Correlation between apolipoprotein A-IV and triglyceride concentrations in human sera

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Abstract Apolipoprotein A-IV concentration was measured by a newly developed competitive enzyme immunoassay in sera from fasted human subjects (n = 105) whose triglyceride concentrations ranged from 20 to 474 mg/dl (total cholesterol below 260 mg/dl) and in which chylomicrons could not be detected. Mean (± SD) apolipoprotein A-IV concentration was 13.0 ± 2.6 mg/dl in sera with triglyceride levels ranging from 20 to 100 mg/dl, 16.9 ± 3.7 mg/dl in sera with triglyceride levels ranging from 101 to 250 mg/dl, and 22.7 ± 6.7 mg/dl in sera with triglyceride levels ranging from 251 to 474 mg/dl. The differences among the three groups were highly significant ($P < 0.001$). Moreover, variations of apolipoprotein A-IV concentrations according to the triglyceride levels were noted within the normotriglyceridemic population. Apolipoprotein A-IV concentration was 12.8 ± 2.1 mg/dl for triglyceride levels ranging from 20 to 75 mg/dl and 16.4 ± 3.8 mg/dl for triglyceride levels ranging from 76 to 150 mg/dl ($P < 0.01$). In the entire population that was studied there was a significant linear correlation ($r = 0.61$, $P < 0.001$) between the concentrations of serum apolipoprotein A-IV and triglyceride. Although the hypothesis of an unknown factor independently influencing both very low density lipoproteins and apolipoprotein A-IV cannot be ruled out, and although no apolipoprotein A-IV was found in the triglyceride-rich lipoprotein fraction after separation by gel filtration, these data suggest that, in fasting subjects, the secretion of very low density lipoproteins could contribute to the plasma apolipoprotein A-IV level.—**Lagrost, L., P. Gambert, S. Meunier, P. Morgado, J. Desgres, P. d'Athis, and C. Lallemant.** Correlation between apolipoprotein A-IV and triglyceride concentrations in human sera. *J. Lipid Res.* 1989. **30:** 701 .710.

Supplementary key words enzyme immunoassay • gel filtration • hypertriglyceridemia • very low density lipoproteins

There is detailed information on the structural and physicochemical properties of human plasma apoA-IV (1–4), but data on its metabolic function and changes in concentration under pathophysiological conditions are scarce. Synthesized, at least partly, in the intestinal endothelium, and secreted in association with chylomicrons (5), apoA-IV is suspected of playing a role in triglyceride transport (6). Indeed, an increase of its plasma concentration has been found after lipid feeding (5, 7) and in circumstances associated with accumulation of remnants of triglyceride-rich lipoproteins, such as chronic renal failure treated by peritoneal dialysis or hemodialysis (8, 9). Conversely, a decrease has been observed in patients with malabsorption syndrome (10) and in human subjects receiving total parenteral nutrition (11). However, no significant alteration of plasma apoA-IV concentration has been found in the major dyslipoproteinemias and no correlation has been noted between the concentration of apoA-IV and those of the main plasma lipid components (12, 13).

The development of a new apoA-IV enzyme immunoassay, which is described in this report, has allowed us to undertake a large scale exploration of the variations of apoA-IV plasma concentration. We report here the results obtained in a population of subjects with various plasma triglyceride levels.

MATERIALS AND METHODS

Blood samples

The study was conducted on blood samples obtained from 105 adult subjects, 65 males and 40 females, with serum cholesterol concentrations below 260 mg/dl and triglyceride levels ranging from 20 to 474 mg/dl. This population was divided into three groups, A, B, and C, with triglyceride ranges 20–100, 101–250, and 251–474 mg/dl, respectively. In group A (n = 19) the subjects were disease-free and did not take drugs other than oral contra-

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; SDS, sodium dodecyl sulfate.

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ceptive drugs. In groups B (n = 65) and C (n = 21) the subjects were out- or in-patients on whom lipoprotein investigations were being carried out. Group B included insulin-dependent diabetics (5%) treated with insulin, non-insulin-dependent diabetics (14%) treated with oral hypoglycemic drugs, patients with chronic renal insuffi ciency (9%) on hemodialysis, and subjects suffering from chronic alcohol intoxication (19%). There was no other pathology with the exception of cardiovascular impairments. In this group 19% of the subjects were treated with beta blockers and 5% with a lipid-lowering drug (fenofibrate). Similarly, group C included insulin-dependent diabetics (10%) treated with insulin, non-insulin-dependent diabetics (24%) treated with oral hypoglycemic drugs, patients with chronic renal insufficiency (10%) on hemodialysis, and subjects suffering from chronic alcohol intoxication (10%). In this group 5% of the subjects were treated with a lipid-lowering drug (fenofibrate) and no subject received therapy with beta blockers.

Blood was drawn, after the subjects had fasted overnight, into plain glass tubes and placed immediately at 4°C. Sera were collected at the same temperature by a 5-min centrifugation at 3000 *g* and kept at 4°C or frozen at −20°C. The absence of chylomicrons in sera was checked after the sera had been standing for 12 h at 4°C and by electrophoresis in a 2–16% polyacrylamide gel (14).

Purification and characterization of human apoA-IV

Human apoA-IV was extracted from human serum by a lipid emulsion (Intralipid) as described by Weinberg and Scanu (15) and purified by the preparative electrophoresis method described by Meunier et al. (16). Briefly, 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 for 5 h at 100 V the portion of gel containing apoA-IV was cut off and placed on the top of a vertical agarose slab gel. The protein was then transferred to the agarose gel by electrophoresis and finally removed from the gel by a 15-min centrifugation at 250,000 *g* in a 100.2 rotor on a TL-100 ultracentrifuge (Beckman, Palo Alto, CA). SDS was removed from the purified protein preparation by using Extracti-Gel D (Pierce, Rockford, IL).

The purity of the protein preparation was checked by electrophoresis in an 80–250 g/l polyacrylamide gradient gel (Phastsystem, Pharmacia, Uppsala, Sweden) for 15 min at 250 V in a 0.55 g/l SDS, 0.20 mol/l Tricine, 0.20 mol/l Tris, pH 7.5, buffer.

The protein molecular weight was determined by comparison with a low molecular weight calibration kit (Pharmacia) after SDS electrophoresis in a 25–300 g/l polyacrylamide gradient gel according to the general procedure of Davis (17). The electrophoresis was performed for 5 h, 1 h at 100 V and 4 h at 200 V, in a 2 g/l SDS, 49 mmol/l Tris, 380 mmol/l glycine, pH 8.3, buffer. The gels were stained with Coomassie Brilliant Blue G-250 (18).

The amino acid composition of apoA-IV was determined after hydrolysis of the protein by 6 M HCl at 110°C for 20 h under nitrogen and separation and quantitation of the amino acids by cation exchange chromatography using a 6300 Beckman amino acid analyzer.

Preparation of anti-apoA-IV antiserum

Polyclonal antibodies to human apoA-IV were raised in a rabbit by four injections, each containing 250 μg of purified protein emulsified with an equal volume of Freund's complete adjuvant, at 15-day intervals. The rabbits were bled 5 days after a last injection of 500 μg.

ApoA-IV enzyme immunoassay

Plate coating. A 100-μl volume of pure apoA-IV solution (2.5 mg/l) in Tris–glycine buffer (6.5 mmol/l, pH 9.2) was pipetted into each well of a polystyrene microwell plate (Immuno 96 F Type I from Nunc, Kamstrup, Denmark). After an overnight incubation at 4°C, the plates were washed three times with a 150 mmol/l NaCl, 0.025% (v/v) Tween 20 wash solution by using a Nunc immunowash. To block nonspecific absorption, each well was then incubated for 30 min at room temperature with 250 μl of a 10 g/l bovine albumin solution 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.

Sample treatment. Samples (total sera and serum fractions) were generally assayed without prior treatment. In some experiments they were delipidated with butanol-diisopropylether 40:60 (v/v) according to the procedure of Cham and Knowles (19). ApoA-IV-containing samples, delipidated or not, and anti-apoA-IV antiserum were diluted to desired concentrations in a 10 g/l human albumin–phosphate buffer (pH 7.2) using a Beckman Accuprep 222A automated diluter. Equal volumes (200 μl) of diluted apoA-IV-containing samples and diluted antiserum were mixed in glass tubes and incubated overnight at 4°C. Aliquots (100 μl) of the mixtures were pipetted into the immunoplate microwells and incubated for 4 h at room temperature. The plates were then washed three times with the Tween 20 wash solution.

Detection of bound anti-apoA-IV antibodies. Peroxidase-conjugated anti-rabbit antibodies (Bio-Rad, Richmond, CA) diluted in a 10 g/l bovine albumin phosphate buffer were pipetted (100 μl) into each microwell and incubated for 1 h at 37°C. After completion of the incubation, the plates were washed three times as before. Freshly prepared 0.4 g/l *o*-phenylenediamine, 0.68 g/l hydrogen peroxide solution in a 6.6 mmol/l sodium phosphate, 3.4 mmol/l citrate buffer (pH 5.2) was pipetted (100 μl) into

each microwell. After 15 min at room temperature in the dark, the reaction was stopped by addition of 30 μl of 2.5 mol/l H_2SO_4. The absorbances were read at 490 nm in a Multiskan Elisa reader (Titertek, Finland).

Calibration. The primary standard curve was constructed from a set of dilutions of purified apoA-IV solution whose protein concentration was determined by the method of Lowry et al. (20) with bovine albumin as a standard. The apoA-IV concentration of a pool of sera from normolipidemic subjects was determined as described. This pool, constituting a secondary standard, was aliquoted into 100-μl samples and stored at – 20°C. Eight dilutions (apoA-IV concentrations from about 0.008 to 1 mg/l) were used to construct a secondary calibration curve for each plate of apoA-IV immunoassay. Standard curves were fit to the data points by polynomial regression analysis. Routinely, four dilutions of each sample were assayed and the apoA-IV concentration was calculated by averaging the four results.

Densitometric quantitation of apoA-IV

ApoA-IV concentrations of $d < 1.006$ g/ml serum fractions from chylomicronemic subjects were quantified by densitometric scanning after apoA-IV electrophoretic separation in an SDS polyacrylamide gradient gel. Samples of ultracentrifugally prepared triglyceride-rich lipoproteins were mixed with 0.5 volume of 100 g/l SDS, 66 g/l dithiothreitol, 0.24 mol/l Tris-HCl, pH 6.7, reducing solution and were incubated for 5 min at 80°C. ApoA-IV was separated from the other proteins by electrophoresis in a 25–300 g/l polyacrylamide gradient gel containing 1 g/l SDS in a 60 mmol/l Tris-HCl, pH 6.7, buffer. The running buffer was 49 mmol/l Tris, 380 mmol/l glycine (pH 8.3) with 1 g/l SDS. After 5 h of electrophoresis at 80 mA, the protein bands were stained with Coomassie Brilliant Blue G-250 and the gel was scanned at 633 nm with a 2202 laser densitometer (LKB, Bromma, Sweden) coupled with an HP 3390A reporting integrator (Hewlett-Packard, Washington, DC). Concentrations were determined by comparing apoA-IV peak area values with a standard curve obtained under the same experimental conditions with a pure apoA-IV solution.

Gel permeation chromatography

Gel permeation chromatography was performed in a Pharmacia Fast Protein Liquid Chromatography system equipped with a 30-cm Superose 6HR column. Serum samples were injected in a volume of 200 μl and eluted at room temperature with a 150 mmol/l NaCl, 1 mmol/ Na_2EDTA, pH 7.4, solution at a flow rate of 24 ml/h in about 45 min. The effluents were continuously monitored at 280 nm and fractions of 0.6 ml were collected. The column was calibrated with ultracentrifugally prepared VLDL, LDL, and HDL, and with bovine albumin solution.

Lipoprotein component assays

Total cholesterol and triglyceride levels were determined by enzymatic methods using Boehringer (Mannheim, FRG) reagents. HDL cholesterol was assayed after selective precipitation of apoB-containing lipoproteins by concanavalin A (21). VLDL + LDL cholesterol concentrations were obtained by difference. ApoA-I was assayed by immunoturbidimetry (22) with anti-apoA-I antibodies purchased from Behring (Marburg, FRG). ApoE concentrations were measured by a competitive enzyme immunoassay using a specific monoclonal antibody (23) and standardized with purified apoE (16).

Apolipoprotein preparations

ApoA-I extracted together with apoA-IV by Intralipid was purified by the same method. ApoE was purified by preparative electrophoresis (16).

Statistical evaluations

Statistical methods were as recommended by Snedecor and Cochran (24). One-way analysis of variance was used to compare the significance of mean differences. Correlations between serum parameters were analyzed by linear regression.

RESULTS

Purification and characterization of apoA-IV

ApoA-IV was completely purified by preparative electrophoresis from the protein mixture extracted by action of the Intralipid emulsion on total serum. In an SDS polyacrylamide gel it gave a single band with an apparent molecular weight of 46,000 (**Fig. 1**) which is in agreement

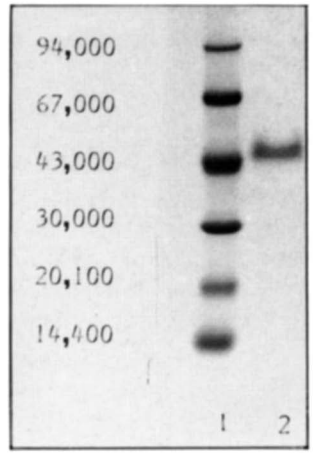

Fig. 1. SDS polyacrylamide gel electrophoresis of 1) 0.5 μl of low molecular weight calibration kit (94,000, phosphorylase b; 67,000, albumin; 43,000, ovalbumin; 30,000, carbonic anhydrase; 20,100, trypsin inhibitor; 14,000, α-lactalbumin); and 2) 20 μg of purified apoA-IV.

TABLE 1. Amino acid composition of apoA-IV

Amino Acid[a]	This Study[b,c]	From Amino Acid Sequence (3)[b]
Alanine	8.8	8.6
Arginine	6.9	7.2
Aspartic acid + asparagine	10.0	10.1
Glutamic acid + glutamine	25.6	25.9
Histidine	2.3	2.6
Isoleucine	1.5	1.4
Leucine	15.2	14.9
Lysine	7.8	7.6
Phenylalanine	3.0	3.2
Proline	3.7	3.5
Serine	5.5	5.2
Threonine	4.4	4.3
Valine	5.3	5.5

[a]Cysteine, methionine, tryptophane, and tyrosine were not determined. Due to the use of a Tris-glycine buffer during the electrophoretic preparative step, values for glycine were not taken into account.
[b]Data expressed in mol/100 mol of determined amino acids.
[c]Values from a single determination.

with values previously reported (3, 15). The amino acid composition of the isolated protein (**Table 1**) was similar to that determined from the amino acid sequence of apoA-IV (3).

ApoA-IV enzyme immunoassay

Optimal coating was found for an apoA-IV concentration of about 2.5 mg/l. Nonspecific absorption was minimal when residual binding sites in the microwells were blocked with 10 g/l bovine serum albumin solution. Optimal dilutions of anti-apoA-IV antiserum and peroxidase-conjugated immunoglobulin solution were 2.8×10^{-4} and 5×10^{-5}, respectively. Under these conditions, the minimum detectable content was 1 ng and the working range of the assay extended from 1 to 10 ng of apoA-IV per well (**Fig. 2**). The specificity of the antiserum was tested against apoE and the main proteins of the Intralipid extract, apoA-I and albumin. Under the conditions of the immunoassay these proteins were unable to compete with the coated apoA-IV (Fig. 2). The inhibition analysis with pure apoA-IV, normolipidemic sera, hypertriglyceridemic sera, delipidated sera, and triglyceride-rich serum fractions showed no preferential binding of isolated apoA-IV compared to the other competitors (**Fig. 3**). The displacement curves were parallel and their polynomial regression coefficients were better than 0.99. The precision of the assay was evaluated by analyzing a serum 10 times in the same microwell plate and by running six assays of the same serum over a 10-day period. Under these conditions, the intra- and inter-assay coefficients of variation were 3.0% and 3.9%, respectively. When the serum was delipidated, the intra-assay coefficient of variation rose to 8.4%. The accuracy of the immunoassay was checked by comparison with an independent method based on the scanning of apoA-IV bands separated by electrophoresis in a polyacrylamide gradient gel. The determinations were performed in nondelipidated $d < 1.006$ g/ml fractions isolated by ultracentrifugation from two chylomicronemic sera. The results (mean ± SD) were not significantly different: 1.20 ± 0.09 mg/dl and 2.10 ± 0.14 mg/dl (n = 3) by enzyme immunoassay compared to 1.24 ± 0.10 mg/dl and 2.00 ± 0.31 mg/dl (n = 3) by densitometry. The influence of delipidation on apoA-IV levels was studied on 69 sera. Delipidation led to results

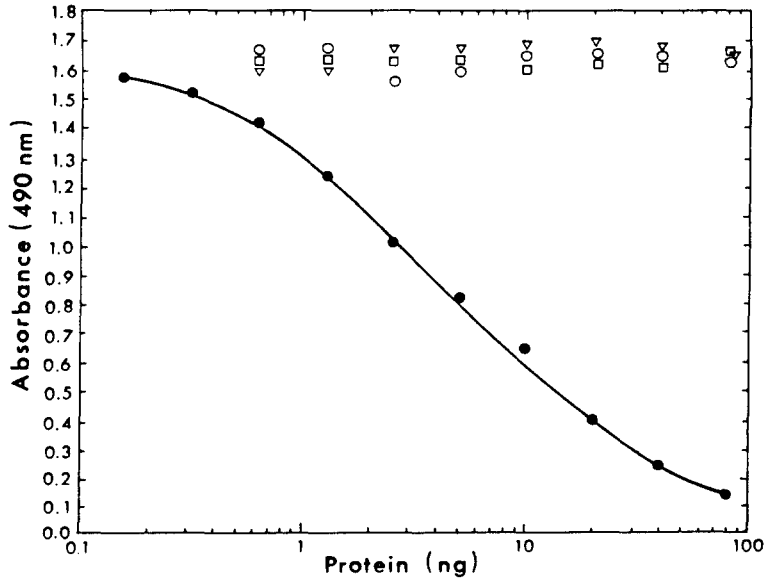

Fig. 2. Calibration curves for apoA-IV quantitation obtained with apoA-IV (●), apoE (○), apoA-I (▽), and human albumin (□). Values are mean of duplicates. On the abscissa is plotted the amount of protein per microwell.

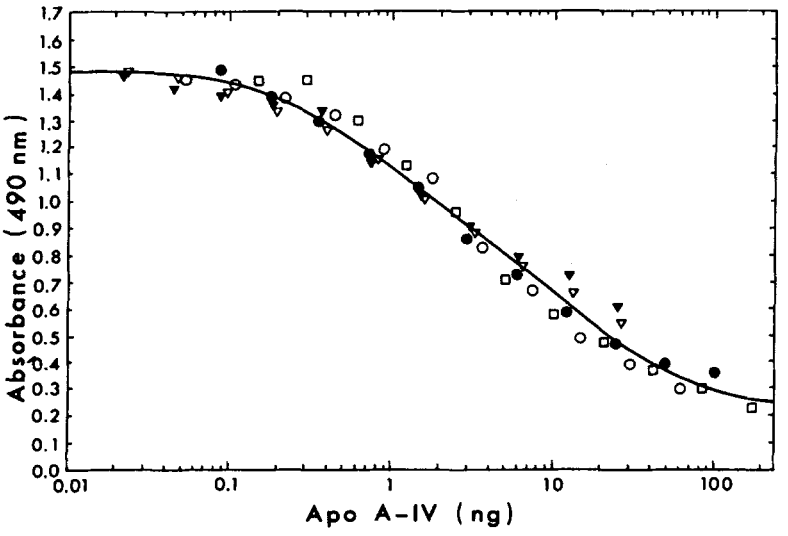

Fig. 3. Inhibition analysis with pure apoA-IV solution (●), nondelipidated serum (○), delipidated serum (▽), hypertriglyceridemic serum (445 mg/dl triglycerides) (□), and d < 1.006 g/ml fraction from chylomicronemic serum (▼). Each sample, in which apoA-IV concentration was determined by comparison with the pure apoA-IV preparation, was diluted from 1/128 to 1/65536. A unique curve was constructed by polynomial regression analysis (r = 0.990).

significantly lower ($P<0.001$), 15.6 ± 5.1 mg/dl (mean ± SD) versus 17.8 ± 4.8 mg/dl without delipidation.

ApoA-IV concentrations were measured by the enzyme immunoassay in nondelipidated sera obtained from 19 healthy subjects, 10 women and 9 men, age ranging from 19 to 58 years. Their serum cholesterol triglyceride levels were below 230 mg/dl and below 100 mg/dl, respectively. The concentration of apoA-IV was 13.0 ± 2.6 (mean ± SD) mg/dl.

ApoA-IV concentrations and serum triglyceride levels

ApoA-IV concentrations were measured in 105 sera whose triglyceride concentrations ranged from 20 to 474 mg/dl and total cholesterol concentrations were below 260 mg/dl, and in which chylomicrons could not be detected. ApoA-IV values obtained in nondelipidated sera were markedly increased in hypertriglyceridemic subjects. Mean (± SD) apoA-IV concentration was 22.7 ± 6.7 mg/dl in sera with triglyceride levels ranging from 251 to 474 mg/dl (group C) compared with 16.9 ± 3.7 mg/dl in sera with triglyceride levels ranging from 101 to 250 mg/dl (group B), and 13.0 ± 2.6 mg/dl in sera with triglyceride levels ranging from 20 to 100 mg/dl (group A). The differences between the three groups were highly significant ($P<0.001$). Moreover, variations of apoA-IV concentrations according to triglyceride levels were noted within the normotriglyceridemic group. ApoA-IV concentration was 12.8 ± 2.1 mg/dl for triglyceride levels in the range of 20–75 mg/dl (n = 13) and 16.4 ± 3.8 mg/dl for triglyceride levels in the range of 76–150 mg/dl (n = 38). This difference was statistically significant ($P<0.01$). In the whole population studied there was a significant linear correlation (r = 0.61, $P<0.001$) between apoA-IV and triglyceride serum concentrations (**Fig. 4A**). When apoA-IV was measured in delipidated sera, the coefficient of correlation was slightly lower but still highly significant (r = 0.49, $P<0.001$) and the parameters of the linear regression were similar (Fig. 4B). ApoA-IV and triglyceride levels were highly significantly correlated in both male (r = 0.60, $P<0.001$) and female (r = 0.65, $P<0.001$) groups (**Fig. 5**). The correlation was not significantly different between the two groups. As there were differences in lipoprotein cholesterol distribution between the three groups, correlations between apoA-IV concentration and other lipid parameters were tested. Mean total cholesterol concentration increased slightly as triglyceride concentration increased, but this difference was significant only between the A and C groups. HDL cholesterol was significantly lower and VLDL + LDL cholesterol was higher in groups B and C compared with group A (**Table 2**). However, linear regression analysis did not reveal any relationship between apoA-IV and HDL or VLDL + LDL cholesterol concentrations. Only a weak correlation with total cholesterol concentration (r = 0.20, $P<0.05$) was observed (**Fig.6**). There was no correlation within any of the three groups. Body mass index, mass in kg to (height in cm)^2 ratio, in A, B, and C groups was 22.4 ± 2.6 (mean ± SD), 26.2 ± 4.9, and 26.2 ± 2.0, respectively. The only difference was between groups A and B ($P<0.05$). Body mass index was not significantly correlated with apoA-IV level.

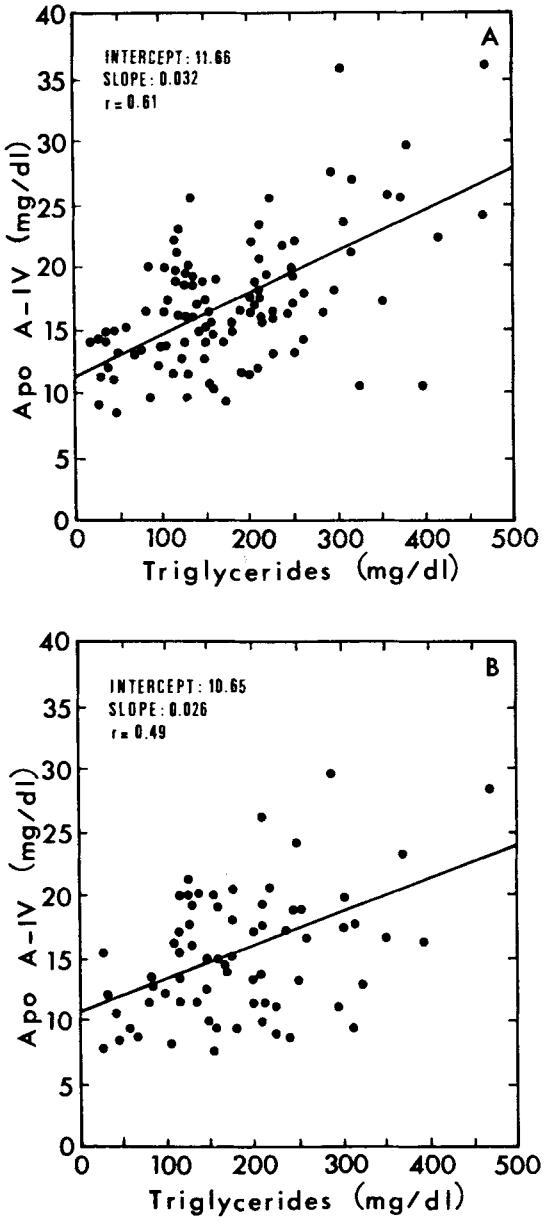

Fig. 4. Correlation of apoA-IV and triglyceride concentrations in 105 native sera (A) and 69 delipidated sera (B).

Distribution of apoA-IV in normo- and hypertriglyceridemic sera

Sera containing different amounts of triglycerides were subjected to gel filtration and the separated protein fractions were analyzed for total cholesterol, apoA-I, apoE, and apoA-IV contents. As serum triglyceride concentration increased, cholesterol and apoA-I increased in the triglyceride-rich fraction and apoE redistributed from small to large particles. On the other hand, the general distribution of apoA-IV did not change and the increase of this apoprotein occurred only in HDL and lipoprotein-free fractions (**Fig. 7**).

DISCUSSION

The present report demonstrates a significant correlation between apoA-IV and triglyceride concentrations in human sera. The apoA-IV competitive enzyme immunoassay developed for this study and reported here has provided a specific and highly sensitive method for measuring apoA-IV concentrations in human sera as well as in serum fractions. Until recently large scale determinations of serum apoA-IV levels were hindered by the lack of a precise, simple, and rapid technique for apoA-IV measurements. Radioimmunoassays (7, 13) and elec-

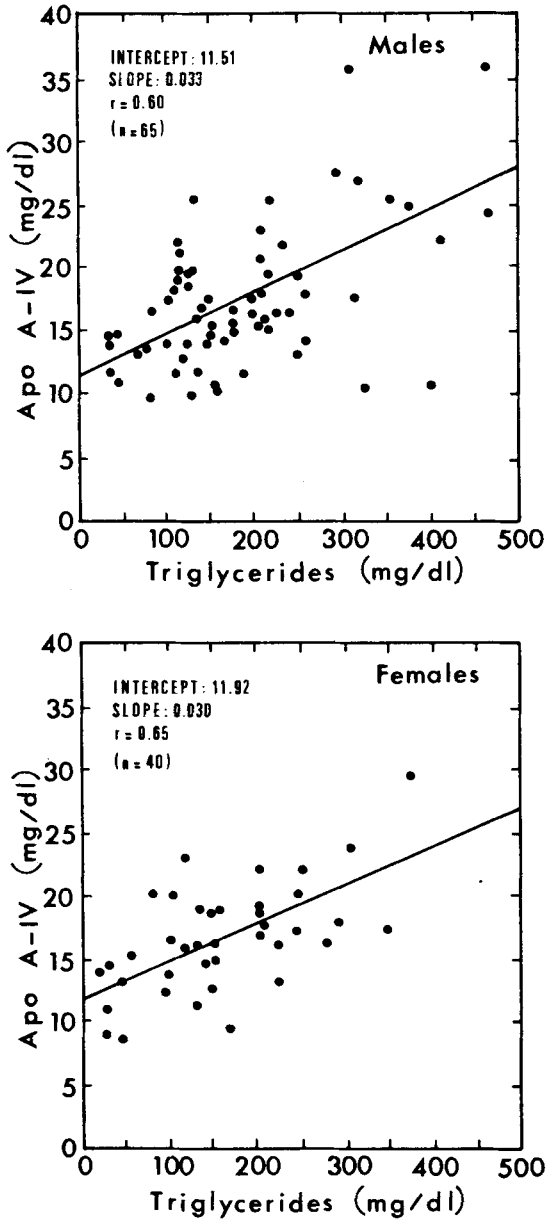

Fig. 5. Correlation of apoA-IV and triglyceride concentrations in native sera from males and females.

TABLE 2. Cholesterol concentration in 105 sera from normo- and hypertriglyceridemic subjects

	Total Cholesterol	HDL Cholesterol	VLDL + LDL Cholesterol
		mg/dl (mean ± SD)	
Triglycerides 20 to 100 mg/dl (A) (n = 19)	184 ± 27	60 ± 23	124 ± 31
Triglycerides 101 to 250 mg/dl (B) (n = 65)	200 ± 34	44 ± 14	156 ± 36
Triglycerides 251 to 474 mg/dl (C) (n = 21)	212 ± 37	32 ± 11	180 ± 31
Significance A vs. B	N.S.	$P < 0.001$	$P < 0.01$
Significance B vs. C	N.S.	$P < 0.01$	$P < 0.05$
Significance A vs. C	$P < 0.05$	$P < 0.001$	$P < 0.001$

troimmunoassays (5,25,26) were the only available methods. The first enzyme immunoassay for apoA-IV was described by Rosseneu et al. (12) when the present work was in the process of completion. Compared with the method of Rosseneu et al. (12), the present method has similar sensitivity and precision and, likewise, it does not require sample pretreatment. The present method is set up more easily since it dispenses with the coupling of anti-apoA-IV antibodies with enzymes and can be carried out with commercially available conjugated antibodies. The values of apoA-IV concentrations obtained with this technique in sera from normolipidemic subjects are very similar to those reported by Rosseneu et al. (12) and are in good agreement with data obtained by electroimmunoassays (5, 25, 26) and radioimmunoassays (13), except for the higher values reported by Bisgaier et al. (7).

Whereas previous studies did not find any significant alteration of serum apoA-IV concentration in dyslipidemic sera (12, 13), the present study showed an increase of apoA-IV in hypertriglyceridemic sera and, moreover, a correlation between apoA-IV and triglyceride concentrations throughout a large range of levels, from low to moderately elevated concentrations. The use of less precise methods of assaying apoA-IV and the exploration of smaller dyslipidemic populations probably explain the negative results reported by other authors. In the present study, the exploration of sera in a large range of triglyceride concentrations implied a heterogeneity of the population with regard to pathology and therapy. Under these conditions one might suspect an indirect relation between triglyceride and apoA-IV levels, with some factors, such as diet, physical activity, or drugs, being able to independently influence both triglyceride and apoA-IV metabolism. This hypothesis was sustained by significant differences in the serum cholesterol distribution and body mass index between the three groups and a correlation between total cholesterol and apoA-IV levels. However, this correlation was weak and there was no correlation of apoA-IV concentration with other parameters, neither lipoprotein cholesterol concentrations nor body mass in-

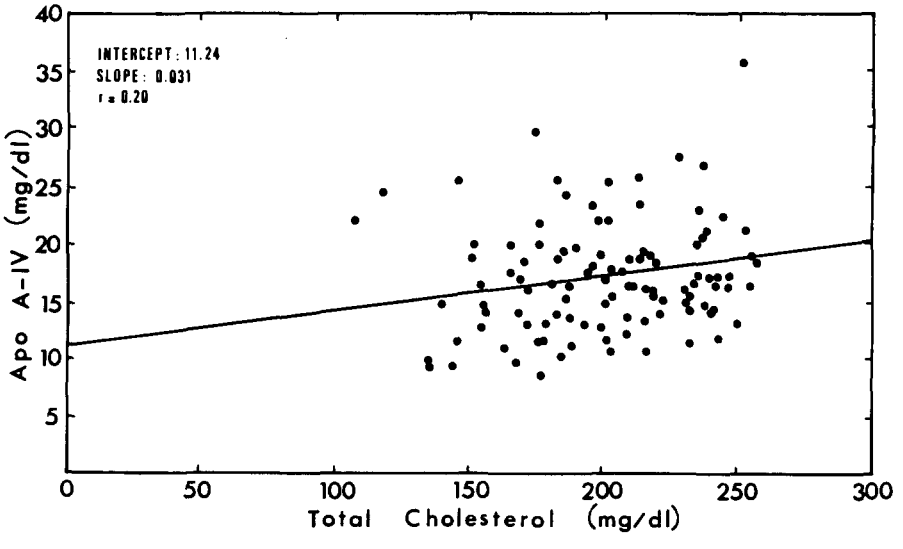

Fig. 6. Correlation of apoA-IV and total cholesterol concentrations in 105 native sera.

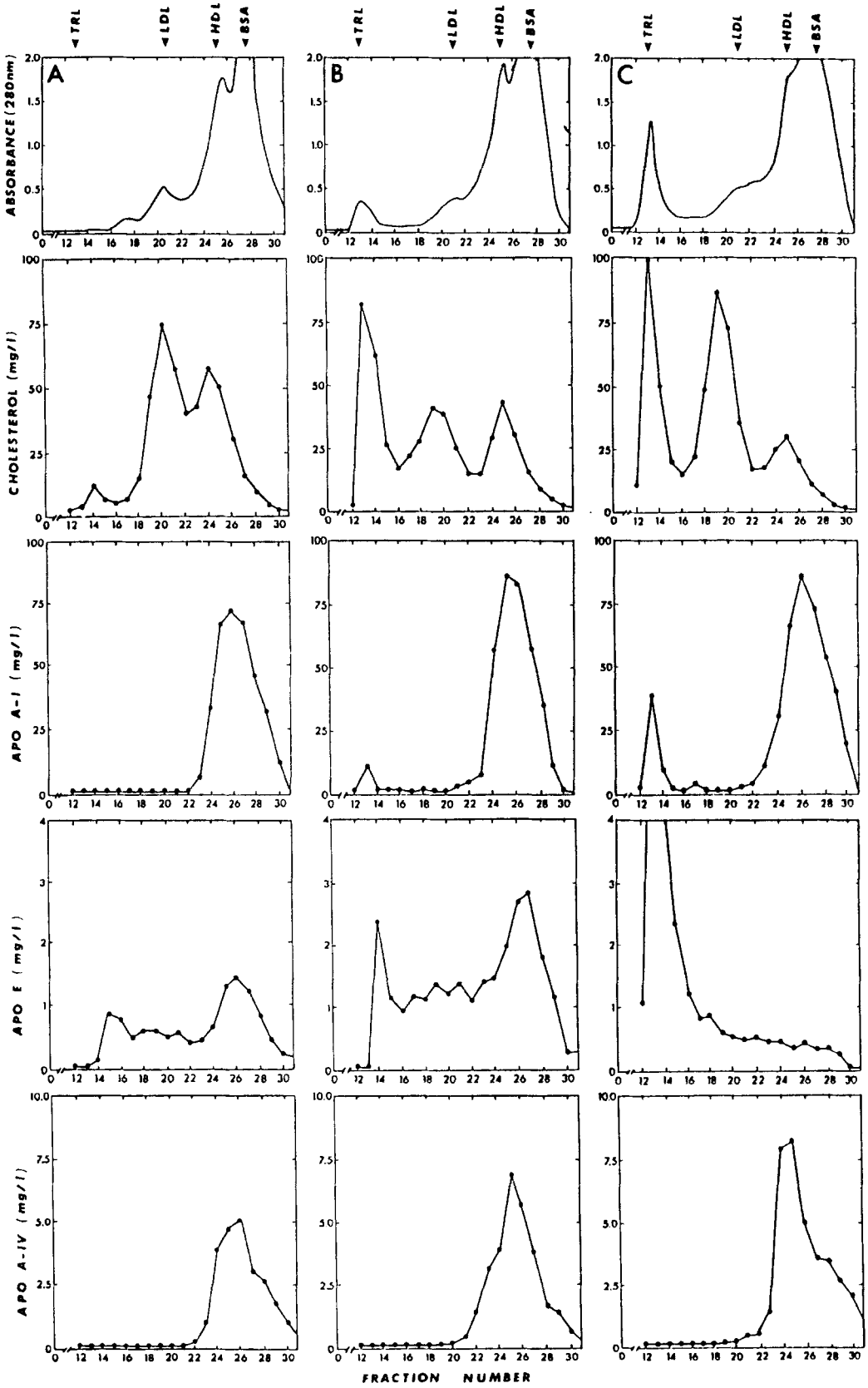

Fig. 7. Gel filtration profiles of normo- and hypertriglyceridemic sera. Triglyceride concentrations were 54 mg/dl in A, 309 mg/dl in B, and 895 mg/dl in C (C was a chylomicronemic serum). Total apo A-IV concentrations were 11.2 mg/dl in A, 20.1 mg/dl in B, and 35.8 mg/dl in C. Elution volumes of isolated human triglyceride-rich lipoproteins, TRL ($d < 1.006$ g/ml), LDL ($1.019 < d < 1.063$ g/ml), HDL ($1.063 < d < 1.21$ g/ml), and bovine serum albumin, BSA, are indicated by arrows.

dex. Although an indirect relation cannot be ruled out, the strength of the correlation between triglyceride and apoA-IV serum concentrations suggests a direct relation between these two parameters. In nonchylomicronemic sera from fasted subjects, such as those we studied, triglycerides are contained mainly in VLDL and the correlation between apoA-IV and triglyceride concentrations can be interpreted as a correlation between apoA-IV concentrations and VLDL levels. Thus, although the serum gel filtration experiments showed that, in agreement with other studies (7, 12, 13), no or almost no apoA-IV was found in the VLDL fraction, one is led to consider the possibility that some apoA-IV is brought into plasma by VLDL particles. It has been demonstrated, in humans as well as in rats, that upon its introduction into plasma, apoA-IV associated with triglyceride-rich lipoproteins is rapidly removed from the carrier lipoproteins and transferred into HDL and lipoprotein-free fractions (13, 27, 28). This rapid dissociation of apoA-IV from triglyceride-rich lipoproteins explains why Bisgaier et al. (7) found only a very minor peak of apoA-IV associated with triglyceride-rich lipoproteins in plasma obtained after fat feeding, in spite of a 25% increase of total apoA-IV serum concentration. Similarly, assuming that VLDL can supply apoA-IV, apoA-IV could be rapidly released from this lipoprotein fraction. One should, then, consider the eventuality of a secretion of apoA-IV by the liver. ApoA-IV is known to be synthesized by the intestine, both in the rat and in humans (5, 29). Synthesis by the liver has been demonstrated in the rat (30) but is still uncertain in humans. In this respect, recent studies of the distribution and expression of apoA-IV mRNA in some human tissues have not been conclusive (3, 4). Our data allow the question of an eventual role of the liver in the formation of the apoA-IV pool of human plasma to remain open.

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