** Plasma metabolism of apolipoprotein A-IV in humans. *J. Lipid Res.* 1986. **27:** 813–827.

Supplementary key words high density lipoproteins • plasma lipoprotein-free fraction • gel filtration • metabolic heterogeneity • cholesteryl ester • chylomicron metabolism

Human apolipoprotein A-IV (apoA-IV) is a glycoprotein of known amino acid sequence with an apparent molecular weight of 46,000 (1–4). In plasma and in the lymph, apoA-IV is polymorphic, having a major isoprotein component along with a number of other minor components with higher and lower isoelectric points (3–6). The chemical basis of this polymorphism is not known. Studies in populations support the idea that apoA-IV polymorphism is genetically determined, being controlled by two common ($A\text{-}IV^1$ and $A\text{-}IV^2$) and at least two rare alleles ($A\text{-}IV^3$ and $A\text{-}IV^4$) which are probably located at the same apoA-IV structural gene locus (6).

ApoA-IV was first identified in rat plasma (7). The liver and the intestine are the major sites of synthesis of apoA-IV in the rat (8, 9). Hepatic synthesis of apoA-IV in humans may be negligible (2). In humans and rats, apoA-IV is synthesized as preapoA-IV, a precursor protein with a 20-amino acid-long NH_2-terminal extension that is cleaved before protein secretion (10–12). In the rat, approximately 50% of the total plasma apoA-IV is synthesized by the intestine at the same rate as apoA-I (8). ApoA-IV is synthesized in the rat's liver, however, at less than half the rate of apoA-I (8, 9). Windmueller and Wu (8) have presented results indicating that the production of apoA-IV (and apoA-I as well) by the small intestine is not regulated by the rate of intestinal triacylglycerol transport and that it is not increased by prolonged fat feeding. They found, however, that there was a marked reduction in the direct release of newly synthesized intestinal apoproteins into the blood during active fat absorption, with a concomitant increase in the proportion appearing in the mesenteric lymph, which could explain the dramatic increase of apoA-IV content seen in the urine of chyluric subjects after ingestion of fat (13). On the other hand, Gordon et al. (11) have shown that apoA-IV mRNA increases in the enterocytes during fat transport, supporting the idea of an increased synthesis of this apoprotein. Fat feeding in humans (14) and in rats (15) slightly increases plasma apoA-IV. In

Abbreviations: HDL, high density lipoproteins; PAGE-SDS, polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate; DTNB,5, 5-dithiobis-(2-nitrobenzoic acid); LFF, lipoprotein-free fraction.

[1]Some of the data contained in this manuscript were presented at the 57th Scientific Session of the American Heart Association in 1984 at Miami, FL.

addition, the proportion of apoA-IV associated to the HDL decreases in rats while the amount in the lipoprotein-free fraction increases, and this shift is more pronounced when cholesterol (2%) is added to the diet (15).

The bulk of the current evidence indicates that the metabolism of apoA-IV is remarkably distinct from that of the other plasma apolipoproteins. In plasma, the majority is not associated to lipoproteins. Rather, in humans, at least 90% is recovered in the d > 1.210 g/ml lipoprotein-free fraction after ultracentrifugation (2–4, 13, 14). ApoA-IV, however, is a major protein constituent of the lymph chylomicrons. Lymph chylomicrons also contain as major protein constituents a form of apoB, denoted B-48 (16), and the precursor of plasma mature apoA-I, proapoA-I (17). In this respect the intestinal chylomicron apoprotein pattern is remarkably distinct from that of any other lymph or plasma lipoprotein. The plasma concentration of apoB-48 and of proapoA-I is negligible due to their rapid catabolism, and they do not accumulate in any lipoprotein classes. Several investigators have proposed that both of these apoproteins might serve a unique function in the plasma catabolism of intestinal lipids and of the newly secreted lipoproteins (16–19), although just what this function is remains to be elucidated. Turnover data in vivo in rats have been interpreted as evidence that chylomicron apoA-IV is first transferred to the lipoprotein-free fraction and then, from this pool, to HDL (20). Recent data (21, 22) support the idea that the transfer of apoA-IV from the lipoprotein-free fraction to HDL may be linked to cholesteryl ester formation. In humans, a small fraction of apoA-IV is associated in plasma with HDL, but it has been found associated also in increased quantities in the d < 1.006 g/ml cholesteryl ester-rich lipoproteins that accumulate in the plasma of subjects genetically deficient in apoE (23) or with chronic renal insufficiency (24). The kinetic behavior of apoA-IV and the functional significance of its compartmentalization in plasma are the subjects of this report.

We determined with a sensitive radioimmunoassay the concentration of apoA-IV in a number of normolipidemic and hyperlipidemic subjects, and the distribution of this apoprotein in plasma was analyzed by molecular sieve chromatography. The turnover rate of apoA-IV in plasma was determined in normal volunteers. The metabolic relationship of the different pools of apoA-IV in plasma was investigated and their metabolic characteristics were assessed.

MATERIALS AND METHODS

Isolation of apolipoprotein A-IV

ApoA-IV was isolated from human thoracic duct lymph chylomicrons. Lymph was collected from a fistula implanted in the thoracic duct lymph of subjects undergoing immunosuppression therapy before kidney transplantation; it was a kind gift from Dr. G. Fish of the University of Texas Medical Branch in Galveston. Lymph was collected under sterile conditions in plastic bags and then usually returned to the patient, depleted of lymphocytes and other plasma cells by centrifugation. Lymph for the isolation of chylomicrons was stored at 4°C and utilized within 3 to 5 days after collection. Chylomicrons were isolated by ultracentrifugation in a Beckman SW 27 swing-bucket rotor, at 27,000 rpm for 60 min at 4°C. Under these conditions the chylomicrons became packed at the top of the tube and could be easily recovered with the aid of a spatula. Chylomicrons were dispersed in sterile saline and washed following the same ultracentrifugation procedure. This operation was repeated twice. The second time, packed chylomicrons were dispersed in 0.08% ammonium bicarbonate containing 0.02% NaN_3. Chylomicrons could be stored in this buffer at −70°C for up to 6 months without detectable alteration of the apolipoprotein pattern as judged by gel electrophoresis. For the isolation of apoA-IV, the chylomicron preparations were lyophilized and lipid was extracted with chloroform–methanol 3:1 (v/v). The extraction procedure was performed the first time overnight at −20°C and then twice subsequently with the same organic solvent mixture for 1 hr at room temperature. The protein was recovered by centrifugation and dried under a stream of N_2. The pellet was solubilized in 0.1% SDS, 0.1% β-mercaptoethanol, and 50 mM Tris-HCl buffer at pH 7.4; it was then loaded on a column (1.5 × 200 cm) packed with Bio-Gel A-1.5m (200–400 mesh, from Bio-Rad) equilibrated with the sample buffer. Flow rate was maintained at 30 ml/hr and 2-ml fractions were collected. The elution of the proteins was monitored at 280 nm and the apoprotein content was checked by PAGE-SDS (25). Fractions containing only apoA-IV were pooled and extensively dialyzed against 0.08% ammonium bicarbonate before being stored at −70°C. Protein purity and identity were determined by two-dimensional gel electrophoresis (26), and by amino acid compositional analysis. The preparation gave a single immunoprecipitation line with anti-apoA-IV, and no reaction was seen against anti-apoA-I, anti-apoA-II, anti-apoB (LDL), anti-apoE, or anti-human serum albumin (27).

Apolipoprotein A-IV radioiodination

ApoA-IV was radioiodinated by a modification of the iodine–monochloride method of McFarlane (28). Briefly, 50 μl of apoA-IV solution in 0.08% ammonium bicarbonate (≅ 0.5 mg/ml) was mixed with 50 μl of 1 M glycine–NaOH and 2–5 mCi of carrier-free Na ^{125}I or Na ^{131}I (NEN). Radiolabeling was done by adding 20 μl of 0.02% ICl in 2 M NaCl. The mixture was immediately passed on a PD10 column (Sephadex G25, from Pharmacia) for the separation of the radioiodinated protein from the

bulk of unbound radioactive iodine. The eluted fractions containing ^{125}I- or ^{131}I-labeled apoA-IV were pooled and extensively dialyzed against sterile, injectable saline (0.15 M NaCl from Travenol). The efficiency of the radioiodination ranged between 8 and 15%. Before utilization of these tracers in the in vivo turnover study, the preparations were diluted 1 to 5 with a concentrated solution (25%) of injectable human serum albumin (Albuminar 25 from Armour) and passed two times through a 0.22-μm Millipore filter. These preparations were checked for sterility and pyrogeneity and directly utilized for the turnover study within no more than 16 hr.

Preparation of polyclonal antibodies against apoA-IV

Polyclonal antibodies against apoA-IV were raised in rabbits (29). The protein (200 μg) was emulsified with complete Freund Adjuvant and injected subcutaneously on the back of female New Zealand rabbits. After this initial immunization, apoA-IV (100 μg) was emulsified with incomplete Freund Adjuvant and injected every other week. After 2 months the animals were exsanguinated by cardiac puncture and the sera were tested for titer by sequential dilution (29) and for specificity by immunodiffusion against apoA-IV, apoA-I, apoA-II, apo(LDL)B, apoE, and apoC-III (27).

Radioimmunoassay of apoA-IV and apoA-I

The radioimmunoassay was performed in 0.125 M Na-borate, 0.1% BSA, 0.35% Tween 20, pH 8.0, buffer. Standards and unknown samples were diluted appropriately in the borate buffer and preincubated overnight. Aliquots of the diluted standard and of the unknowns were brought to 1 ml with the same buffer to achieve final dilutions, and 0.1 ml of ^{125}I-labeled apoA-IV (20,000 cpm; 1–2 ng) and 0.1 ml of anti-apoA-IV antibody (diluted 1 to 300) were added. This concentration of antibody precipitated 50% of the labeled material in a preliminary binding study (30). Labeled and unlabeled apoA-IV were equilibrated with the antibody for 2 days at 4°C. At the end of this incubation period, 0.1 ml of normal rabbit serum (diluted 1 to 100) and 0.1 ml of goat anti-rabbit IgG (diluted 1 to 15), both from Pel-Freez, were added and the radioimmunoassay cocktail was incubated for another 2 days at 4°C. The immunoprecipitate was pelleted by centrifugation and the supernatant was removed. The radioactivity associated to the pellet was counted in a gamma counter for a time sufficient to reach 5,000 cpm. The intraassay coefficient of variation was determined for each assay by analyzing in quintuplicate a normolipidemic and a hypertriglyceridemic control serum. The interassay coefficient of variation was determined by averaging the mean value of the control sera concentrations for all of the assays performed. The concentration of the primary apoA-IV standard was determined by amino acid analysis and routinely with the Bradford method (31); bovine serum albumin was utilized as standard. ApoA-I was quantitated by radioimmunoassay as previously described (32).

Validation of the radioimmunoassay procedure: quantitation of the apoA-IV-to-apoA-I mass ratio in plasma by gel electrophoresis

An electrophoretic method was developed for the quantitation of the apoA-IV-to-apoA-I mass ratio directly in plasma samples. In this way the plasma apoA-IV-to-apoA-I mass ratio could be calculated and compared with the value determined in the same plasma samples by radioimmunoassays. Chromogenicity of apoA-IV and apoA-I was determined by loading known amounts of apolipoprotein (1 to 50 μg) onto isoelectrofocusing gels suitable for two-dimensional gel electrophoresis (26). Following the electrophoretic run, the proteins were stained with Coomassie Blue R250, destained, and the portion of the gel corresponding to the protein spot was cut out for extraction. The dye was extracted for 72 hr with 25% pyridine in water (33). Absorbance of the extracted dye was read at 605 nm. Readings were corrected for the procedural losses by adding known amounts of ^{125}I-labeled apoA-IV and ^{131}I-labeled apoA-I to the proteins before electrophoresis and counting the radioactivity recovered with the different apolipoproteins in the final stained gel. For the quantitation of the apoA-IV-to-apoA-I mass ratio, plasma samples (20 μl) were treated with 40 μl of a buffer containing 8.6 M urea and 10% (v/v) of pH 4 to 6 ampholines (5) and electrophoresed. The stained protein spots were cut out and the eluted dye solution was read at 605 nm.

Fractionation of plasma lipoproteins by column chromatography

Plasma lipoproteins were fractionated by molecular sieve chromatography on an AcA34 (LKB) column (1.5 × 150 cm) equilibrated with 0.15 M NaCl, 50 mM Tris-HCl buffer at pH 7.4. Flow rate was maintained at 30 ml/hr and 2-ml fractions were collected. This column could be loaded with 2 to 5 ml of plasma without detectable changes in the elution pattern of the peaks or their resolution. The columns were calibrated with a d < 1.063 g/ml lipoprotein fraction, HDL_2 (d 1.063–1.100 g/ml), and HDL_3 (d 1.100–1.210 g/ml), separated by sequential ultracentrifugation, and with human serum albumin. For the determination of the radioactivity distribution in plasma in samples collected during the turnover study, a series of smaller columns was utilized (1.5 × 100 cm). These columns were operated at a flow rate of 45 ml/hr and 5 ml of plasma was routinely loaded. The chromatographic run, in this case, could be completed in less than 5 hr. The chromatographic separations were carried out at 4°C.

ApoA-IV in vivo turnover studies in humans

The subjects for this study were normolipidemic males, ages 25 to 32. Complete physical examinations before admission to the study protocol verified that all the subjects had normal liver, thyroid, and kidney functions. All protocols for the turnover studies were approved by the Institutional Review Board of the Baylor College of Medicine and The Methodist Hospital. The normal volunteers followed an outpatient protocol and were on an ad libitum diet during the study. All subjects were given 1 g/day of SSKI beginning 3 days before the injection of radioiodinated apoA-IV and thereafter for the entire duration of the study. The plasma concentrations of cholesterol, triglycerides, and HDL cholesterol and the body weight were determined daily. Plasma samples were collected at 10 min and at 3, 6, and 9 hr after the injection of the radiolabeled preparation and daily thereafter in the morning. Urine samples were collected between the plasma sampling times.

In the initial experiments, radioiodinated apoA-IV was injected as a free protein in three volunteers. When this was done, ^{125}I-labeled apoA-IV preparations were adjusted to an activity of 8–10 μCi/ml by dilution, if necessary, with a 5% solution of injectable human serum albumin (Albuminar 5 from Armour). Subjects A and B received a different radioiodinated preparation than subject C. In a second series of experiments, apoA-IV was injected into three other volunteers in association with lipoproteins. For this purpose, 2–4 μg of radiolabeled apoA-IV (5–20 mCi) was added to 2 ml of the volunteer postprandial plasma and the sample was immediately fractionated on an AcA34 column (1.5 × 50) equilibrated with injectable saline. Prior to column packing, the column and the fittings were autoclaved, and only freshly opened batches of AcA34 were utilized. This column was operated under a sterile hood. The profile of the eluate was monitored by determining the protein concentration with the Bradford method (31) and by counting the radioactivity in aliquots of the collected column fractions. Small cuts of the peak corresponding to the triglyceride-rich lipoproteins (VLDL plus LDL), HDL, and the plasma proteins (the lipoprotein-free fraction here denoted LFF) were dialyzed against injectable saline and diluted with Albuminar 5 prior to filtration through a 0.22-μm Millipore filter. The injected preparations were sterile and pyrogen-free. One of the volunteers (D) was injected with ^{125}I-labeled apoA-IV triglyceride-rich lipoproteins and ^{131}I-labeled apoA-IV HDL. A second volunteer (E) received ^{125}I-labeled apoA-IV LFF and ^{131}I-labeled apoA-IV HDL. A third subject (F) received ^{125}I-labeled apoA-IV triglyceride-rich lipoproteins and ^{131}I-labeled apoA-IV LFF. The same radioiodinated apoA-IV preparations were used for subjects D and E. In all the studies, when radioiodinated apoA-IV was injected either as a free protein or associated to lipoproteins, the plasma samples collected at 10 min and 3, 24, and 72 hr after the injection were analyzed for the radioactivity distribution by column chromatography. Plasma radioactivity decay curves were analyzed by a graphic procedure (34) and the area under the curve was calculated by integration. Protein residence time and degradation rate were determined by input–output analysis (35).

RESULTS

Apolipoprotein isolation and characterization

Apolipoprotein A-IV, isolated from lymph chylomicrons by molecular sieve chromatography in the presence of 0.1% SDS, was pure by a variety of criteria. The protein gave a single band on PAGE-SDS with apparent molecular weights of 46,000, and on the two-dimensional gel electrophoretogram it showed polymorphism with a distribution pattern virtually identical to that shown by apoA-IV in the original chylomicron preparation (see **Fig. 1**). ApoA-IV reacted with only the corresponding specific antibody in the immunodiffusion plate when tested against anti-apoA-IV, anti-apoA-I, anti-apoA-II, anti-apo(LDL)B, and anti-apoE. No displacement of the radiolabeled antigen was detected when apoA-IV was added in excess amounts to the radioimmunoassay cocktail for apoA-I (32), apo(LDL)B (36), or apoE (37). Amino acid analysis of the protein gave results consistent with the published composition for apoA-IV (1–3, 13).

Anti-apoA-IV antisera raised in rabbits were characterized by immunodiffusion and gave no reaction when tested

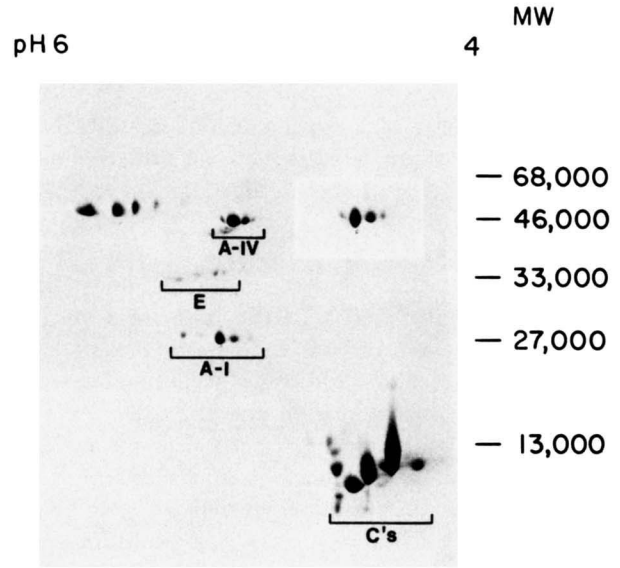

Fig. 1. Two-dimensional gel electrophoretograms of the thoracic duct lymph chylomicron apolipoproteins, and of column chromatography purified apoA-IV (in the insert). Two-dimensional gel electrophoresis was performed as described in the Methods section. The gels were loaded with approximately 50 μg of chylomicron apoprotein or 8 μg of purified apoA-IV.

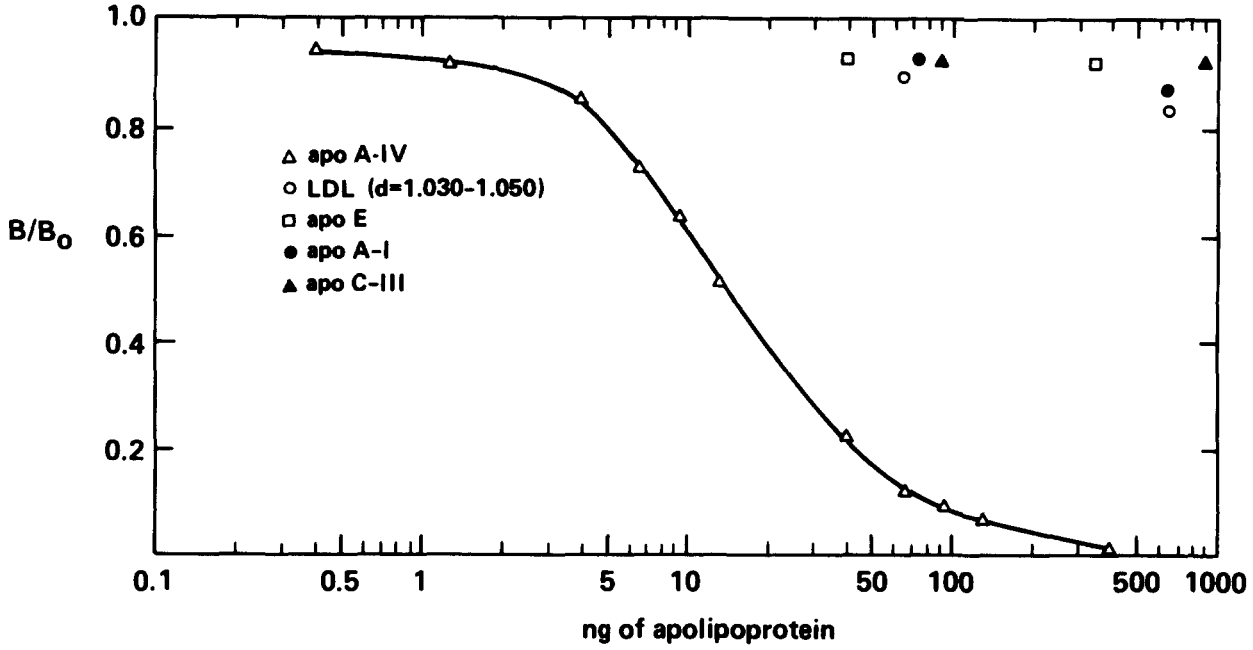

Fig. 2. Human apoA-IV radioimmunoassay competitive displacement curve. The curve shows the displacement of radioiodinated apoA-IV by purified human apoA-IV, apoA-I, apoC-III, apoE, and apo(LDL)B. See the Methods section for the procedural details.

against apoA-I, apoA-II, apo(LDL)B, apoE, and apoC-III. A single arc of identity was formed against whole serum or purified apoA-IV, consistent with the monospecificity of the antisera.

Apolipoprotein A-IV radioimmunoassay

Optimal conditions for the radioimmunoassay of apoA-IV were found when Tween 20 (0.35%) was added to the cocktail. Up to 92% of radioiodinated apoA-IV could be immunoprecipitated by an excess of anti-apoA-IV antibody. Nonspecific binding, determined in a competitive assay with radioiodinated apoA-IV and saturating amounts of cold pure apoA-IV, was less than 7%.

ApoA-I, apo(LDL)B, apoE, and apoC-III were unable to compete with radioiodinated apoA-IV in the apoA-IV radioimmunoassay. The displacement curve of radioiodinated apoA-IV resulting from increasing amounts of cold pure apoA-IV is shown in **Fig. 2.** The assay was sensitive up to 5 ng and the usable portion of the displacement curve was within the 5-to-50 ng range. The results of the displacement experiments of the tracer using serial dilution of normolipidemic plasma (Chol = 203 mg/dl; TG = 105), hypertriglyceridemic plasma (Chol = 323; TG = 732), hypertriglyceridemic VLDL, and the 1.210 g/dl ultracentrifuge bottom from normolipidemic plasma are, shown in **Fig. 3.** In these assays, the apoA-IV secondary standards displaced radioiodinated apoA-IV indistinguishably from purified apoA-IV and gave logit-log curves virtually superimposable to those of the primary standard. These results showed that, under the conditions chosen for the assay, the expression of the apoA-IV epitope in the different lipoprotein and plasma fractions was independent of the dilution.

Further validation of the assay required that identical values for the apoA-IV mass be obtainable by the radioimmunoassay method and by an independent method.

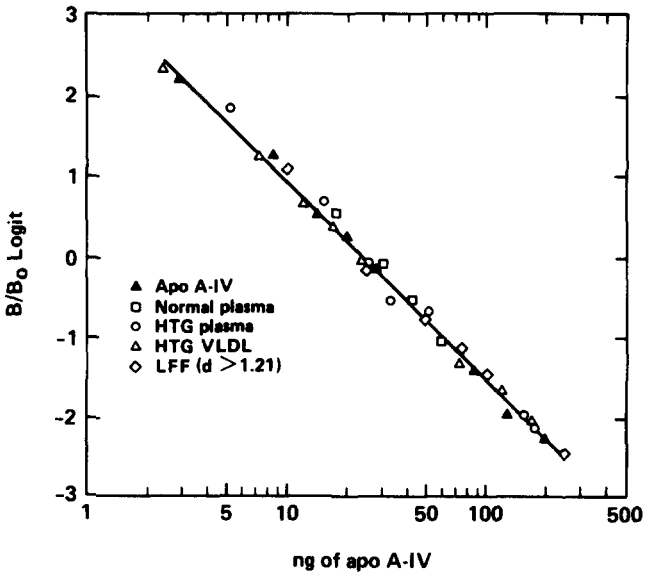

Fig. 3. *Human apoA-IV radioimmunoassay competitive log-logit displacement curve.* ApoA-IV concentration was measured in normolipidemic plasma, hypertriglyceridemic plasma, VLDL isolated from hypertriglyceridemic plasma, and the d > 1.210 g/ml ultracentrifuge bottom of normolipidemic plasma utilizing purified apoA-IV as primary standard. These secondary standards were then added in increasing amounts to the radioimmunoassay cocktail and the B/Bo logit was calculated. The linear correlation coefficient for all the sets of data is better than 0.96.

For this purpose, the absolute values of apoA-IV and apoA-I were measured in ten plasma samples by radioimmunoassay and the mass ratios were compared to those obtained by a method based on the separation and quantitation of plasma apoA-IV and apoA-I by gel electrophoresis. For this second method, chromogenicity of apoA-IV and apoA-I was determined by electrophoresing known amounts of purified apoA-IV and apoA-I. The mass ratio for the two apolipoproteins in the plasma samples, as determined by radioimmunoassay and by electrophoresis, was (mean ± SD) 0.14 ± 0.04 and 0.19 ± 0.03, respectively, with the two populations of values being statistically equivalent.

The intraassay and interassay coefficients of variation for the apoA-IV and apoA-I radioimmunoassays were, respectively, 7% and 5%, and 12% and 10%.

Apolipoprotein A-IV levels and distribution in plasma

ApoA-IV concentration was determined by radioimmunoassay in 105 plasma samples drawn from normal subjects and subjects with different forms of hyperlipoproteinemia. The mean concentration of apoA-IV in the plasma withdrawn from normal subjects was 17.2 ± 3.2 mg/dl. No statistical difference was found between the apoA-IV levels in the normal subjects and in the groups of subjects with type IIa, type IIb, type IV, and type V hyperlipidemia. In a type III subject, apoA-IV concentration was 23.4 mg/dl (see **Table 1**). No linear correlation could be found between the apoA-IV plasma concentrations and the plasma cholesterol, triglyceride, or HDL cholesterol levels.

In order to evaluate whether gross changes in the individual lipoprotein levels affect apoA-IV distribution in plasma, as has been reported for other apolipoproteins, the distribution of apoA-IV in the plasma of several normolipidemic and hypertriglyceridemic subjects was next examined. The plasma was loaded on a 1.5 × 200 cm AcA34 column and the mass of apoA-IV and apoA-I in the eluted fractions was quantitated by specific radioimmunoassays. This column allowed resolution into distinct peaks of VLDL together with LDL, HDL_2, HDL_3, as well as a distinct peak containing the bulk of the plasma protein. This last plasma fraction (LFF) contained most of the plasma apoA-IV (more than 70%). The remaining apoA-IV eluted in the HDL region, and the peak had an elution volume slightly but consistently larger than that of apoA-I in HDL_3. The elution pattern was similar in all the normolipidemic and hypertriglyceridemic plasma samples analyzed. The frac-

TABLE 1. Lipid and and apoA-IV plasma levels in normal and hyperlipoproteinemic subjects

	Plasma Concentration			
Type (n)	Chol	TG	HDL Chol	ApoA-IV
		mg/dl		
Normal (30)	178 ± 8[a]	115 ± 8	38 ± 3	17 ± 1
IIa (6)	300 ± 17	178 ± 10	35 ± 4	16 ± 3
IIb (20)	277 ± 15	340 ± 33	33 ± 2	14 ± 2
III (1)	332	620	15	23
IV (43)	214 ± 8	478 ± 39	28 ± 1	17 ± 1
V (5)	328 ± 20	1,231 ± 113	27 ± 2	18 ± 1

[a] Mean ± SEM.

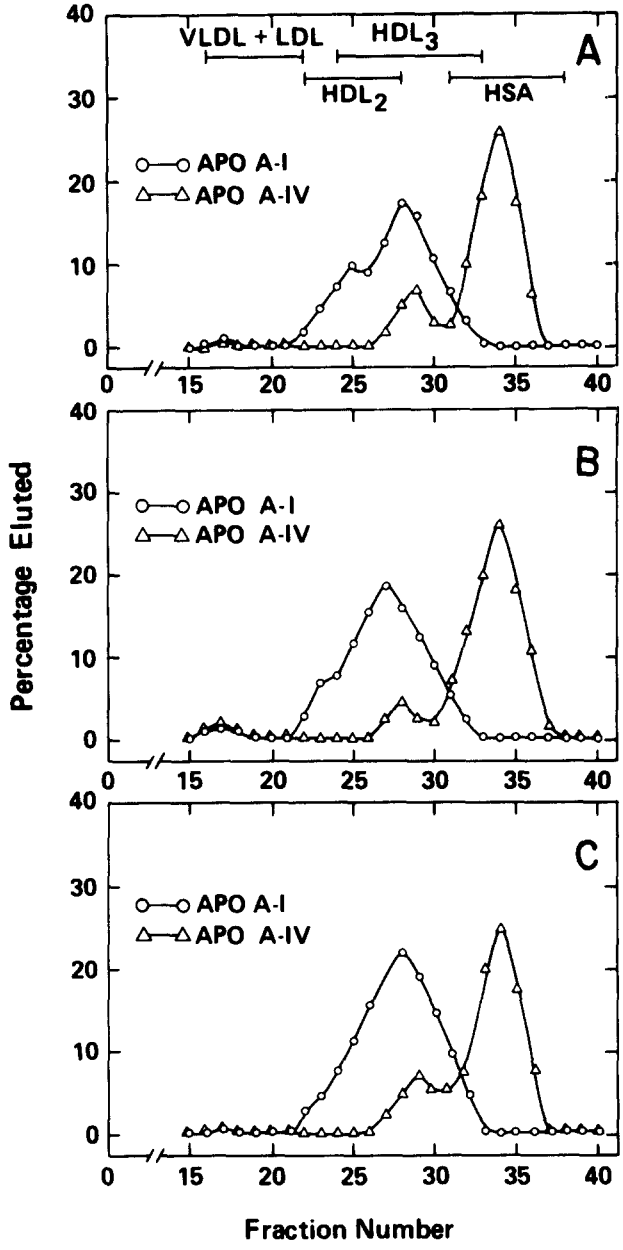

Fig. 4. Distribution of apoA-IV and apoA-I in human plasma. Five ml of plasma was subjected to molecular sieve chromatography on an AcA34 column (1.5 × 200). The eluted fractions were monitored for their content in apoA-IV and apoA-I by specific radioimmunoassays. Panel A, normolipidemic plasma (Chol, 186 mg/dl; TG, 98; apoA-IV, 15.3); panel B, type V hypertriglyceridemic plasma (Chol, 361; TG, 1,460; apoA-IV, 16.8); and panel C, type III hypertriglyceridemic plasma (Chol. 332; TG, 620; apoA-IV, 23.4).

tion of apoA-IV associated to the triglyceride-rich lipoproteins never exceeded 8% of the total plasma mass, even in a grossly hypertriglyceridemic plasma sample (see **Fig. 4**).

Apolipoprotein A-IV metabolism in plasma

The compartmentalization of apoA-IV in plasma among the lipoprotein fractions and the LFF was investigated through in vivo turnover studies. In the first sets of experiments, ^{125}I-labeled apoA-IV was injected as a free protein into three normal volunteers. Before injection, the tracer was tested for the ability to behave in plasma as native apoA-IV. For this purpose, ^{125}I-labeled apoA-IV (2–4 μg) was added to 5 ml of plasma freshly drawn from a normolipidemic subject. The plasma contained 0.01% of DTNB to inhibit the cholesterol esterification reaction. The mixture was immediately applied to a 1.5 × 200 cm AcA34 column and the radioactivity and the apoA-IV mass in the eluate were quantitated. The column elution profiles show that apoA-IV specific activity (the ratio of ^{125}I-labeled apoA-IV radioactivity to the A-IV mass) was similar in the lipoproteins as well as in the LFF (**Fig. 5**). Longer periods of incubation (up to 60 min) prior to gel filtration did not substantially modify these protein and radioactivity distributions.

The radiotracers were injected in the morning into subjects after an overnight fast. After 10 min the first plasma sample was withdrawn, followed by plasma withdrawals at 3, 6, and 9 hr postinjection and then daily in the fasting state up to the sixth day of the study. The disappearance curves of the ^{125}I-labeled apoA-IV-associated radioactivity from plasma for the three subjects studied are shown in **Fig. 6.** In these subjects, the mean residence time for apoA-IV in plasma was 0.64 ± 0.01 days (mean ± SE) and the degradation rate was 8.69 ± 0.23 mg/kg · day. The observed kinetic parameters of apoA-IV in the three subjects together with the relevant plasma lipid levels are tabulated in **Table 2.** During the course of this same experiment, ^{125}I-labeled apoA-IV radioactivity distribution was analyzed in plasma at different times after the injection (see **Fig. 7**). A sufficient number of columns were run simultaneously to allow rapid processing of the plasma samples as soon as 20 min after withdrawals. Ten minutes after the injection, most of the radioactivity in plasma was recovered in the LFF peak and approximately 33% was associated to HDL. At the subsequent times, an increasing proportion of the radioactivity remaining in plasma was recovered with HDL. At 24 hr, more than 60% of the plasma radioactivity was associated to HDL and only 28% with the LFF.

Two possible mechanisms were considered as explanations of the progressive increase of ^{125}I-labeled apoA-IV in HDL with time. First, there could be a net transfer of apoA-IV in the direction of LFF to HDL or, second, apoA-IV could have different turnover rates in HDL and LFF. In order to distinguish between these two possibilities and to establish the metabolic relationship between the plasma pools of apoA-IV in the triglyceride-rich lipoproteins, HDL, and the LFF, a new series of in vivo turnover studies was carried out. Radioiodinated ^{125}I-labeled apoA-IV or ^{131}I-labeled apoA-IV (2–4 μg) was added to the recipient's own plasma collected under sterile conditions after a fatty meal,

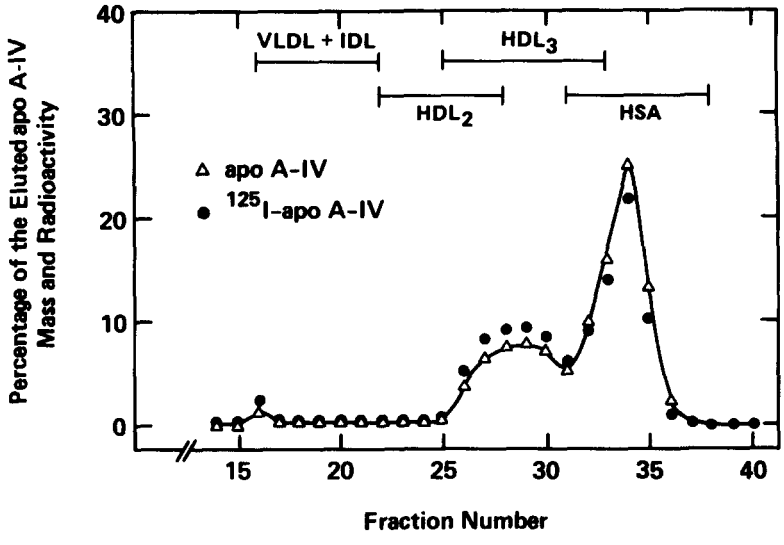

Fig. 5. Distribution of apoA-IV and radioiodinated apoA-IV in human plasma. A trace amount of ^{125}I-labeled apoA-IV (approximately 700,000 cpm) was added to 5 ml of freshly drawn normolipidemic plasma containing 0.01% DTNB, and the mixture was loaded immediately afterwards over an AcA34 column (1.5 × 200 cm) for the plasma fractionation. ApoA-IV mass on the eluate was measured by a specific radioimmunoassay. HSA, human serum albumin.

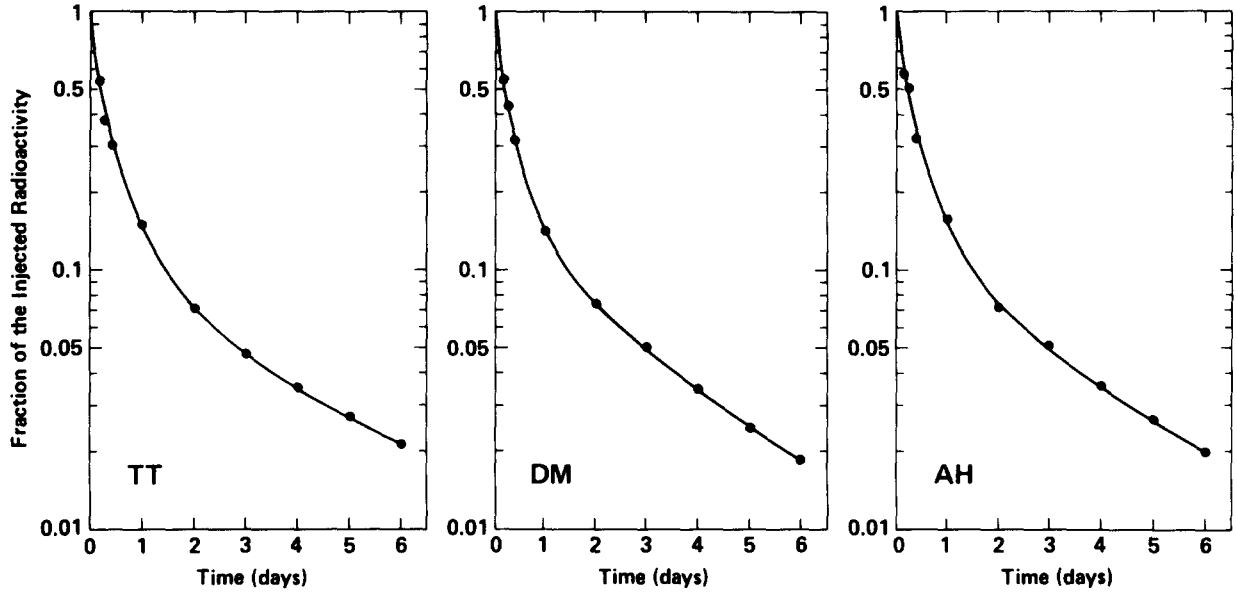

Fig. 6. Decay of the radioactivity in the plasma of three normolipidemic subjects after injection of ^{125}I-labeled apoA-IV or ^{131}I-labeled apoA-IV. Radioiodinated apoA-IV (5 to 10 μCi) was injected as free protein into three normolipidemic volunteers. Plasma samples were collected 10 min and 3, 6, and 9 hr after the injection and thereafter in the mornings for 5 additional days. The fractions of the activity at 10 min are plotted.

and the sample (2 ml) was immediately chromatographed on a 1.5 × 50 cm AcA34 column. Fractions of the column eluate were selected to correspond to the peaks of the triglyceride-rich lipoproteins, HDL, and LFF. In vitro incubation (at 37°C for 60 min in the presence of 0.01% DTNB) of these fractions with plasma revealed that radioactivity could be transferred from the triglyceride-rich lipoproteins to the HDL and LFF fractions. On the contrary, when radioiodinated apoA-IV-labeled HDL were incubated, virtually all of the radioactivity remained within this fraction only. The same occurred when labeled LFF was incubated. It was however noted that, when the LFF was stored at 4°C for more than 72 hr, subsequent incubation of this fraction with plasma resulted in a transfer of radioactivity, more evidently in the HDL not matched by a corresponding transfer of mass. For these reasons radioiodinated apoA-IV-labeled fractions were immediately sterilized after isolation and utilized for the turnover studies within 16 hr. The three volunteers for this study received, respectively, ^{125}I-labeled apoA-IV triglyceride-rich lipoproteins at the same time as ^{131}I-labeled apoA-IV HDL or ^{125}I-labeled apoA-IV triglyceride-rich lipoproteins together with ^{131}I-labeled apoA-IV LFF or ^{125}I-labeled apoA-IV LFF together with ^{131}I-labeled apoA-IV HDL (see **Table 3**). Plasma samples were obtained at 10 min and at 3, 6, and 9 hr postinjection and thereafter daily in the morning for 3 more days. The plasma was counted for the radioactivity and a portion (5 ml) was quickly chromatographed through the 1.5 × 100 cm AcA34 columns. When radiolabeled apoA-IV associated to the triglyceride-rich lipoproteins was injected, an almost complete transfer of the radioactivity to HDL and LFF was evident at 10 min after the injection. Approximately 53% of the counts in plasma could be recovered with LFF and 37% with HDL (**Fig. 8**). At 3 hr after injection, most of the plasma radioactivity could again be re-

TABLE 2. Human apolipoprotein A-IV: metabolic parameters in plasma (I)

					Plasma Concentration				ApoA-IV	
Subject	Sex	Age	Height	Weight	Chol	TG	HDL Chol	ApoA-IV	Residence Time	Degradation Rate
			cm	*kg*	*mg/dl*				*d*	*mg/kg · d*
A	M	25	173	67	186 ± 7[a]	82 ± 5	31 ± 2	14.3 ± 3.1	0.62	8.65
B	M	26	181	70	175 ± 5	86 ± 3	37 ± 4	16.0 ± 1.2	0.65	9.03
C	M	28	176	70	201 ± 4	90 ± 4	36 ± 3	14.4 ± 1.4	0.64	8.40
Mean ± SD									0.64 ± 0.02	8.69 ± 0.32

[a]Mean ± SD from 11 independent determinations performed on plasma samples collected at admission and at the selected time point during the turnover study.

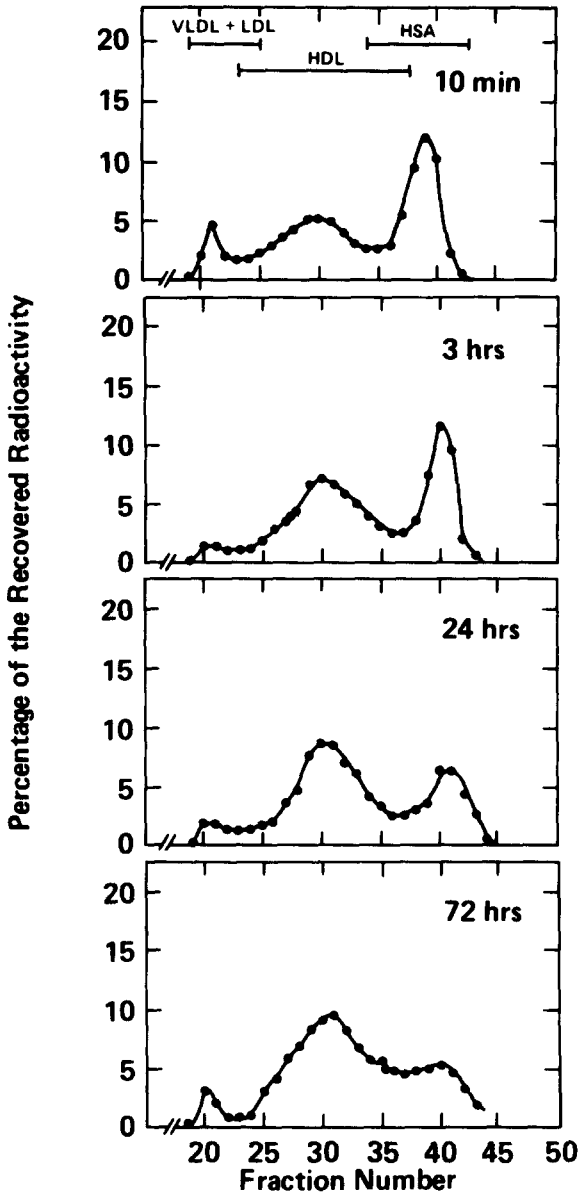

Fig. 7. Distribution of the radioactivity in plasma after injection of radioiodinated apoA-IV as free protein. After the injection of radioiodinated apoA-IV, plasma was sampled at different times and subjected (5 ml) to molecular sieve chromatography on an AcA34 column (1.5 × 100 cm) for the determination of the radioactivity distribution among the triglyceride-rich lipoproteins, HDL, and the lipoprotein-free plasma fraction. The elution curves are those obtained for the turnover study in subject A. Distributions for the other two subjects are similar.

covered with HDL and LFF; however, the proportion associated with HDL had increased.

Ten minutes after the HDL fraction had been injected, virtually all of the plasma radioactivity could be recovered in the HDL peak. At all of the subsequent withdrawal times up to 72 hr no transfer of the radioactivity to the other apoA-IV plasma pools could be detected and more than 90% of the radioactivity remaining in plasma was associated with HDL (**Fig. 9**). In this metabolic situation, the decay in plasma of the radioactivity actually represented exclusively the disappearance of the radioactivity from the pool of apoA-IV in HDL alone. Thus the kinetic parameters of apoA-IV in HDL could be calculated from the plasma radioactivity disappearance curve (Table 3). The residence time of apoA-IV associated to HDL in plasma determined in two subjects was 1.61 days and the apoA-IV degradation rate through the HDL pool was 0.86 mg/kg·day assuming that 25% of apoA-IV in plasma was in this fraction at all the times.

After injection of radioiodinated apoA-IV-labeled LFF, virtually all of the plasma radioactivity was recovered within the chromatographic region of the injected fraction (see **Fig. 10**) at all of the times from 10 min to 72 hr. These results were similar to those for the experiments with apoA-IV associated with HDL. Thus the kinetic parameters of apoA-IV in LFF could be calculated from the plasma radioactivity disappearance curves (**Fig. 11** and Table 3). The residence time and the degradation rate were, respectively, 0.55 days and 7.67 mg/kg·day as a mean in the two subjects studied.

DISCUSSION

An increasing body of evidence suggests that apoA-IV may have specific functions in the lipid transport and in the metabolism of the lipoproteins in plasma. The content of apoA-IV in the lipoproteins from human lymph rises during fat absorption and the postprandial levels of apoA-IV in plasma are increased (13, 14, 38). Similarly, in rats, a single fatty meal elevates apoA-IV plasma levels above the prefeeding values and produces detectable changes in the relative distribution of apoA-IV among HDL and the lipoprotein-free plasma fraction (15). Green et al. (14) have found low apoA-IV plasma levels in subjects with abetalipoproteinemia, a finding consistent with the idea that impaired intestinal chylomicron formation affects apoA-IV lymph transport. Other in vitro studies with rat plasma suggest that apoA-IV in the HDL and LFF pools is in a dynamic state and that mass transfer of apoA-IV from LFF to HDL is linked to cholesterol esterification (21, 22). While this series of observations implicates apoA-IV in intestinal lipid absorption, in lipoprotein formation, and in the metabolism of plasma cholesteryl esters, several aspects of the kinetic behavior of apoA-IV remain obscure. In particular, there is incomplete knowledge of the distribution of apoA-IV in plasma, whether or not such compartmentalization reflects metabolic heterogeneity and, ultimately, whether it is related to specific functions of apoA-IV.

The plasma distribution of apoA-IV in humans is notably different from that of the other apolipoproteins in that most of the circulating apoA-IV is not associated to any of the major lipoprotein classes. Several authors (4, 14, 21,

TABLE 3. Human apolipoprotein A-IV: metabolic parameters in plasma (II)

Subject	Sex	Age	Height	Weight	Injected Tracers	Plasma Concentration				ApoA-IV HDL		ApoA-IV LFF	
						Chol	TG	HDL Chol	ApoA-IV	Residence Time[c]	Degradation Rate	Residence Time	Degradation Rate
			cm	*kg*		*mg/dl*				*d*	*mg/kg · d*	*d*	*mg/kg · d*
D	M	25	170	68	^{125}I-A-IV TRL/^{131}I-A-IV HDL[a]	175 ± 6[b]	81 ± 5	38 ± 2	14.3 ± 2.0	1.73	0.77		
E	M	27	169	72	^{125}I-A-IV LFF/^{131}I-A-IV HDL	183 ± 5	104 ± 6	45 ± 2	14.6 ± 2.3	1.48	0.95	0.53	7.70
F	M	32	180	75	^{125}I-A-IV TRL/^{131}I-A-IV LFF	166 ± 5	115 ± 4	42 ± 3	16.2 ± 1.4			0.57	7.64
Mean										1.61	0.86	0.55	7.67

[a] TRL, triglyceride-rich lipoproteins; HDL, high density lipoproteins; LFF, lipoprotein-free fractions.
[b] Mean ± SD from nine independent determinations performed on plasma samples collected at admission and at the selected time points during the turnover study.
[c] Due to the rapid transfer of the radioactivity from the triglyceride-rich lipoproteins to HDL and LFF, the metabolic parameters for ^{125}I-apoA-IV TRL could not be calculated.

38) have pointed out that apoA-IV distribution in plasma can be grossly altered by ultracentrifugation. In fact, a significantly higher proportion of apoA-IV is recovered associated with HDL when the plasma is fractionated by a less disruptive procedure, such as column chromatography. The latter procedure was utilized throughout our study. A column was selected that allowed discrimination of the HDL into HDL_2 and HDL_3 subfractions. Moreover, the plasma fraction free of lipoproteins eluted from the chromatographic column as a single peak well separated from those containing lipoproteins. With this column technique, only 1 to 5% of apoA-IV was found associated to the triglyceride-rich lipoprotein fraction in the normolipidemic fasting plasma. Approximately one-fifth of the plasma apoA-IV eluted together with the HDL. Within the HDL fraction, the lipoprotein peak containing apoA-IV had a retention volume higher than that of the apoA-I in HDL_3, which suggests that apoA-IV is associated in plasma to HDL of small size. It is believed that these HDL particles also contain apoA-I, apoA-II, and the C peptides in addition to apoA-IV and that they might have an intracellular origin, being characterized by a high ratio of saturated to unsaturated cholesteryl ester (38, 39). The majority (80%) of apoA-IV eluted from the column together with the bulk of the plasma proteins. Otha, Fidge, and Nestel (39) have used an affinity chromatography column complexed with anti-apoA-IV to isolate from the lipoprotein-free plasma fraction a lipoprotein complex containing apoA-IV, apoA-I, a peptide with a molecular weight of 59,000, and lipids.

Others have measured the plasma concentration of apoA-IV by immunoelectrophoresis (3, 4, 14, 24) and the results are similar to those obtained with our radioimmunoassay method. These concentrations, however, are lower than those reported by Bisgaier et al. (38), who also have utilized a radioimmunoassay method. The discrepancy likely reflects differences in methodology. In our study, the absolute value of the apoA-IV concentrations has been validated by a thoroughly independent method based on gel electrophoresis and the measurement of the apoA-IV-to-apoA-I mass ratio in different plasma samples. In order to assess a possible relationship between apoA-IV

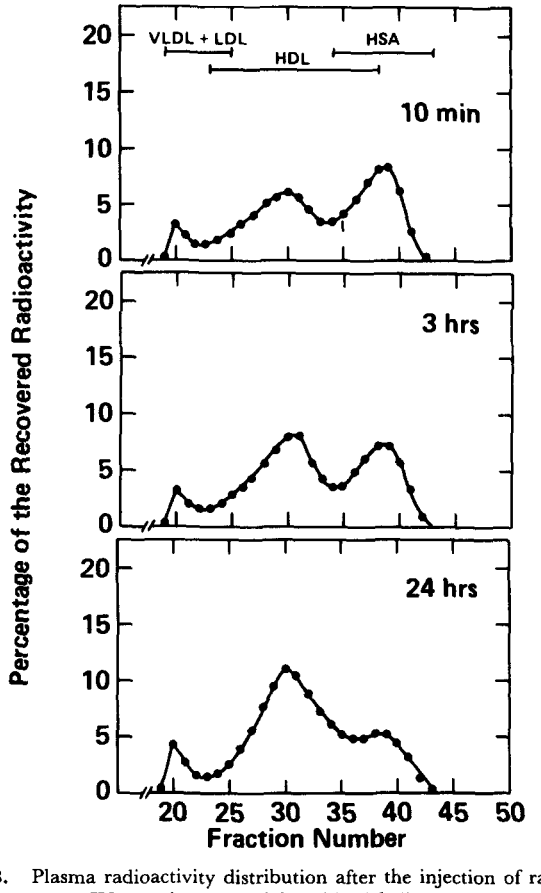

Fig. 8. Plasma radioactivity distribution after the injection of radioiodinated apoA-IV associated to triglyceride-rich lipoproteins. Radioiodinated apoA-IV was incubated with the recipient subject's own plasma collected after a fatty meal, and the mixture was subjected to molecular sieve chromatography (see the Methods section). The triglyceride-rich lipoprotein fraction that eluted from this column was then utilized for the turnover study. Plasma samples were obtained at different times after the injection and chromatographed over an AcA34 column for determination of the radioactivity distribution in plasma.

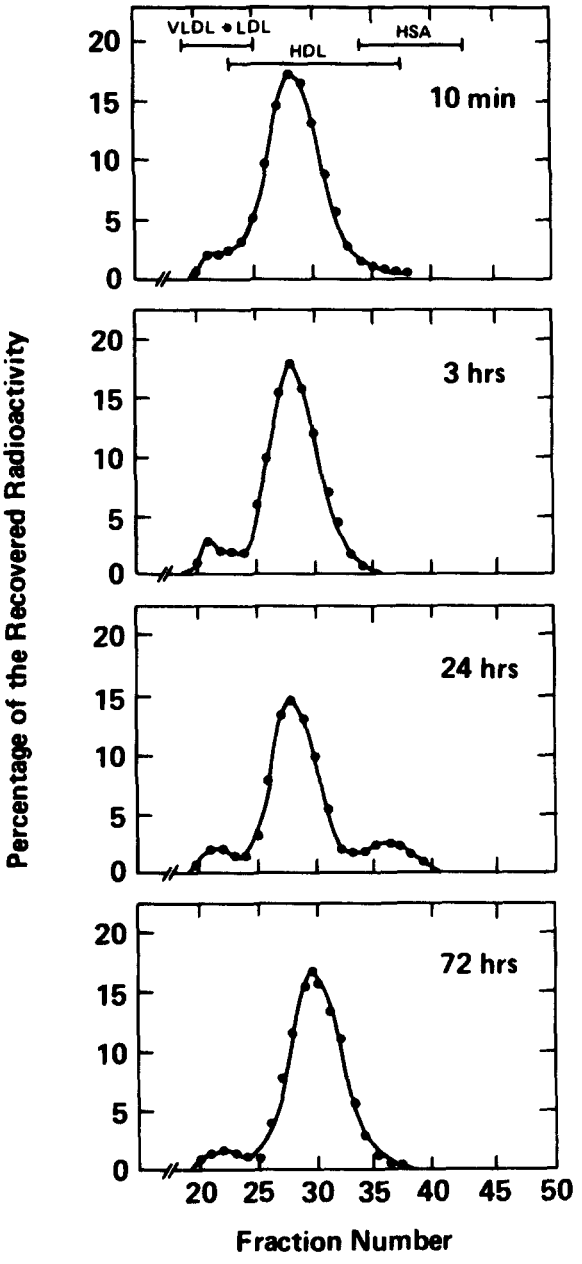

Fig. 9. Plasma radioactivity distribution after the injection of radioiodinated apoA-IV associated to HDL. Radioiodinated apoA-IV was incubated with the recipient subject's own plasma and radiolabeled HDL recovered by molecular sieve chromatography. The plasma samples obtained at different times after the injection of radiolabeled HDL were then chromatographed over an AcA34 column for determination of the radioactivity distribution in plasma.

plasma concentrations and the levels of the various lipoproteins in plasma, apoA-IV was measured in a number of normal and dyslipoproteinemic plasma samples. Abnormal concentrations have been reported for virtually all the other human apolipoproteins in the different hyperlipoproteinemic states (40). A previous study by Utermann and Beisiegel (4) showed that apoA-IV levels are virtually identical in normolipidemic and hypertriglyceridemic subjects. In this study, apoA-IV levels were also normal in subjects with hypercholesterolemia. Moreover, apoA-IV distribution did not appear to be correlated with the degree of hyperlipidemia. This might simply reflect the fact that the majority of apoA-IV is not associated to the lipoproteins in plasma.

The results from the turnover experiments indicate that apoA-IV is among the apolipoproteins with the highest turnover rate; the fractional catabolic rate is, in fact, 1.56

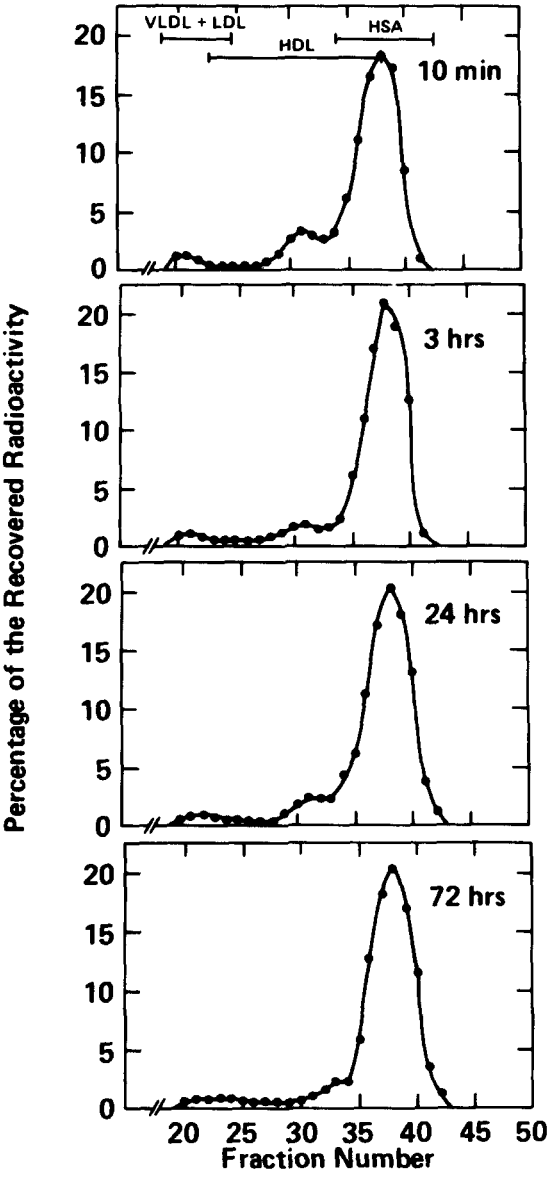

Fig. 10. Plasma radioactivity distribution after injection of radioiodinated apoA-IV carried in the lipoprotein-free plasma fraction (LFF). Radioiodinated apoA-IV was incubated with the recipient subject's own plasma and the mixture was subjected to molecular sieve chromatography to separate radiolabeled LFF. The plasma samples obtained at different times after the injection of the tracer were then chromatographed over an AcA34 column for determination of the radioactivity distribution in plasma.

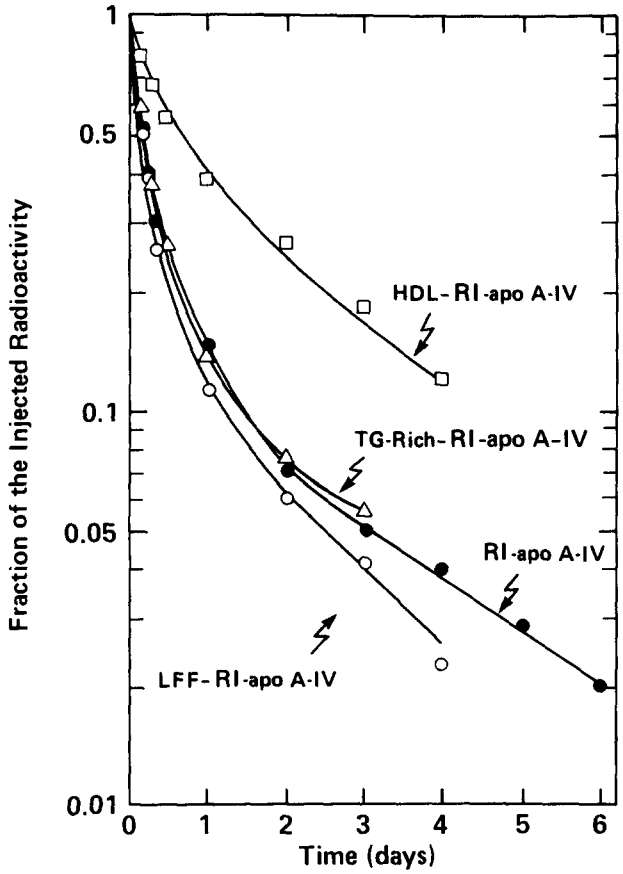

Fig. 11. Plasma radioactivity decay curves generated by the injection of radioiodinated apoA-IV free or associated to the triglyceride-rich lipoproteins, the HDL, or the lipoprotein-free plasma fraction (LFF). Radioiodinated (RI) apoA-IV was incubated with the recipient subject's own plasma and the mixture was subjected to molecular sieve chromatography over an AcA34 column (1.5 × 50 cm). The eluted triglyceride-rich lipoprotein, HDL, and lipoprotein-free plasma fractions were then utilized for the turnover studies whose radioactivity distribution results are shown in Figs. 9, 10, and 11. Since radioiodinated apoA-IV injected associated to HDL or LFF did not transfer to another apoA-IV pool while in circulation, the generated plasma radioactivity decay curves actually monitored the disappearance rate of apoA-IV in HDL and LFF, allowing calculation of the apolipoprotein catabolic rates in these two pools. Due to the rapid transfer of triglyceride-rich lipoprotein radioiodinated apoA-IV to HDL and LFF soon after injection, the decay curve denoted TG-Rich-RI-apoA-IV simply illustrates the decay in plasma of the radioactivity originally associated to the injected triglyceride-rich lipoprotein. It should be regarded as a composite of the decay curves of the radioiodinated apoA-IV in HDL and LFF. The decay curve for free apoA-IV shown in this figure is the mean of the curves of Fig. 6 (see also Table 2). The curves for apoA-IV associated to lipoproteins are the mean of the values from two subjects (see Table 3).

pools per day. Similar results have been reported by others (41). In order to meet such a catabolic demand, apoA-IV must be synthesized at the remarkable rate of more than 8 mg/kg · day.[2] This approaches the rate of synthesis of apoA-I, which is one of the most abundant plasma apolipoproteins (28, 32). The same studies support the view that apoA-IV is metabolically compartmentalized in plasma among three major pools, i.e., the triglyceride-rich lipoprotein, the HDL, and the LFF pools. Subsequent to the injection of the triglyceride-rich lipoproteins labeled with radioiodinated apoA-IV, radioactivity rapidly transferred from this fraction to HDL and LFF. This behavior lends support to the idea that the HDL and LFF apoA-IV pools are maintained, at least in part, by an influx from the plasma triglyceride-rich lipoproteins. Extrapolation of this catabolic model to apoA-IV carried by lymph chylomicrons entering the vascular space, however, may not be warranted as apoA-I, with which apoA-IV shares many structural similarities (2), is transferred directly from the chylomicrons to HDL (42–44). When radioiodinated apoA-IV-labeled HDL and LFF were injected, on the other hand, no exchange of radioactivity between the different plasma pools could be detected. The behavior of apoA-IV in LFF appears noteworthy. If apoA-IV in this fraction is, in fact, a free protein, a redistribution of the radioactivity among the different apoA-IV pools would be expected after the injection of radioiodinated apoA-IV-labeled LFF as occurred when radioiodinated apoA-IV was injected as a free protein (see Figs. 8 and 11). The lack of this redistribution suggests that apoA-IV in LFF does not behave as a free protein. Indeed, complexes of apoA-IV with cholesterol and phospholipid have been isolated from the LFF of humans (35) and rats (45). Association of apoA-IV with lipids may be a constraint for free movement. Dimers of apoA-IV have also been identified in plasma (46) and may affect apoA-IV distribution. However, their relative concentration may be low and dimerization per se may not preclude the formation of apoA-IV-lipid complexes, reassessing the lipid association as a potential major constraining factor. LFF-apoA-IV may be of directly synthesized (8). Alternatively, some of the excess lipids that form at the surface of chylomicrons and triglyceride-rich lipoproteins when these are acted upon by lipoprotein lipase may be specifically removed together with apoA-IV and transferred to the LFF for final catabolism (39, 44, 47, 48). ApoA-IV-rich lipoproteins have been observed in the plasma of subjects with hereditary apoE deficiency (23) or chronic renal failure (24) and have the characteristics of chylomicron remnants suggesting that apoA-IV may be implicated in the catabolism of the intestinal triglyceride-rich lipoproteins.

In additional support to the idea that apoA-IV is metabolically compartmentalized in plasma is the finding that the residence times of apoA-IV in LFF and in HDL are different (0.55 days vs. 1.61 days, respectively). Transfer of apoA-IV from LFF to HDL has been observed during in vitro incubation of human and rat plasma; it appears to be linked to the cholesterol esterification reaction (21, 22). The question arises whether this pathway is also operative in vivo. We have observed that in freshly drawn

[2]In two hypertriglyceridemic subjects (TG > 700 mg/dl), the apoA-IV residence time and degradation rate were 0.85 and 0.96 days, and 6.42 and 7.00 mg/kg · day, respectively.

human plasma the mass transfer of apoA-IV in the direction of LFF to HDL occurs in vitro at a noticeably lower rate than that reported in the rat (data not shown). Clearly there may be species differences accounting for this result. In the rat, in contrast to humans, a greater portion of apoA-IV is found in the HDL than in the LFF (22). The results from the in vitro incubation studies, however, may be confusing, since that system is dynamically closed and allows the progressive build-up of cholesteryl ester-rich lipoproteins which are usually rapidly cleared in vivo and for which apoA-IV may have a high affinity.[3] If in vivo LFF apoA-IV was actually converted into HDL apoA-IV, it should have resulted in an accumulation of radiolabeled apoA-IV in HDL because, in this pool, apoA-IV has a slower turnover rate. Since this was not seen, the fact that apoA-IV in HDL and in LFF have different turnover rates rather suggests that the HDL and LFF apoA-IV pools have different catabolic fates. Perhaps the HDL and LFF apoA-IV pools have distinct metabolic functions and this is responsible for their different catabolism.

In a recent report, Otha, Fidge, and Nestel (41) observed specific activity of apoA-IV in HDL up to 30 times higher than that in LFF after injection of radioiodinated apoA-IV. These results are said by these authors to mimic the behavior of radioiodinated apoA-IV in vitro. Similar findings, however, could not be obtained in our laboratory. As illustrated in Fig. 7, the distribution of the radioactivity in vivo at 10 min after the injection was similar to that predicted by the distribution of the mass of apoA-IV. Similar results could be obtained in vitro. The reason for the discrepancies between our results and those obtained by Otha et al. (41) may depend in part upon differences in the methods utilized for apoA-IV isolation and radioiodination. In addition, in our study, apoA-IV distribution in plasma was determined by molecular sieve chromatography while ultracentrifugation was used by Otha et al. (41). As discussed previously, apoA-IV distribution is plasma may be grossly affected during the ultracentrifugation procedure, and radioiodinated apoA-IV may have sheared from HDL at a different rate than native apoA-IV, yielding erroneous measurements of the specific activity. Finally, different subject populations were studied.

The high rate of turnover of apoA-IV may reveal other major functions of this apolipoprotein. The residence time of apoA-IV in HDL, even if it is longer than that of apoA-IV in LFF, is, in fact, much shorter than that of apoA-I (approximately 4.5 days) which is the major apolipoprotein in HDL (28, 32, 49). If apoA-IV and apoA-I in human plasma reside on the same lipoprotein particle as the compositional studies of Otha, Fidge, and Nestel (39) suggest, then it may occur that HDL lipids, or a selected portion of these lipids, are directly catabolized together with apoA-IV without the whole HDL particle being irreversibly degraded. It has been reported (50) that in the rat the uptake of HDL cholesteryl ester by the liver, adrenal, and gonads is dissociated from that of apoA-I. HDL are extremely dynamic lipoproteins (49). Lipids derived from the breakdown of lipoproteins with lower density or from the peripheral tissues are quickly accommodated with changes in composition, size, and hydrated density (44, 47, 48, 51–54). Cholesteryl ester is formed on HDL and transferred to lipoproteins of lower density (55). ApoA-IV may function in a catabolic system bypassing complete HDL destruction and allowing excess lipids to leave HDL and the lipoprotein to recirculate. Further studies are necessary, however, to assess the full validity of this hypothesis.

In conclusion, the data presented provide evidence that apoA-IV is metabolically compartmentalized in plasma into three distinct pools. ApoA-IV in the triglyceride-rich lipoprotein pool is transferred to the HDL and LFF apoA-IV pools. In HDL, apoA-IV is carried by lipoprotein particles smaller than typical HDL_3. On this lipoprotein particle, the catabolism of apoA-IV appears dissociated from that of apoA-I. The kinetic behavior of apoA-IV circulating in the LFF suggests that in this plasma fraction apoA-IV is not a free protein but rather is lipid-associated or is in a different physical conformation. The fate of apoA-IV in HDL and LFF appears to be that of final catabolism, since no transfer of radioactivity could be .detected between these pools and the other apoA-IV plasma pools during in vivo turnover studies. The facts that apoA-IV has a rapid plasma catabolism and that its residence times in HDL and LFF are different may be due to a specific catabolic processing of apoA-IV at some level. This process may discriminate between HDL and LFF apoA-IV.

We are indebted to Dr. Sarada Prasad for the generous help given in establishing the radioimmunoassay assays, and to Ms. Sandy White and her staff for the nursing care provided to the volunteers while at the General Clinical Research Center of the Methodist Hospital.

Manuscript received 11 October 1985.

REFERENCES

1. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apolipoprotein from the d < 1.006 lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.

[3]Preliminary results suggest that the differences between rat and human in the magnitude of the HDL apoA-IV pool, and in the rate of mass transfer of apoA-IV from LFF to HDL observed in vitro, may be accounted for by the absence in rat plasma of the cholesteryl ester transfer system.

2. Elshourbagy, N. A., D. W. Walker, M. S. Boguski, J. L. Gordon, and J. M. Taylor. 1986. The nucleotide and derived amino acid sequence of human apolipoprotein A-IV mRNA and the close linkage of its gene to the genes of apolipoprotein A-I and C-III. *J. Biol. Chem.* **26:** 1998–2002.
3. Weinberg, R. B., and A. M. Scanu. 1983. Isolation and characterization of human apolipoprotein A-IV from lipoprotein-depleted serum. *J. Lipid Res.* **24:** 52–59.
4. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma: isolation and quantitation. *Eur. J. Biochem.* **99:** 333–343.
5. Menzel, H-J., R. G. Kladetzky, and G. Assmann. 1982. One-step screening method for the polymorphism of apolipoproteins A-I, A-II, and A-IV. *J. Lipid Res.* **23:** 915–922.
6. Utermann, G. 1983. Screening for apolipoprotein mutants by electrophoretic procedures. *In* Proceedings of the Workshop on Apolipoprotein Quantification. K. Lippel, editor. NIH publication no. 83-1266, Bethesda, MD. 101–122.
7. Swaney, J. B., H. Reese, and H. A. Eder. 1974. Polypeptide composition of rat high density lipoproteins: characterization by SDS-gel electrophoresis. *Biochem. Biophys. Res. Commun.* **59:** 513–519.
8. Windmueller, H. G., and A. L. Wu. 1981. Biosynthesis of plasma apolipoproteins by rat small intestine without dietary or biliary fat. *J. Biol. Chem.* **256:** 3012–3016.
9. Ghiselli, G., H. B. Brewer, Jr., and H. G. Windmueller. 1982. Apolipoprotein A-I isoprotein synthesis by the perfused rat liver. *Biochem. Biophys. Res. Commun.* **107:** 144–149.
10. Gordon, J. I., D. P. Smith, D. H. Alpers, and A. W. Strauss. 1982. Proteolytic processing of the primary translation product of rat intestinal apolipoprotein A-IV in RNA: comparison with preproapolipoprotein A-I processing. *J. Biol. Chem.* **257:** 8418–8423.
11. Gordon, J. I., D. P. Smith, D. H. Alpers, and A. W. Strauss. 1982. Cloning of a complementary deoxyribonucleic acid encoding a portion of rat intestine preapolipoprotein A-IV messenger ribonucleic acid. *Biochemistry.* **21:** 5424–5431.
12. Gordon, J. I., C. L. Bisgaier, H. G. Sims, O. Sachdev, R. M. Glickman, and A. W. Strauss. 1984. Biosynthesis of human preapolipoprotein A-IV. *J. Biol. Chem.* **259:** 468–474.
13. Green, P. M. R., R. M. Glickman, C. O. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins: studies in chyluric subjects. *J. Clin. Invest.* **64:** 233–242.
14. Green, P. M. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV: intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
15. DeLamatre, J. G., and P. S. Roheim. 1983. The response of apolipoprotein A-IV to cholesterol feeding in rats. *Biochim. Biophys. Acta.* **751:** 210–217.
16. Kane, J. P., D. Hardman, and H. E. Paulus. 1980. Heterogeneity of apolipoprotein B: isolation of a new species from human chylomicrons. *Proc. Natl. Acad. Sci. USA.* **77:** 2465–2469.
17. Ghiselli, G., E. J. Schaefer, J. A. Light, and H. B. Brewer, Jr. 1983. Apolipoprotein A-I isoforms in human lymph: effect of fat absorption. *J. Lipid Res.* **24:** 731–736.
18. Stalenhoef, A. F. M., M. J. Malloy, J. P. Kane, and R. J. Havel. 1984. Metabolism of apolipoproteins B-48 and B-100 of triglyceride-rich lipoproteins in normal and lipoprotein lipase-deficient humans. *Proc. Natl. Acad. Sci. USA.* **81:** 1839–1843.
19. Ghiselli, G., A. M. Gotto, Jr., S. Tanenbaum, and B. C. Sherrill. 1985. Proapolipoprotein A-I conversion kinetics in vivo in human and rat. *Proc. Natl. Acad. Sci. USA.* **82:** 874–878.
20. Fidge, N. H. 1980. The redistribution and metabolism of iodinated apolipoprotein A-IV in rats. *Biochim. Biophys. Acta.* **619:** 129–141.
21. DeLamatre, J. G., C. A. Hoffmeier, A. G. Lacko, and P. S. Roheim. 1983. Distribution of apolipoprotein A-IV between the lipoprotein and the lipoprotein-free fractions of rat plasma: possible role of lecithin:cholesterol acyltransferase. *J. Lipid Res.* **24:** 1578–1585.
22. Weinberg, R. B., and M. S. Spector. 1986. Lipoprotein affinity of human apolipoprotein A-IV during cholesterol esterification. *Biochem. Biophys. Res. Commun.* **135:** 756–763.
23. Ghiselli, G., E. J. Schaefer, P. Gascon, and H. B. Brewer, Jr. 1981. Type III hyperlipoproteinemia associated with apolipoprotein E deficiency. *Science.* **214:** 1239–1241.
24. Nestel, P. J., N. H. Fidge, and M. H. Tan. 1982. Increased lipoprotein-remnant formation in chronic renal failure. *N. Engl. J. Med.* **307:** 329–333.
25. Weber, K., and H. Osborn. 1969. The reliability of molecular weight determination by dodecylsulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* **244:** 4406–4412.
26. Anderson, N. G., and N. L. Anderson. 1978. Analytical techniques for cell fractionation. XXII. Two-dimensional analysis of serum and tissue proteins: multiple gradient slab-gel electrophoresis. *Anal. Biochem.* **86:** 341–354.
27. Ouchterlony, O. 1958. Diffusion-in-gel methods for immunological analysis. *Prog. Allergy* **5:** 1–78.
28. Schaefer, E. J., C. B. Blum, R. I. Levy, L. L. Jenkins, P. Alaupovic, D. M. Foster, and H. B. Brewer, Jr. 1978. Metabolism of high density lipoprotein apolipoproteins in Tangier disease. *N. Engl. J. Med.* **229:** 905–910.
29. Harboe, N., and A. Ingild. 1973. Immunization, isolation of immunoglobulins, estimation of antibody titer. *In* A Manual of Quantitative Immunoelectrophoresis. N. H. Axelsen, J. Kroll, and B. Wecke, editors. Universitetsforlaget, Oslo. 161–164.
30. Rodbard, D., and D. M. Hutt. 1974. Statistical analysis of radioimmunoassays and immunoradiometric (labeled antibody) assays. *In* Radioimmunoassays and Related Procedures in Medicine. International Atomic Energy Agency, Vienna. **1:** 165–192.
31. Bradford, M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Anal. Biochem.* **72:** 248–254.
32. Ghiselli, G., M. G. Rohde, S. Tanenbaum, S. Krishnan, and A. M. Gotto, Jr. 1985. Origin of apolipoprotein A-I polymorphism in plasma. *J. Biol. Chem.* **260:** 15662–15668.
33. Fenner, C., R. R. Traut, D. T. Mason, and J. Wikman-Coffelt. 1975. Quantification of Coomassie Blue-stained proteins in polyacrylamide gels based on analysis of eluted dye. *Anal. Biochem.* **63:** 595–602.
34. Shipley, R. A., and R. E. Clark. 1972. Tracer Methods for in Vivo Kinetics: Theory and Applications. Academic Press, New York. 24–28.
35. Lieberman, S., and P. Samuel. 1982. Determination of total body cholesterol: input-output analysis versus compartmental analysis. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 332–336.
36. Karlin, J. B., D. J. Juhn, A. M. Scanu, and A. M. Rubenstein. 1978. Measurement of serum apolipoprotein B by radioimmunoassay. *Eur. J. Clin. Invest.* **8:** 19–26.
37. Karlin, J. B., S. C. Prasad, A. M. Gotto, Jr., and W. A.

Bradley. 1983. Immunochemical heterogeneity of human apolipoprotein E as measured by radioimmunoassay. *In* Proceedings of the Workshop on Apolipoprotein Quantification. K. Lippel, editor. NIH Publ. no. 83-1266, Bethesda, MD. 212-221.
38. Bisgaier, C. L., O. P. Sachdev, L. Megna, and R. M. Glickman. 1985. Distribution of apolipoprotein A-IV in human plasma. *J. Lipid Res.* **26:** 11-25.
39. Otha, T., N. H. Fidge, and P. J. Nestel. 1984. Characterization of apolipoprotein A-IV complex and apoA-IV isoforms in human lymph and plasma lipoproteins. *J. Biol. Chem.* **259:** 14888-14893.
40. Alaupovic, P. 1983. Serum apolipoprotein profiles of dyslipoproteinemias. *In* Dietary Fat and Health. E. G. Perkins and W. J. Visek, editors. American Oil Chemists' Society, Champaign, IL. 574-597.
41. Otha, T., N. H. Fidge, and P. J. Nestel. 1985. Studies on the in vivo and in vitro distribution of apolipoprotein A-IV in human plasma and lymph. *J. Clin. Invest.* **76:** 1252-1260.
42. Schaefer, E. J., L. J. Jenkins, and H. B. Brewer, Jr. 1978. Human chylomicron apolipoprotein metabolism. *Biochem. Biophys. Res. Commun.* **80:** 405-412.
43. Imaizumi, K., M. Fainaru, and R. J. Havel. 1978. Composition of proteins of mesenteric lymph chylomicrons in the rat and alterations produced upon exposure of chylomicrons to blood serum and serum proteins. *J. Lipid Res.* **19:** 712-722.
44. Tall, A. R., P. H. R. Green, R. M. Glickman, and J. W. Riley. 1979. Metabolic fate of chylomicron phospholipids and apolipoproteins in the rat. *J. Clin. Invest.* **64:** 977-989.
45. Dallinga-Thie, G. M., P. H. E. Groot, and A. van Tol. 1985. Distribution of apolipoprotein A-IV among lipoprotein subclasses in rat serum. *J. Lipid Res.* **26:** 970-976.
46. Weinberg, R. B., and M. S. Spector. 1985. The self-association of human apolipoprotein A-IV. Evidence for an in vivo circulating dimeric form. *J. Biol. Chem.* **260:** 14279-14286.
47. 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.
48. Redgrave, T. G., and D. M. Small. 1979. Quantitation of the transfer of surface phospholipid of chylomicrons to the high density lipoprotein fraction during the catabolism of chylomicrons in rat. *J. Clin. Invest.* **64:** 162-171.
49. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* **25:** 1017-1058.
50. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435-5439.
51. Havel, R. J. 1957. Early effect of fat ingestion on lipids and lipoproteins of serum in man. *J. Clin. Invest.* **36:** 848-854.
52. Havel, R. J., J. P. Kane, and M. L. Kashyap. 1973. Interchange of apolipoproteins between chylomicrons and high density lipoproteins during alimentary lipemia in man. *J. Clin. Invest.* **52:** 32-38.
53. Eisenberg, S., and D. Rachmilewitz. 1975. Interaction of rat plasma very low density lipoprotein with lipoprotein lipase-rich (postheparin) plasma. *J. Lipid Res.* **16:** 341-351.
54. Schaefer, E. J., M. G. Wetzel, G. Bengtsson, R. O. Scow, H. B. Brewer, Jr., and T. Olivecrona. 1982. Transfer of human lymph chylomicron constituents to other lipoprotein density fractions during in vitro lipolysis. *J. Lipid Res.* **23:** 1259-1273.
55. Oschry, Y., and S. Eisenberg. 1982. Rat plasma lipoproteins: re-evaluation of a lipoprotein system in an animal devoid of cholesteryl ester transfer activity. *J. Lipid Res.* **23:** 1099-1106.