Evidence for high density lipoproteins as the major apolipoprotein A-IV-containing fraction in normal human serum

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Abstract The distribution of human apolipoprotein A-IV was studied in sera from normolipidemic fasting subjects by high performance gel filtration on a Superose 12 HR column. The major part of apolipoprotein A-IV eluted in the range of the apolipoprotein A-I peak, and distributed mainly in the large-size high density lipoprotein subfractions. Only a small peak or a shoulder on the main fraction appeared in the elution volume of free apolipoprotein A-IV. To investigate the relation of apolipoprotein A-IV with high density lipoprotein particles, serum high density lipoproteins were precipitated by incubating human serum with anti-apolipoprotein A-I immunoglobulins. At optimal concentrations, inducing a precipitation of 90 to 95% of serum apolipoprotein A-I, about 70% of serum apolipoprotein A-IV was precipitated. It was concluded that, in fasting human serum, apolipoprotein A-IV was mainly associated with high density lipoprotein particles. This high degree of association to high density lipoproteins did not result from the known in vitro redistribution of apolipoprotein A-IV induced by lecithin: cholesterol acyltransferase activity since it was observed in sera in the presence of inhibitors of this enzyme. The comparison of gel filtration profiles of total serum and of serum fractions separated by ultracentrifugation showed that the apolipoprotein A-IV–high density lipoprotein association was a weak one, easily dissociated by the ultracentrifugation process. The existence in fasting human serum of a predominant high density lipoprotein-associated form of apolipoprotein A-IV should stimulate more studies of the general function and metabolism of this protein. —**Lagrost, L., P. Gambert, M. Boquillon, and C. Lallemant.** Evidence for high density lipoproteins as the major apolipoprotein A-IV-containing fraction in normal human serum. *J. Lipid Res.* **30:** 1525–1534.

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ApoA-IV, a 46,000 dalton protein described for the first time in 1974 in the rat (1) is still a little known apolipoprotein. It has been shown to be an LCAT activator (2), a cofactor in the HDL conversion process (3), to bind specifically to rat hepatocytes (4, 5) and to bovine aortic endothelial cells (6, 7), and to play a role in reverse cholesterol transport (8). However, its precise metabolic function remains obscure. Moreover, its plasma distribution is still uncertain and the reported proportions of apoA-IV associated with lipoproteins, both in rat and in human, vary widely.

Apolipoprotein A-IV was found initially in rat HDL and chylomicrons (1, 9) and later in human chylomicrons, VLDL and in the $d > 1.21$ g/ml plasma fraction (10–14). It has been reported that apoA-IV enters the blood circulation on triglyceride-rich particles and rapidly dissociates from them to constitute thereafter an unassociated pool (10, 12, 15, 16). Whereas it is known that, in rat, most of this protein subsequently reassociates with HDL (15), in human, very little apoA-IV has been found on lipoprotein particles isolated by traditional methods such as density-gradient or sequential ultracentrifugations (11, 13), agarose gel filtration (13), or crossed immunoelectrophoresis (11,13). However, the presence of some apoA-IV on human HDL has been demonstrated by using plasma gel filtration (17,18) and immunoprecipitation of gel-filtered plasma HDL with anti-apoA-I antibodies (17). An association of apoA-IV with apoA-I, lipids, and other peptides has been shown in HDL and in the $d > 1.21$ g/ml fraction (19) and an LCAT-dependent redistribution of apoA-IV from the lipoprotein-free fraction to HDL has been demonstrated in the rat (20) and more recently in humans (21, 22). In view of these conflicting results, it has been suggested that the absence of apoA-IV on lipoproteins in plasma from fasting individuals could be artifac-

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; LCAT, lecithin:cholesterol acyltransferase; PCMPS, *p*-chloromercuriphenylsulfonic acid; DTNB, 5,5'-dithiobis-2-nitrobenzoic acid; SDS, sodium dodecyl sulfate.

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tual and due to a redistribution of the apolipoprotein towards the lipoprotein-free fraction induced by serum fractionation techniques (13, 23, 24).

Convinced that knowing the real status of human apoA-IV in plasma would be an important step to the understanding of the role of this apolipoprotein, we undertook the study of its distribution in normal fasting serum by gel filtration on a high performance system and by HDL immunoprecipitation.

MATERIALS AND METHODS

Sera

Human blood was drawn from fasting normal adults into plain glass tube and placed immediately at 4°C. Sera were collected by a 5-min centrifugation at 3000 *g*, stored at 4°C, and analyzed within the next 8 h. In some experiments aliquots of sera were immediately supplemented with PCMPS (2 mmol/l) or DTNB (1 mmol/l).

Rate-zonal ultracentrifugation

Rate-zonal ultracentrifugation was performed as previously described by Patsch et al. (25) in an L2-65B ultracentrifuge (Beckman, Palo Alto, CA) equipped with a Beckman Ti-14 zonal rotor. Sera were fractionated in a 1.00–1.40 g/ml nonlinear NaBr gradient formed with the program cam A of Patsch et al. (25). A 7-ml aliquot of serum was injected at d 1.16 g/ml. The ultracentrifugation was performed at 45,000 rpm for 24 h at 4°C. At the end of the run, 10-ml fractions were collected and their absorbance was measured at 280 nm. The density of the collected fractions was determined using a DMA 35 digital densitymeter (Paar, Graz, Austria).

Sequential ultracentrifugation

Sera were brought to the desired density by addition of solid NaBr. The ultracentrifugation was carried out for 48 h at 5°C and 45,000 rpm (176,000 *g*) in an L7-65 ultracentrifuge equipped with a 65 rotor (Beckman). The separated fractions were collected by aspiration.

Gel permeation chromatography

Gel permeation chromatography was performed in a Pharmacia (Uppsala, Sweden) Fast Protein Liquid Chromatography system equipped with a 30 cm Superose 12-HR column (molecular weight exclusion limit 2×10^6). Serum samples were injected in a volume of 50 or 100 μl and eluted at room temperature with a 150 mmol/l NaCl, 1 mmol/l Na_2EDTA, pH 7.4, solution at a flow rate of 12 ml/h, with a column pressure of 0.4 MPa. The elution was completed in about 75 min. The effluents were continuously monitored at 280 nm and fractions of 0.4 ml were collected. The column was calibrated with bovine serum albumin, aldolase, purified apoA-IV and ultracentrifugally prepared VLDL + LDL ($d < 1.063$ g/ml), HDL_2 ($1.063 < d < 1.125$ g/ml), and HDL_3 ($1.125 < d < 1.210$ g/ml) fractions.

HDL immunoprecipitation

ApoA-I-containing lipoproteins were immunoprecipitated with an anti-human apoA-I γ-globulin fraction from sheep (Boehringer, Mannhein, FRG). Nonfractionated sera and anti-apoA-I antiserum were diluted with a 40 g/l polyethylene glycol 6800 (Aldrich Chemie, Steinheim, FRG) solution supplemented or not with 2 mmol/l PCMPS and containing 10 mmol/l Na_2HPO_4, 5 mmol/l NaH_2PO_4, 150 mmol/l NaCl, and adjusted at pH 7.2 with NaOH. Aliquots (100 μl) of diluted human serum (1:20) were mixed with 100 μl of antiserum dilutions ranging from 1:1 to 1:256. After 1 h incubation at 37°C the mixtures were centrifuged for 15 min at 10,000 *g*. Concentrations of cholesterol, apoA-I, and apoA-IV were measured in the supernatants and compared with those in control mixtures where antiserum was replaced by dilution buffer.

ApoA-IV purification

Human apoA-IV was extracted from human serum using a lipid emulsion (Intralipid) and purified by preparative electrophoresis as previously described (26). The Intralipid protein extract was applied to a linear gradient of polyacrylamide ranging from 25 to 300 g/l and containing 1 g/l of SDS. After electrophoresis, the portion of gel containing apoA-IV was cut off, the protein was transferred into an agarose gel and was finally removed from the gel by centrifugation. SDS was removed from the purified protein preparation by Extracti-gel D (Pierce, Rockford, IL).

ApoA-IV immunoassay

ApoA-IV concentrations were measured by a competitive enzyme immunoassay standardized with purified apoA-IV as previously described (26). Briefly, plate coating was prepared by pipetting a 100-μl volume of pure apoA-IV solution (2.5 mg/l) into each well of a polystyrene microwell plate (Immuno 96F Type I from Nunc, Kamstrup, Denmark). After an overnight incubation at 4°C, the plates were washed and nonspecific absorption was blocked by incubation for 30 min at room temperature with 250 μl of a 10 g/l bovine albumin solution. Mixtures of apoA-IV-containing samples and diluted rabbit antiserum were incubated overnight at 4°C. Then, 100-μl aliquots were pipetted into the immunoplate microwells. After a 4-h incubation at room temperature, the plates were washed and bound anti-apoA-IV antibodies were detected using peroxidase-conjugated anti-rabbit antibodies and a solution of *O*-phenylenediamine (0.4 g/l) and hydrogen peroxide (0.68 g/l).

Lipoprotein component assays

Total cholesterol, free cholesterol, and triglyceride levels were determined by enzymatic methods using Boehringer (Mannheim, FRG) reagents. Esterified cholesterol was calculated as the difference between total and free cholesterol. HDL cholesterol was assayed after selective precipitation of apoB-containing lipoproteins by concanavalin A (27). Phospholipid levels were enzymatically determined using bioMerieux (Charbonnieres les Bains, France) reagents. ApoA-I was assayed by immunoturbidimetry (28) with Behring (Marburg, FRG) anti-apoA-I antibodies.

RESULTS

ApoA-IV distribution after serum ultracentrifugation

When human serum was submitted to rate zonal ultracentrifugation, serum proteins were fractionated into four distinct fractions, VLDL + LDL, HDL_2, HDL_3, and lipoprotein-free fractions (**Fig. 1A**). Under these conditions the bulk of apoA-IV (about 90%) was found in the lipoprotein-free fraction (Fig. 1B). This distribution contrasted with that of apoA-I, which, as expected, appeared mainly in the HDL_2 and HDL_3 fractions. Similar results were obtained after serum fractionation by sequential ultracentrifugation. About 80% of total apoA-IV was present in the $d > 1.25$ g/ml infranatant whereas more than 90% of apoA-I was recovered in the lipoprotein fraction (**Table 1**).

ApoA-IV distribution after serum gel filtration

The Superose 12 HR gel permeation column was calibrated with VLDL + LDL, HDL_2 and HDL_3 preparations, pure apoA-IV, and two proteins, albumin and aldolase, whose molecular weights are close to those of the monomeric and dimeric forms of apoA-IV, respectively. As shown in **Fig. 2**, VLDL and LDL eluted in a single peak in the void volume. HDL_2 and HDL_3 were well separated from the latter fraction and partially separated from each other. When pure apoA-IV was injected onto the column at concentrations ranging from 5.5 to 22.0

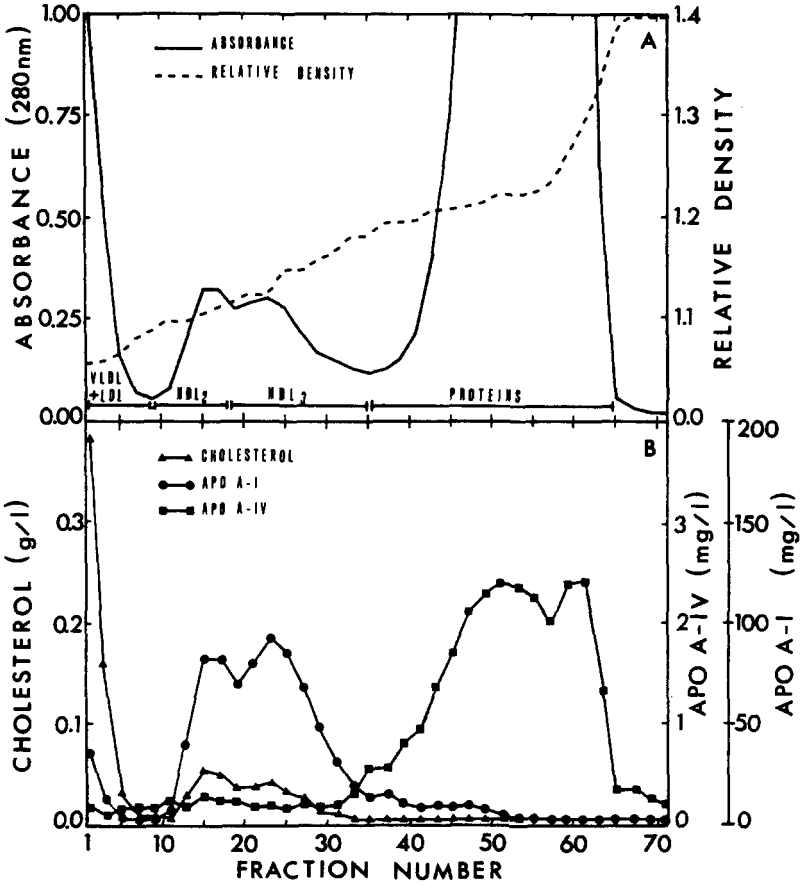

Fig. 1. Serum fractionation and apoA-IV distribution after rate-zonal ultracentrifugation: 80.5% of total apoA-IV was recovered. The serum was from a 34-yr-old woman (total cholesterol, 220 mg/dl; HDL cholesterol, 86 mg/dl; triglycerides, 41 mg/dl; apoA-IV, 11.2 mg/dl).

TABLE 1. ApoA-IV and lipoprotein component concentrations in a total serum and in serum fractions separated by sequential ultracentrifugation

	Total Serum	Lipoproteins (d < 1.25 g/ml)		Infranatant (d > 1.25 g/ml)	
		mg/dl[a]	%[b]	mg/dl[a]	%[b]
	mg/dl				
Total cholesterol	195	165	98.2	3	1.8
Phospholipids	217	170	93.9	11	6.1
Triglycerides	47	38	100	0	0
ApoA-I	196	129	92.8	10	7.2
ApoA-IV	14.9	2.8	18.2	12.6	81.8

[a]Per dl of nonfractionated serum.
[b]Percent of the sum of the concentrations in both fractions.

mg/dl, it eluted in a single peak distinct from that of HDL_2, and corresponded to the descending portion of the HDL_3 peak. Its elution volume was that of albumin (mol wt 67,000). The poor separation of aldolase (mol wt 158,000) from albumin suggested that monomeric and dimeric apoA-IV could not be separated under our chromatographic conditions.

Sera from 10 normal fasted subjects were fractionated on the same column. Their elution profiles are shown in **Fig. 3**. In contrast with results of the ultracentrifugation studies, the main part of apoA-IV appeared in the HDL fraction and eluted in the range of the apoA-I peak (**Fig. 4**). Within the HDL range, apoA-IV was predominantly localized in the larger-sized HDL particles. Only a small peak or a shoulder on the main fraction appeared in the elution volume of free apoA-IV. The amount of apoA-IV recovered in the VLDL + LDL fraction was minimal and never exceeded 6%.

To rule out potential artefactual effects, the different steps of the analytical process were checked. ApoA-IV distribution was not dependent on the pressure applied on the column. As shown in **Fig. 5**, a decrease from 0.4 to 0.2 MPa of the column pressure did not significantly change the apoA-IV elution profile. We verified that the estimation of apoA-IV by the enzyme immunoassay did not vary with the elution fraction. Inhibition curves obtained with serum fractions in the HDL elution range, serum fractions in the free apoA-IV elution range and total serum, paralleled that obtained with purified apoA-IV (**Fig. 6**). A gel filtration total recovery ranging from 80 to 100% confirmed that apoA-IV immunoassays were not likely under- or overestimated according to the nature and composition of the gel filtration fractions.

To eliminate a potential influence of LCAT activity on the distribution of apoA-IV after collection of the samples, experiments were conducted on sera supplemented, upon their collection, with LCAT inhibitors. The addition of DTNB or PCMPS at concentrations known to inhibit completely the activity of LCAT did not modify the elution profile of apoA-IV. In the absence or in the presence of inhibitors the main peak of apoA-IV coeluted with the HDL fraction (data not shown). This point was especially important to check since we confirmed that incubation of total serum at 37°C induced notable alterations of the gel filtration pattern of human apoA-IV.

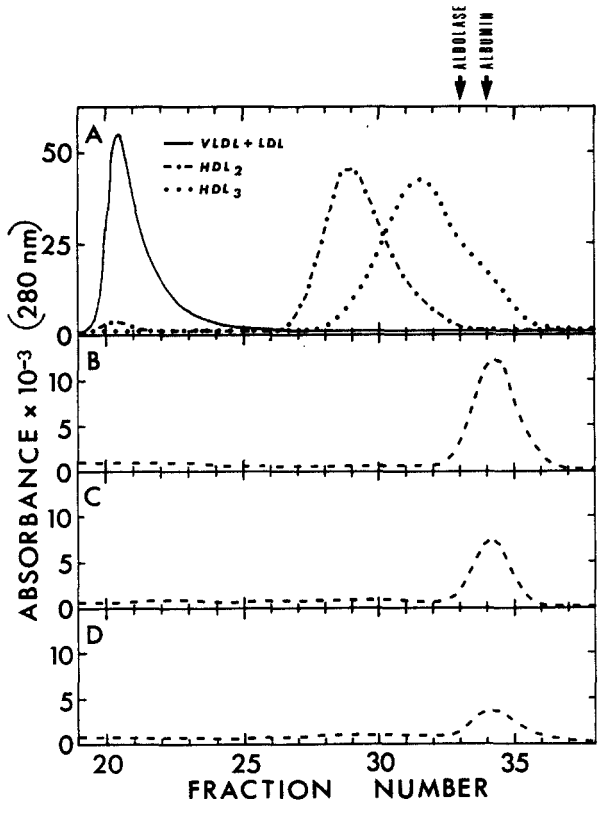

Fig. 2. Gel filtration elution profiles of ultracentrifugally isolated VLDL + LDL, HDL_2 and HDL_3 fractions (A) and pure apoA-IV (B, C and D). Pure apoA-IV (22.0 mg/dl, B; 11.0 mg/dl, C; 5.5 mg/dl, D), VLDL + LDL (protein concentration, 50 mg/dl), HDL_2 (protein concentration, 130 mg/dl), and HDL_3 (protein concentration, 150 mg/dl) were injected on a 50 μl volume on a Superose 12 HR column. Arrows indicate the maxima of absorption at 280 nm of pure proteins injected onto the column.

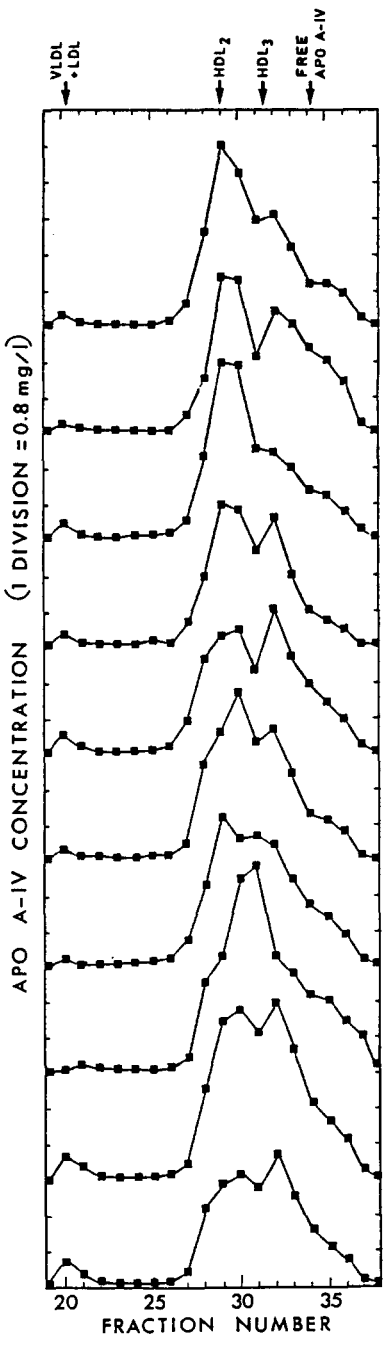

Fig. 3. ApoA-IV gel filtration profiles of 10 sera from fasted normolipidemic subjects. A 50-μl aliquot of serum was injected on the column. Subjects (four males and six females) were 24–40 years of age. Serum concentrations (mean ± SD) were: total cholesterol, 178 ± 18 mg/dl; HDL cholesterol, 66 ± 9 mg/dl; triglycerides, 52 ± 20 mg/dl; apoA-IV, 12.7 ± 2.4 mg/dl. Arrows indicate the maxima of absorption at 280 nm of isolated fractions injected onto the column.

When sera were incubated for 3 to 6 h in the absence of LCAT inhibitors, a significant shift of apoA-IV occurred from the lipoprotein-free elution fractions towards the larger HDL-containing fractions (**Fig. 7B, 7C**). Conversely, incubation in the presence of LCAT inhibitors induced a displacement of the bulk of apoA-IV towards the lipoprotein-free fraction (Fig. 7D).

Alteration of apoA-IV distribution induced by ultracentrifugation.

The alteration of apoA-IV distribution induced by ultracentrifugation was demonstrated by comparing the elution profiles of total serum and of serum fractions obtained by ultracentrifugation at d 1.25 g/ml (**Fig. 8**). Whereas cholesterol and apoA-I elution profiles of total serum and of the $d < 1.25$ g/ml supernatant were very similar, only a small amount of apoA-IV was recovered in the HDL fraction of the supernatant. The bulk of apoA-IV was present in the lipoprotein-free fraction and eluted as free apoA-IV. When a unique elution profile was reconstructed by cumulating the apoA-IV amounts recovered in the corresponding elution volumes obtained from the ultracentrifugation fractions, a shift of apoA-IV from the HDL fraction to the lipoprotein-free fraction, was evident, suggesting a dissociation of apoA-IV from the HDL particles induced by the ultracentrifugation step.

HDL immunoprecipitation

To investigate the relation of apoA-IV with HDL particles, serum HDL were precipitated by incubating human serum with anti-apoA-I immunoglobulins. At optimal concentration of anti-apoA-I antibodies, 90 to 95% of serum apoA-I was precipitated (**Fig. 9**). The same proportion of the HDL cholesterol content, as determined after precipitation of apoB-containing lipoproteins with concanavalin A, was found in the precipitate. Under these conditions about 70% of serum apoA-IV was precipitated with HDL, whether in the presence or in the absence of PCMPS in the reaction mixture. This amount was considerably lower (less than 10%) when 0.1% Tween was added to the serum dilution prior to the immunoprecipitation step (**Fig. 10**). Control experiments with pure apoA-IV demonstrated the absence of precipitation of the apolipoprotein by anti-apoA-I antibodies (Fig. 10).

DISCUSSION

The present study shows evidence that, in normal fasted human sera, a major part of apoA-IV is associated with the HDL fraction.

This conclusion is not in accord with results presented in previous reports. Indeed, the first studies, based on ultracentrifugation, showed that in fasted human plasma more than 95% of apoA-IV was present in the $d > 1.21$ g/ml lipoprotein-free fraction (13, 23). However, more recently, the use of gel permeation chromatography on agarose columns has brought slightly different results. Although the bulk of apoA-IV was found to elute with pro-

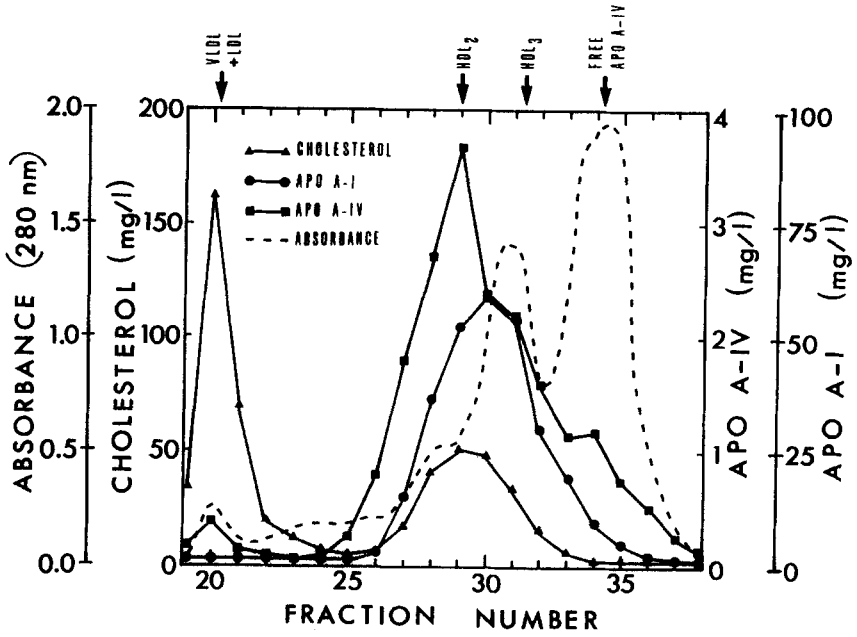

Fig. 4. ApoA-IV, apoA-I, and cholesterol profiles after gel filtration of total serum. A 100-μl aliquot of serum was injected on the column. The serum was that shown in Fig. 1.

tein fractions not associated with lipoproteins, a significant amount, 25–35%, of apoA-IV was recovered in lipoprotein-, mainly HDL-, containing fractions (17, 18, 29). The discrepancy between ultracentrifugation and gel

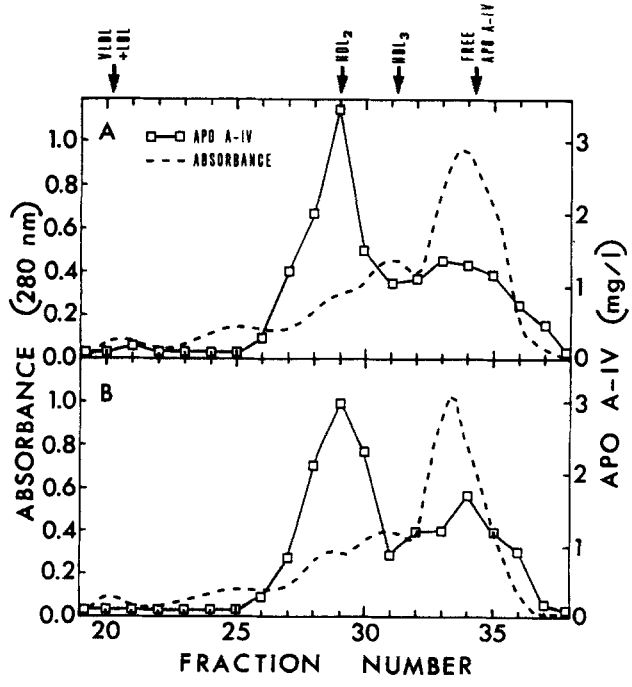

Fig. 5. Effect of flow rate on the gel filtration profile of serum apoA-IV. A: Flow rate 0.1 ml/min, column pressure 0.2 MPa. B: Flow rate 0.2 ml/min, column pressure 0.4 MPa. The serum was from a 23-yr-old woman (total cholesterol, 185 mg/dl; HDL cholesterol, 73 mg/dl; triglycerides, 83 mg/dl; apoA-IV, 13.1 mg/dl).

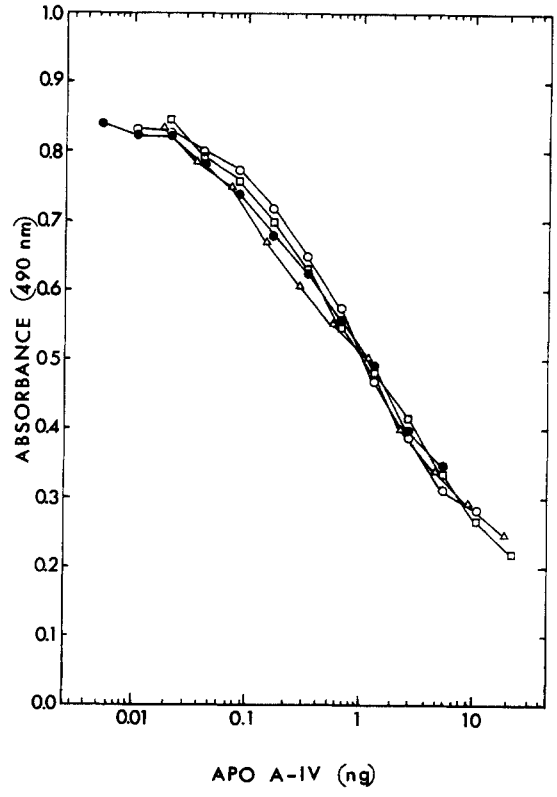

Fig. 6. Inhibition analysis with pure apoA-IV (△), total serum (□), and gel filtration fractions 29 (○) (HDL elution range) and 34 (●) (free apoA-IV elution range). ApoA-IV concentrations in serum and gel filtration fractions were determined by comparison with a pure apoA-IV preparation. Serum was diluted from 1/512 to 1/524288 and gel filtration fractions from 1/20 to 1/20480. Abcissa: amount of apoA-IV per well of the microtitration plate.

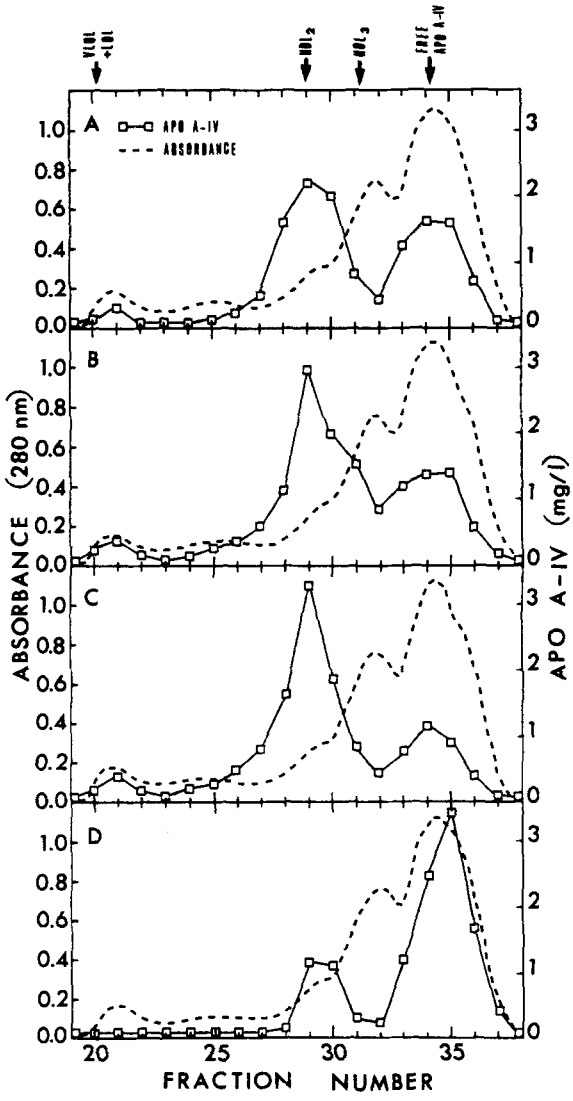

Fig. 7. Effect of incubation of total serum on the gel filtration profile of apoA-IV. A: control maintained at 4°C. B: serum incubated at 37°C for 3 h in the absence of PCMPS. C: Serum incubated at 37°C for 6 h in the absence of PCMPS. D: Serum incubated at 37°C for 6 h in the presence of 2 mmol/l PCMPS. The proportions of esterified cholesterol were 70.0% (A), 71.9% (B), 74.4 (C), and 69.0% (D). The serum was from a 31-yr-old woman (total cholesterol, 236 mg/dl; HDL cholesterol, 100 mg/dl; triglycerides, 33 mg/dl; apoA-IV, 11.2 mg/dl).

filtration data drew attention to the potential disruptive effects of the separation techniques, especially ultracentrifugation. In the rat, Dallingha-Thie, Groot, and van Tol (30) reported that significant amounts of apoA-IV as well as apoA-I or apoE might be stripped from HDL during ultracentrifugation. This loss of apolipoproteins could be attributable to the combined effects of high ionic strength and high sheering forces (31).

The present study took advantage of recent progress in gel permeation chromatography, more particularly the development of new extensively cross-linked agarose gel matrixes. As shown by Ha and Barter (32) the use of such matrixes, with small size agarose beads of increased rigidity, greatly improves the resolution of lipoprotein gel filtration and notably reduces the separation time. It is conceivable that, in these conditions, lipoproteins are less exposed to denaturation conditions and that weak intermolecular bindings may remain intact. By using a high performance Superose 12 HR column we were able not only to confirm the presence apoA-IV in the HDL fraction that had been shown by conventional agarose gel chromatography, but to demonstrate that, in fasting human sera, a majority of apoA-IV eluted with the HDL fraction, mainly within the HDL_2 size-range. Experiments reported in this study show that coelution of apoA-IV and HDL was not likely the result of an artifactual association of apoA-IV with lipoprotein particles. Indeed, the elution pattern was not dependent on the column pressure, a parameter that could have been a factor of ar-

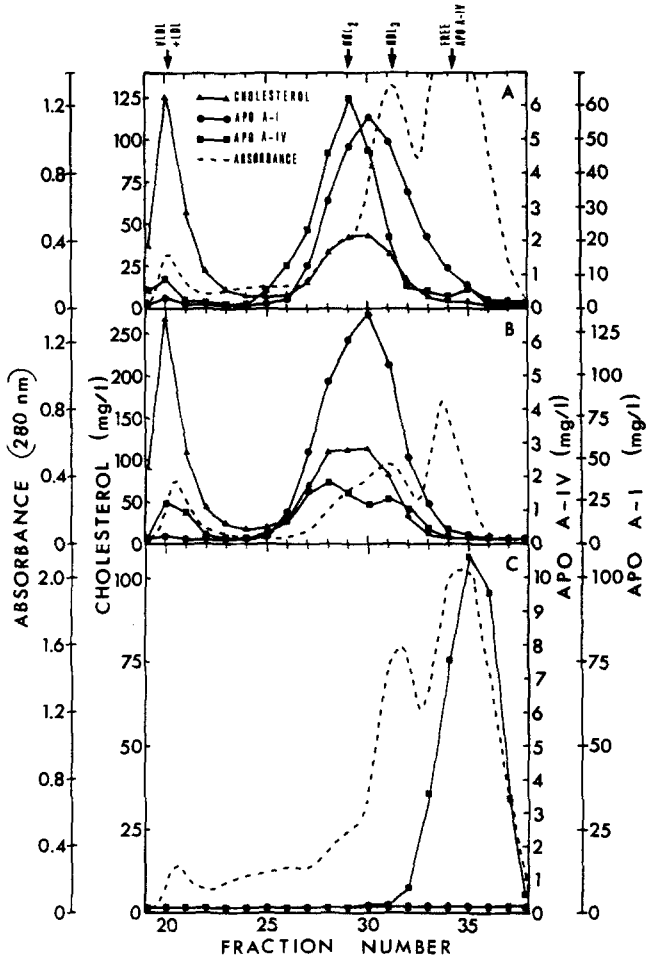

Fig. 8. ApoA-IV distribution after gel filtration of total serum (A), $d < 1.25$ g/ml (B) and $d > 1.25$ g/ml (C) serum fractions separated by sequential ultracentrifugation. The serum was from a 30-yr-old woman (total cholesterol, 195 mg/dl; HDL cholesterol, 79 mg/dl; triglycerides, 49 mg/dl; apoA-IV, 13.8 mg/dl). A 100-µl aliquot of serum or serum fraction was injected on the column.

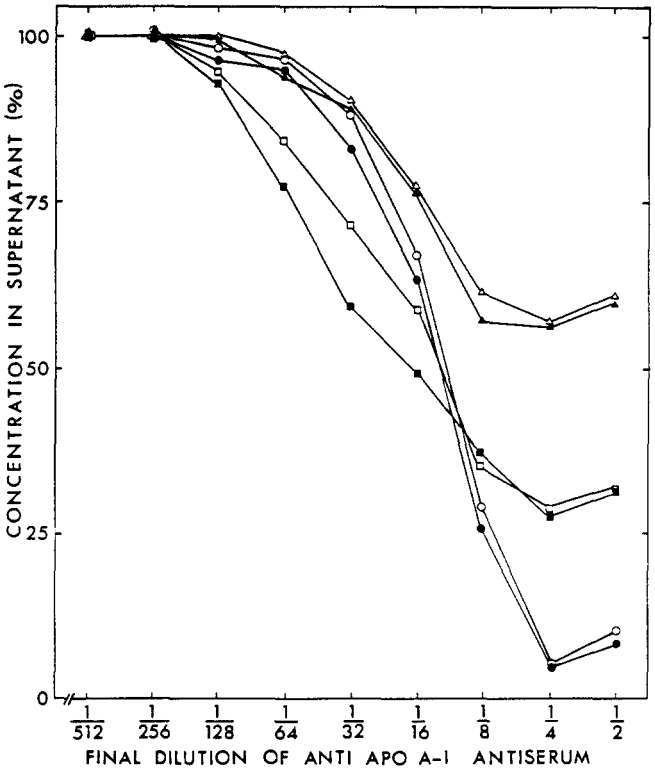

Fig. 9. Effect of HDL immunoprecipitation on serum apoA-IV: influence of LCAT inhibitor. Total serum was incubated with various dilutions of anti-apoA-I immunoglobulins in the absence (close symbols) or in the presence (open symbols) of 2 mmol/l PCMPS. No significant changes in the proportion of esterified cholesterol (less than 1%) were observed in incubated mixtures, supplemented or not with PCMPS. Concentrations of apoA-I (● and ○), cholesterol (▲ and △), and apoA-IV (■ and □) were measured in the supernatant and expressed as percent of those in control mixture in which antiserum was replaced by dilution buffer. The serum was from a 25-yr-old woman (total cholesterol, 222 mg/dl; HDL cholesterol, 108 mg/dl; triglycerides, 57 mg/dl; apoA-IV, 15.2 mg/dl).

tificial association. Moreover, the gel filtration procedure allowed the detection of in vitro displacements of apoA-IV between HDL and lipoprotein-free fractions induced by serum incubations. On the other hand, the peak of apoA-IV in the HDL range could not represent a dimeric form of the protein since aldolase, a protein with a molecular weight close to that of dimeric apoA-IV, eluted much later than HDL. Concurrently, it was demonstrated by immunoprecipitation experiments that, upon serum incubation with anti-apoA-I antibodies, most of serum apoA-IV coprecipitated with apoA-I. Such a coprecipitation, inhibited by the dissociation of HDL particles, strongly suggests that serum apoA-IV is, for the most part, carried by apoA-I-containing HDL, although the existence of HDL particles containing apoA-IV but not apoA-I, as those described in rat serum (30), cannot be excluded. This predominant HDL association is consistent with the high specific radioactivities measured in the HDL fraction following the in vitro or in vivo addition of ^{125}I-labeled A-IV to normal plasma (33). In our study, it was not the result of an in vitro redistribution of apoA-IV induced by LCAT after blood sampling, since it was observed, without any change, when sera were supplemented upon their collection with LCAT inhibitors. The apoA- IV-HDL complexes can be related to associations of lipids, apoA-IV, apoA-I, and other peptides that have been described in human plasma HDL and $d > 1.21$ g/ml fractions (19) and can be put together with HDL particles containing apoA-IV and apoA-I, recently described in interstitial fluid (34). The comparison of gel filtration profiles of total serum and of serum fractions separated by ultracentrifugation explains the discrepancy between the apoA-IV distribution we obtained by gel permeation chromatography and that shown by our ultracentrifugation studies and those of other investigators. The displacement of the peak of apoA-IV towards the lipoprotein-free fraction induced by ultracentrifugation implies that the apoA-IV-HDL association is a weak one, easily dissociated by the ultracentrifugation process.

The physico-chemical properties of human apoA-IV could explain the lability of apoA-IV-HDL complexes. It has the weakest lipid affinity of any human apolipoprotein and, compared to rat apoA-IV, it presents a self association of high affinity which induces a relative hydrophilicity (35, 36). On the other hand, Dvorin et al. (37) have suggested that the affinity of apoA-IV for HDL, in rats as well as in humans, could be linked to the lipid and apoli-

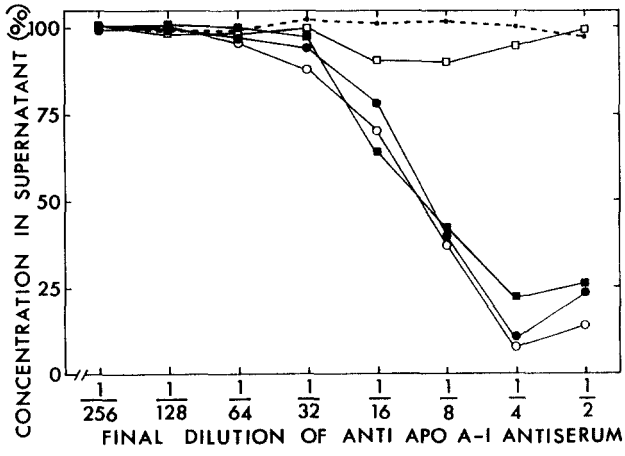

Fig. 10. Effect of HDL immunoprecipitation on serum apoA-IV: influence of detergent. Total serum, supplemented (open symbols) or not (closed symbols) with 0.1% Tween 20, was incubated with various dilutions of anti-apoA-I immunoglobulins. Concentrations of apoA-I (● and ○) and apoA-IV (■ and □) were measured in the supernatant and expressed as in Fig. 9. The serum was from a 31-yr-old woman (total cholesterol, 236 mg/dl; HDL cholesterol, 100 mg/dl; triglycerides, 33 mg/dl; apoA-IV, 11.4 mg/dl). As a control experiment, immunoprecipitation was conducted in the absence of Tween on a solution of pure apoA-IV (11.0 mg/dl) (discontinuous line).

poprotein composition of lipoproteins rather than to the structure of apoA-IV itself. The differences in HDL pattern between rat and human sera would induce variations in the strength of the HDL-apoA-IV binding. A weaker interaction with lipoprotein particles would increase the probability for human apoA-IV to be displaced by techniques of serum fractionation.

The remodeling of lipoprotein surfaces by enzymatic activities or by lipid and apolipoprotein transfers can modulate the association of apoA-IV with HDL. As previously described by other authors for rat (20, 39) and human (21, 22) plasma, we observed that a 37°C incubation of human serum induced an increase of apoA-IV in the HDL-associated pool, whereas a similar incubation in the presence of an LCAT inhibitor led to an inverse displacement, from HDL to the lipoprotein-free fraction. Thus, apoA-IV distribution between HDL and the lipoprotein-free fraction could be the result of a dynamic equilibrium. A loss of surface components from circulating HDL, due to LCAT activity or to transfers of surface lipids or apolipoproteins to nascent triglyceride-rich lipoproteins, would induce an HDL-apoA-IV association (39). On the other hand, lipolysis and lipid transfer activities would imply the reacquisition of surface components and, thus, a decrease in the affinity of HDL for apoA-IV (39). In addition, as demonstrated in the rat, the equilibrium could be susceptible to displacement by other indirect factors such as nutritional status (30), diet (40), or age (41).

The present study provides evidence that in fasted normal human sera apoA-IV is preferably associated with HDL. Further studies should focus on the physiopathological variations of apoA-IV-HDL complexes, the conditions of their dissociation, and the metabolic consequences of apoA-IV transfers from one plasma compartment to another.

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