In vivo metabolism of apolipoprotein A-IV in severe hypertriglyceridemia: a combined radiotracer and stable isotope kinetic study

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Abstract Apolipoprotein (apo) A-IV is an intestinally derived apolipoprotein that plays a potentially important role in lipoprotein metabolism and reverse cholesterol transport. However, the factors that regulate its plasma concentrations are not well understood. Plasma apoA-IV levels have been previously shown to correlate with fasting triglyceride (TG) levels in humans with TG levels less than 300 mg/dl (Lagrost et al. 1989. *J. Lipid Res.* **30:** 701–710). In this study, we established that apoA-IV levels were significantly elevated (mean 29.3 mg/dl) in a group of 15 hypertriglyceridemic patients (TG > 300 mg/dl) compared with normolipidemic controls (mean 13.4 mg/dl). In order to investigate the relationship between hypertriglyceridemia and apoA-IV metabolism, we then studied the in vivo kinetics of apoA-IV in two healthy hypertriglyceridemic patients (mean TG = 1297 mg/dl) compared with normolipidemic control subjects. Combined studies using endogenous stable isotope labeling (with a primed constant infusion of deuterated L-leucine) and exogenous radiolabeling (with ^{125}I) of apoA-IV were performed. Both stable isotope and radiotracer studies demonstrated substantially decreased apoA-IV fractional catabolic rates (FCR) in the hypertriglyceridemic patients (1.24 ± 0.13 day^{-1}) compared with controls (2.33 ± 0.08 day^{-1}). The apoA-IV production rate was not significantly different between the two groups. Gel filtration chromatography of plasma indicated an increased proportion of apoA-IV in the triglyceride-rich lipoproteins (TRL) of the hypertriglyceridemic patients compared with controls and delayed catabolism of this TRL-associated apoA-IV. The rate of apoA-IV catabolism from the lipid deficient fraction was not different between the hypertriglyceridemic patients and controls. In summary, plasma levels of apoA-IV are significantly elevated in hypertriglyceridemic patients due to delayed catabolism of apoA-IV as demonstrated by both endogenous stable isotope labeling and exogenous radiotracer techniques.—**Vergès, B., D. Rader, J. Schaefer, L. Zech, M. Kindt, T. Fairwell, P. Gambert, and H. B. Brewer, Jr.** In vivo metabolism of apolipoprotein A-IV in severe hypertriglyceridemia: a combined radiotracer and stable isotope kinetic study. *J. Lipid Res.* 1994. **35:** 2280–2291.

Supplementary key words very low density lipoprotein • low density lipoprotein • very high density lipoprotein • high density lipoprotein • fractional catabolic rate • fractional synthetic rate • lecithin:cholesterol acyltransferase

Human apolipoprotein (apo) A-IV is a 46-kDa plasma apolipoprotein which is synthesized predominantly in the small intestine (1–4). Although the metabolic functions of apoA-IV remain uncertain, it has been reported that apoA-IV binds to bovine aortic endothelial cells (5), stimulates cholesterol efflux from adipose cells (6, 7), activates lecithin:cholesterol acyltransferase (LCAT) (8, 9), participates in HDL particle conversion by cholesteryl ester transfer protein (CETP) (10, 11), and binds specifically to hepatic tissue (12–14). These data have led to the suggestion that apoA-IV plays a role in reverse cholesterol transport. In addition, apoA-IV modulates the activation of lipoprotein lipase in the presence of apoC-II (15), suggesting a role in the metabolism of triglyceride-rich lipoproteins (TRL). Furthermore, Weinberg, Ibdah, and Phillips (16) demonstrated that apoA-IV exhibits labile reversible binding to HDL_3 and proposed that apoA-IV helps to maintain optimal surface pressure for LCAT and CETP activities.

The distribution of apoA-IV in the plasma is not well defined, as the majority of apoA-IV has been found in the d > 1.21 g/ml lipoprotein-deficient fraction (LDF) after ultracentrifugation (2–4). By traditional gel filtration chromatography, approximately 15–25% of plasma apoA-IV has been found in the HDL size range (4, 17–20). In

Abbreviations: apo, apolipoprotein; TRL, triglyceride-rich lipoproteins; HDL, high density lipoproteins; LDF, lipid-deficient fraction; LPL, lipoprotein lipase; LCAT, lecithin:cholesterol acyltransferase; IEF, isoelectric focusing; BSA, bovine serum albumin; PBS, phosphate-buffered saline; VLDL, very low density lipoproteins; LDL, low density lipoproteins; VHDL, very high density lipoproteins; RT, residence time; FCR, fractional catabolic rate; PR, production rate; FSR, fractional synthetic rate.

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contrast, Lagrost et al. (21), using Superose 12-HR on FPLC, found approximately 70% of apoA-IV associated with HDL. The same investigators found that 70% of apoA-IV immunoprecipitated with an antibody to apoA-I (21). A small fraction of apoA-IV appears to be associated with triglyceride-rich lipoproteins (TRL) in fasting normolipidemic plasma (22, 23).

Factors that regulate plasma apoA-IV concentrations are poorly understood. We recently found that in normolipidemic humans the apoA-IV production rate is the major determinant of the apoA-IV concentration (24). Plasma apoA-IV levels have been reported to be elevated in chronic renal failure (25) and are positively correlated with plasma triglyceride levels (22), although other investigators have not found apoA-IV concentrations to be elevated in hypertriglyceridemia (26). In this study, we first established that apoA-IV levels were significantly elevated in a group of patients with triglyceride levels greater than 300 mg/dl compared with a control population. We then investigated the mechanism for increased apoA-IV levels in hypertriglyceridemia by performing in vivo kinetic studies of apoA-IV in two patients with type V hyperlipoproteinemia and in normolipidemic controls using both exogenous radiotracer and endogenous stable isotope labeling techniques.

METHODS

Study subjects

ApoA-IV levels were measured in 15 hypertriglyceridemic patients with triglyceride levels greater than 300 mg/dl (7 type IV hyperlipoproteinemia and 8 type V hyperlipoproteinemia) and in a group of 50 normotriglyceridemic controls. All the hypertriglyceridemic patients and controls had normal renal function.

Two patients with severe hypertriglyceridemia and four normolipidemic controls were selected for kinetic studies. Both patients had a primary type V hyperlipoproteinemia based on significantly elevated plasma levels of both chylomicrons and VLDL and both had a history of hypertriglyceridemia in other family members. Both had normal thyroid, renal, and liver function. One patient (#1) had a mild fasting hyperglycemia (glucose 184 mg/dl) treated by diet only. The other patient had normal fasting glucose levels. All control subjects had cholesterol, triglyceride, HDL cholesterol, apoA-I, and apoA-IV levels within the normal range and were in good health. The apoA-IV phenotype was A-IV 1/0 in one hypertriglyceridemic patient (#1) and apoA-IV 1/1 in the other (#2). In the four controls, the apoA-IV phenotype was A-IV 1/1. All subjects were placed on a controlled isoweight diet containing 47% of calories as carbohydrate, 37% as fat, and 16% as protein, with 200 mg cholesterol per 1000 kcal and a polyunsaturated to saturated fat ratio of 0.3. This diet was started 3 days before the beginning of the kinetic study and continued for the duration of the study. Subjects were weighed daily and body weights remained in steady state. Triglycerides remained at a constant level throughout the kinetic study, in both patients and controls.

The two hypertriglyceridemic patients had simultaneous endogenous stable isotope and exogenous radiotracer kinetic studies performed. Two controls underwent radiotracer studies in parallel with the two patients; two other controls underwent endogenous labeling stable isotope studies. Both study protocols were approved by the internal Review Board of the National Heart, Lung, and Blood Institute, and written informed consent was obtained from each of the participants.

Endogenous stable isotope study protocol

D_3-leucine was obtained from MSD Isotopes (St. Louis, MO), dissolved in 0.9% NaCl, sterile-filtered, and tested for pyrogenicity and sterility prior to use. Study subjects were given a priming bolus injection (1.2 mg/kg) of D_3-leucine, immediately followed by a constant infusion (24 μg/kg-min) of D_3-leucine for 16 h. During the infusion, subjects were on the controlled isoweight diet described above, but meals were provided in small equal portions every 2 h. Blood samples were drawn from the opposite arm into tubes containing 0.01% EDTA before the priming bolus and then at 10 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, 14 h and 16 h, and placed at 4°C. Plasma was separated by centrifugation (2300 rpm) for 30 min at 4°C. Inhibitors of protease and bacterial growth (aprotinin 200 U/ml and 0.05% sodium azide) were added to each sample. The two type V patients had the radiotracer and the stable isotope studies performed simultaneously.

Free plasma amino acids were isolated from 0.5 ml plasma by cation exchange column chromatography as previously described (27). ApoA-IV was isolated from whole plasma using immunoaffinity chromatography. Anti-apoA-IV immunosorbent was prepared by coupling purified rabbit polyclonal anti-apoA-IV antibodies prepared in our laboratory to Affigel 15 (Bio-Rad, Richmond, CA). Two ml of plasma from selected time points was applied to the anti-apoA-IV column equilibrated with PBS/0.05% EDTA at a flow rate of 10 ml/h. Bound lipoproteins were eluted with 3 M NaSCN at a flow rate of 60 ml/h. The recovery of apoA-IV, assessed using the ratio of ^{125}I-labeled apoA-IV in the retained fraction to ^{125}I-labeled apoA-IV in the plasma applied to the column, was 96 ± 2%. The retained fraction of the apoA-IV column was dialyzed against 10 mmol ammonium bicarbonate, lyophylized, delipidated using ethanol–ether 2:1 (v/v) and stored at 4°C. ApoA-IV was isolated by preparative discontinuous SDS-PAGE as previously described (28). Briefly, the delipidated apoA-IV containing material was solubilized in 4% SDS containing 0.5 M Tris, 8% sucrose, and 1% dithiothreitol and applied to a

1.5-mm-thick vertical slab gel (12 cm × 14 cm). After staining with Coomassie blue R-250 (Bio-Rad Laboratories) apoA-IV was cut from the gels and the gel slices were dried in a 90°C oven. Apolipoproteins were hydrolyzed in 6 N HCl (Sequanal Grade, Pierce, Rockford, IL) for 24 h at 110°C under nitrogen vacuum. After removal of HCl by speed vac lyophilization, the samples were dissolved in 50% acetic acid, applied to cation exchange columns, and amino acids were recovered by elution with 4 N NH_4OH.

Plasma free amino acids and apoA-IV-derived amino acids were derivatized to the N-heptafluorobutyryl isobutyl esters (29). Samples were analyzed by gas chromatography–mass spectrometry (GC–MS) on a Finnigan MAT 4500 (Finnigan MAT, San Jose, CA) in the positive chemical ionization mode, using isobutane as the reagent gas. The selected positively charged ions of 384 *m/z* for leucine and 387 *m/z* for D_3-leucine were monitored and the isotope ratio was determined. Each sample was analyzed at least three times. The enrichment was calculated from the isotope ratio using the method of Cobelli, Toffolo, and Foster (30). The tracer/tracee ratio was determined using the formula: tracer/tracee ratio = $e(t)/(e_i - e(t))$, where e(t) is the enrichment of each sample at time t, and e_i is the enrichment of the infusate (e_i = 99% for this study). An attempt was made to fit the tracer/tracee curves to a monoexponential function using SAAM 31 (31), however this analysis was unable to adequately fit the data. Therefore, fractional synthetic rates (FSR) were determined by linear regression analysis of the apoA-IV tracer/tracee ratio curves, similar to the method reported for determination of apoA-I FSR (32, 33). In the two controls, the apoA-IV tracer/tracee ratios reached plateau within 12 h and this value was used as the estimate of precursor tracer/tracee ratio for apoA-IV synthesis. In the two hypertriglyceridemic patients, the apoA-IV tracer/tracee ratios did not reach plateau within the infusion period. However, in the controls there was a consistent strong relationship between the apoA-IV tracer/tracee ratios and the free plasma leucine tracer/tracee ratios (2.47%/5.07% = 0.487 in control #1; 2.65%/5.65% = 0.469 in control #2), and therefore this fraction (0.48) was used to estimate the apoA-IV precursor tracer/tracee ratios in the two hypertriglyceridemic patients by multiplying it by the free plasma leucine tracer/tracee ratios. The slopes of the tracer/tracee ratios curves were determined by linear regression. The apoA-IV FSR was determined by the slope of the apoA-IV tracer/tracee ratio curve divided by the apoA-IV precursor tracer/tracee ratio. At steady state, fractional catabolic rate (FCR) is equal to FSR. Production rates (PR), expressed in mg/kg-day were determined according to the formula: PR = (FCR × apolipoprotein pool size)/body weight, where apolipoprotein pool size equals apolipoprotein concentration times plasma volume (assumed to be 4% of the body weight).

Exogenous radiotracer study protocol

ApoA-IV was isolated from the infranatant after ultracentrifugation of human serum adjusted to a density of 1.25 g/ml, according to a method previously described by Steinmetz et al. (34). ApoA-I was isolated from the HDL of a healthy donor with normal lipid and apolipoprotein levels using gel permeation and ion exchange chromatography as previously described (35).

Both apoA-IV and apoA-I were homologous. Purified apolipoproteins were iodinated by a modification of the iodine monochloride method as previously reported (36). Briefly, lyophilized apoA-IV and apoA-I were dissolved in a 6 M guanidine-HCl, 1 M glycine (pH 8.5) buffer; 5 mCi of $Na^{125}I$ or $Na^{131}I$ was added, then the iodide monochloride was added during light vortexing of the sample. Approximatively 0.5 mole iodine was incorporated per mole of apolipoprotein. Radiolabeled apolipoproteins were reassociated with autologous plasma and samples were dialyzed extensively against PBS/0.01% EDTA at 4°C to remove free iodide. Human serum albumin was added to a final concentration of 5% (wt/vol), and samples were sterile-filtered through a 0.22-μm filter and tested for pyrogens and sterility. Autoradiography of SDS-PAGE established that ^{125}I-labeled apoA-IV and ^{131}I-labeled apoA-I migrated to the expected positions.

One day prior to injection, subjects were given oral potassium iodide at a dose of 900 mg per day in divided doses, and this was continued throughout the study. After an overnight fast, subjects were injected intravenously with 1 μCi/kg of ^{125}I-labeled apoA-IV and 0.35 μCi/kg of ^{131}I-labeled apoA-I. Blood samples were drawn into tubes containing 0.01% EDTA at 10 min and at 1, 3, 6, 12, 16 h and then on days 2, 3, 4, 5, 7, 9, 11, and 14 and placed at 4°C. Plasma was treated as described above for the stable isotope studies. Urine was collected continuously throughout the study. Radioactivity in plasma and urine was quantitated in a Packard Cobra gamma counter (Packard Instrument Company, Downers Grove, IL).

Plasma radioactivity disappearance curves were constructed by dividing the plasma radioactivity at each time point by the radioactivity in the 10-min plasma sample. Plasma residence times (RT) were obtained from the area under the full 14-day plasma curves using a multiexponential computer curve-fitting technique (31). Fractional catabolic rates (FCR) are the reciprocals of the residence times. Production rates (PR) were calculated according to the formula described above.

Gel filtration

Lipoproteins were separated according to size from plasma obtained at multiple time points after injection using gel filtration chromatography. Three ml of plasma was applied through a flow adaptor to a 1.5 × 75 cm glass

column packed with 6% agarose beads (Bio-Gel A-5 m, 200–400 mesh, Bio-Rad), using an elution buffer of 0.9% NaCl, 0.01% EDTA, and 0.01% NaN_3, pH 7.4. The flow rate was 6 ml/h and fractions of 1.5 ml were collected over 24 h at 4°C. The effluents were monitored at 280 nm. The column was calibrated with VLDL, LDL, HDL, VHDL (d 1.21–1.25 g/ml), and bovine serum albumin. ApoA-IV mass and radioactivity were determined in the column fractions.

Analytical methods

Plasma apoA-IV concentrations were measured using a competitive enzyme immunoassay standardized with purified apoA-IV as previously described (22). Briefly, plate coating was prepared by pipetting a 100 μl volume of pure A-IV solution (2.5 mg/l) into each well of a polystyrene microwell plate (Immuno 96F Type 1, 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 serum albumin solution. Diluted samples and diluted rabbit antiserum were incubated overnight at 4°C. Then 100-μl aliquots of the mixtures were pipetted into the immunoplate microwells. After 4 h incubation at room temperature, the plates were washed and bound anti-apoA-IV antibodies were detected using a peroxidase-conjugated anti-rabbit IgG antiserum and a solution of O-phenylenediamine (0.4 g/l) and hydrogen peroxide (0.68 g/l). After 15 min at room temperature in the dark, the reaction was stopped by addition of 30 μl of 2.5 mol/l H_2S0_4. The absorbances were read at 490 nm in a multiskan Elisa reader (Titertek, Finland). Each sample was measured in triplicate at four different dilutions. The coefficients of variation for this apoA-IV ELISA were 3.0% within runs and 3.9% between runs. Results compared favorably with a second method, that of a noncompetitive ELISA similar to that previously described (37). Plasma cholesterol and triglycerides were quantitated by automated enzyme techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol was determined in plasma following dextran sulfate precipitation (38). Plasma apoA-I and apoA-II were quantitated by immunoturbidometric assay (Boehringer-Mannheim, Mannheim, Germany). ApoA-IV phenotyping was performed by isoelectric focusing (pH range: 5–6) of delipidated samples (39). Then proteins were transferred to nitrocellulose by Western blotting followed by immunoblotting with a polyclonal antibody.

Comparisons of means between hypertriglyceridemic patients and controls were made with Student's unpaired *t* test after verification of a normal distribution for the data.

TABLE 1. Mean triglyceride and apoA-IV levels in a group of hypertriglyceridemic patients and in a group of controls

	Hypertriglyceridemic Patients n = 15	Controls n = 50	
Age, y	40.6 ± 12.4	43.5 ± 11.5	NS
Triglycerides, mg/dl	1112 ± 778	58 ± 20	$P = 0.001$
ApoA-IV, mg/dl	29.3 ± 10	13.4 ± 2.7	$P = 0.001$

MODEL SELECTION

The radiotracer data were fit to three exponentials using the SAAM program, which has been extensively reported in the literature (31).

For the stable isotope study, we attempted to fit the tracer/tracee curves to a monoexponential function with a delay. However, this analysis was unable to adequately fit the data and therefore FSR were determined using the linear regression approach, as previously reported by others (32, 33). As recently reviewed by Foster et al. (40), linear regression analysis is not the optimal way to determine FSR of plasma apolipoproteins from stable isotope data, and we are aware that compartmental modelling is the preferred approach. Had we performed the stable isotope alone, we would not have had total confidence in our kinetic data obtained from linear regression analysis. However, the data compare favorably with the radiotracer kinetic data simultaneously obtained and are therefore useful in a confirmatory way.

RESULTS

The mean plasma triglyceride and apoA-IV levels in the hypertriglyceridemic patients and controls are shown in **Table 1.** Hypertriglyceridemic patients had significantly higher levels of apoA-IV than controls, confirming the association of elevated fasting triglycerides with elevated apoA-IV concentrations.

The clinical characteristics of the subjects for the kinetic studies are presented in **Table 2.** Values are the mean of five fasting determinations made while the patients were on a controlled metabolic diet; levels remained in steady state throughout the course of the study.

Endogenous labeling of apoA-IV with D_3-leucine established that the turnover of apoA-IV was much slower in the hypertriglyceridemic subjects than in controls. In **Fig. 1** are shown the plasma free leucine tracer/tracee ratio curves, which remained constant during the 16-h infusion. The mean plasma free leucine tracer/tracee ratios and ranges were 5.07 ± 0.20% (4.83–5.21%) in control 1, 5.65 ± 0.25% (5.45–6.17%) in control 2, 6.0 ± 0.39% (5.61–6.52%) in patient 1, and 6.09 ± 0.24% (5.76–6.26%) in patient 2. The tracer/tracee ratio curves of total plasma apoA-IV are shown in **Fig. 2.** The apoA-IV tracer/tracee ratio curves in the two controls reached plateau by 12 h with a mean tracer/tracee ratio of

TABLE 2. Clinical characterization of the study subjects

Subjects	Sex	Age	BMI	TG	TC	HDL-C	ApoA-I	ApoA-IV
		yr				*mg/dl*		
Type V patients								
#1	M	54	28.3	1451 ± 108	342 ± 29	32 ± 1	121 ± 07	22.1 ± 3.1
#2	M	44	26.3	1143 ± 168	390 ± 24	40 ± 3	131 ± 15	23.7 ± 2.9
Controls								
#1	M	23	21	82 ± 6	164 ± 8	33 ± 2	147 ± 21	10.5 ± 1.2
#2	M	22	26.2	89 ± 8	167 ± 6	35 ± 2	150 ± 16	10.7 ± 2.0
#3	M	22	22.4	80 ± 14	181 ± 9	42 ± 3	122 ± 21	12.4 ± 1.2
#4	F	20	18.3	57 ± 11	147 ± 6	44 ± 2	144 ± 16	11.5 ± 1.8

BMI, body mass index; TG, triglycerides; TC, total cholesterol; HDL-C, HDL-cholesterol.

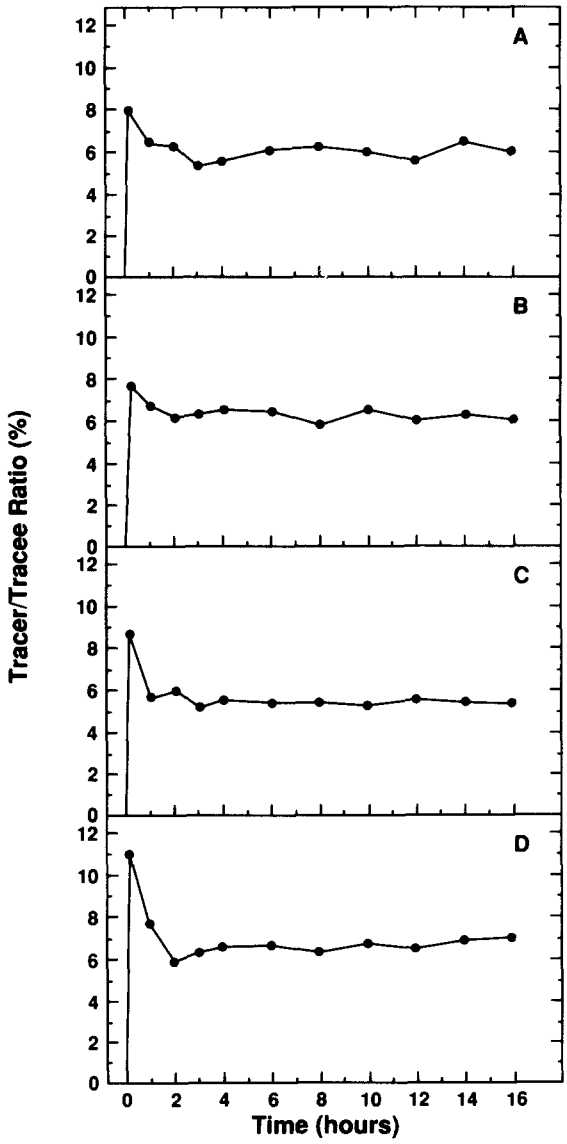

Fig. 1. Tracer/tracee ratios of plasma free leucine during the constant infusion period in patient 1 (A), in patient 2 (B), in control 1 (C), and in control 2 (D).

2.47 ± 0.03% in control 1 and 2.65 ± 0.02% in control 2. In contrast, the apoA-IV tracer/tracee ratio curves in the hypertriglyceridemic subjects had much shallower slopes and did not reach plateau by the end of the infusion, indicating much slower turnover of apoA-IV.

These results were supported by simultaneous injection of exogenously radiolabeled apoA-IV. In **Fig. 3** are shown the ^{125}I-labeled apoA-IV plasma curves demonstrating the delayed apoA-IV catabolism in the hypertriglyceridemic patients compared with control subjects studied at the same time.

The kinetic parameters of apoA-IV and apoA-I metabolism are shown in **Table 3.** The kinetic data obtained independently from the endogenous stable isotope and exogenous radiotracer studies were remarkably similar. By both methods, the FCR of apoA-IV was substantially slower in the hypertriglyceridemic patients than in the controls. ApoA-IV production rates were not different between the patients and the controls. In contrast to apoA-IV, the catabolism of ^{125}I-labeled apoA-I was somewhat faster in the two hypertriglyceridemic patients compared with the controls.

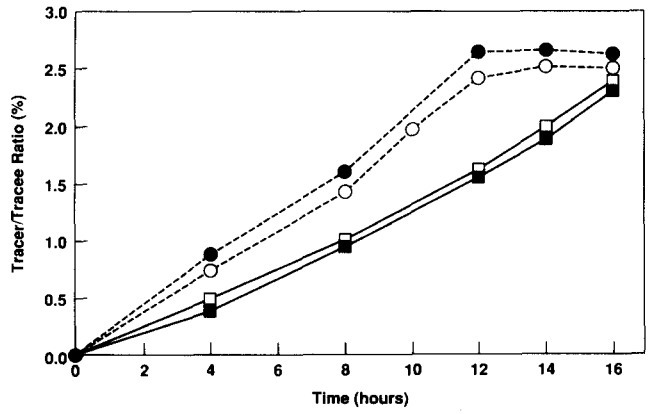

Fig. 2. Tracer/tracee ratios of total plasma apoA-IV with D3-leucine in two patients (squares) and two controls (circles).

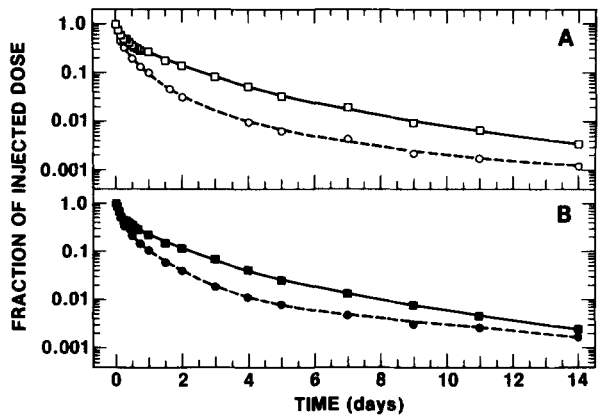

Fig. 3. A: Plasma ^{125}I-labeled apoA-IV radioactivity decay curves following administration of ^{125}I-labeled apoA-IV in patient 1 (squares) and in control 3 (circles). B: Plasma ^{125}I activity decay curves following administration of ^{125}I-labeled apoA-IV in patient 2 (squares) and in control 4 (circles).

The urinary radioactivity data (**Fig. 4**) indicated that the difference in apoA-IV metabolism between patients and controls was not due simply to differential extravascular distribution. Cumulative urinary apoA-IV radioactivity increased much more rapidly in controls than in hypertriglyceridemic patients, with the largest difference within the first 4 days. The urine/plasma (U/P) radioactivity ratios, which reflect fractional catabolic rate, were substantially less in the hypertriglyceridemic patients than the controls especially within the first 4 days.

ApoA-IV distribution was investigated by separating lipoproteins according to size using gel filtration chromatography. Representative column profiles of ^{125}I-labeled apoA-IV and ^{125}I-labeled apoA-I at 10 min and 12 h are shown in **Fig. 5** for one hypertriglyceridemic and one control subject. At the initial time point, substantially more radiolabeled apoA-IV was found in the TRL of the hypertriglyceridemic patients than in the controls. Furthermore, by 12 h, 23% and 48% of the total apoA-IV tracer remained in the TRL of the two hypertriglyceridemic patients, whereas less than 4% of the apoA-IV tracer was found in the TRL of controls. The apoA-IV mass distribution among three major lipoprotein fractions is shown in **Table 4.** ApoA-IV mass was also higher in the TRL of the hypertriglyceridemic patients than the controls.

The apoA-IV specific activity curves of the three apoA-IV-containing lipoprotein fractions are shown in **Fig. 6.** At the initial time point, the specific activities were highest in the TRL and lowest in the LDF. As the apoA-IV specific activity in the TRL decreased that in the LDF increased, suggesting transfer of labeled apoA-IV from TRL to LDF. The decrease in TRL apoA-IV specific activity was slower in the hypertriglyceridemic patients than in controls. By 6 h the specific activities in TRL and the LDF were almost equal in all four subjects. In contrast, the HDL apoA-IV specific activity remained lower than that of TRL and the LDF at all time points after 10 min, suggesting relatively little exchange of apoA-IV between HDL and the other two compartments.

The ^{125}I-labeled apoA-IV plasma decay curves were each fit to three different exponentials, with fractional rate constants L1, L2, and L3, and intercepts α1, α2, and α3 (**Table 5**). The main difference is reduced rate constant L2 in hypertriglyceridemic patients, indicating slower catabolism in this compartment, which may represent the TRL. Moreover, some differences are observed in the intercepts, which represent the apparent distribution of the radiolabeled apolipoprotein among the three components as a fraction of the injected dose. The hypertriglyceridemic patients appear to have more tracer in α2 and α3, which turn over more slowly. Therefore, the slower turnover of apoA-IV in hypertriglyceridemia is caused both by distribution of the apoA-IV to slower turning over compartments and by slower turnover in the L2 compartment.

TABLE 3. Kinetic parameters of apoA-IV and apoA-I metabolism

	ApoA-IV				ApoA-I		
Subjects	Conc	FSR Endog	FCR Exog	PR	Conc	FCR Exog	PR
	mg/dl	*day^{-1}*	*day^{-1}*	*mg/kg-d*	*mg/dl*	*day^{-1}*	*mg/kg-d*
Hyper TG							
#1	22.1	1.29	1.22	10.8	121	0.318	15.4
#2	23.7	1.20	1.04	9.9	131	0.302	15.8
Controls							
#1	10.5	2.39		10.0			
#2	10.7	2.37		10.1			
#3	12.4		2.24	11.1	122	0.261	12.7
#4	11.5		2.26	10.4	144	0.256	14.7

Conc, concentration; FSR, fractional synthetic rate; FCR, fractional catabolic rate; PR, production rate; endog, endogenous stable isotope labeling method; exog, exogenous radiotracer labeling method.

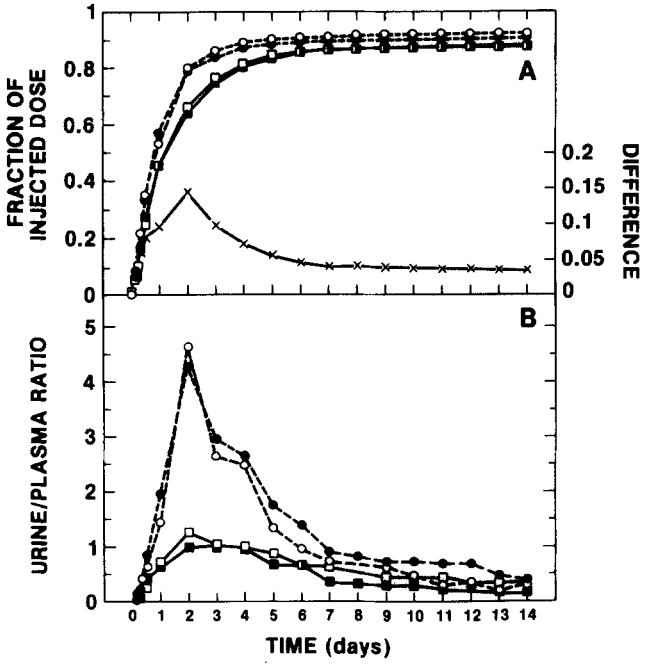

Fig. 4. A: Relative cumulative urinary apoA-IV radioactivity in two patients (squares) and two controls (circles). The Xs represent the difference between controls and patients at each time point. B: Urine/plasma (U/P) radioactivity ratios for apoA-IV in two patients (squares) and two controls (circles).

DISCUSSION

The factors that modulate the in vivo metabolism of apoA-IV in humans are poorly understood. In normolipidemic persons, apoA-IV levels are largely determined by production rate and not by catabolic rate (24). Plasma apoA-IV levels have been reported by Lagrost et al. (22) to be correlated with fasting plasma TG levels. In this report, we extend these findings by demonstrating significantly elevated levels of apoA-IV in a group of 15 hypertriglyceridemic patients with TG levels greater than 300 mg/dl. The purpose of the kinetic studies was to determine whether the increased levels of apoA-IV associated with hypertriglyceridemia are due to increased production or delayed catabolism of apoA-IV. Using both endogenous labeling of apoA-IV with D_3-leucine as well as exogenously radiolabeled apoA-IV, we demonstrated that the catabolism of apoA-IV in two hypertriglyceridemic subjects was substantially delayed, whereas their apoA-IV production rates were normal. The kinetic parameters obtained from these two independent methods were remarkably similar, confirming this result.

The apoA-IV U/P curves in the control subjects had a high early peak followed by a lower plateau, indicating heterogeneity of apoA-IV metabolism and consistent with a previous report (41). This early peak in the U/P ratios

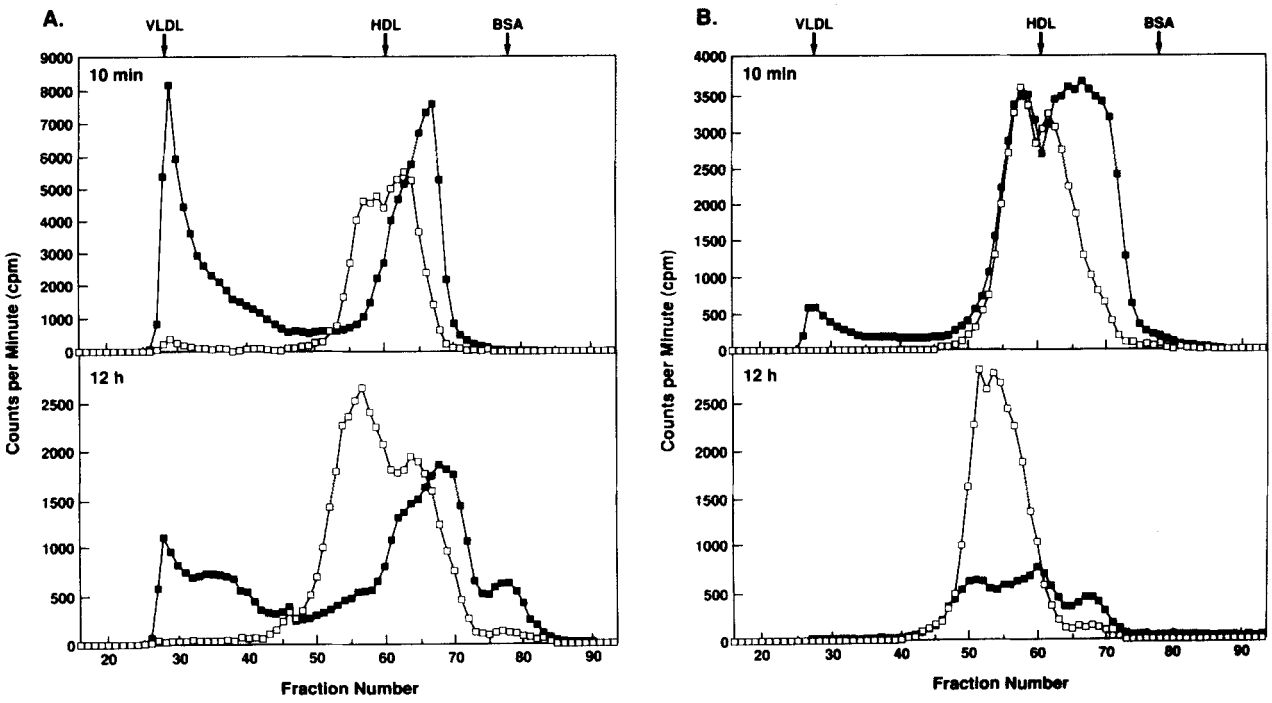

Fig. 5. Representative gel filtration column profiles of ^{125}I-labeled apoA-IV (solid squares) and ^{131}I-labeled apoA-I (open squares) at 10 min and 12 h after injection in one hypertriglyceridemic patient (A) and one control (B).

TABLE 4. Distribution of apoA-IV mass in lipoprotein fractions

Subjects	TRL	HDL	LDF
		mg/dl (% distribution)	
Hyper TG			
1	2.0 ± 0.3 (9.2%)	18.2 ± 1.1 (82.3%)	1.9 ± 0.5 (8.4%)
2	1.6 ± 0.6 (6.8%)	19.1 ± 2.8 (80.5%)	3.0 ± 0.7 (12.7%)
Controls			
3	0.02 ± 0.01 (0.2%)	11.0 ± 0.7 (95.9%)	0.5 ± 0.1 (3.8%)
4	0.03 ± 0.01 (0.3%)	10.8 ± 0.5 (94.7%)	0.6 ± 0.2 (5%)

was significantly blunted in the hypertriglyceridemic patients, consistent with delayed apoA-IV catabolism. Because the U/P ratio peak occurred at the same time (day 2) in controls as in patients, the delayed urinary radioactivity excretion noted in the patients is not due to the retention of ^{125}I-labeled apoA-IV in an extravascular compartment and represents true delayed catabolism of apoA-IV in these patients.

The in vivo distribution of apoA-IV among plasma lipoproteins in humans has not been fully elucidated. It is generally agreed that plasma apoA-IV exists in at least three different pools: TRL, HDL, and the LDF (2, 4, 20-22, 42). However, the quantitative steady state distribution and the rates of apoA-IV exchange among these compartments remain uncertain. Both in vivo and in vitro studies have shown that apoA-IV in TRL can be transferred to HDL and the LDF (20, 42). ApoA-IV on HDL was shown to exchange with apoA-IV in TRL and the LDF in one study (42), while no transfer of HDL apoA-IV to TRL or the LDF was observed in another study (20). It has been demonstrated that most apoA-IV in the LDF does not exchange with the two other fractions (20, 42). The kinetic behavior of apoA-IV circulating in the LDF suggest that in this plasma fraction apoA-IV is not a free protein but rather in complexes that do not permit free exchange of apoA-IV. Indeed, evidence suggests that at least some apoA-IV exists as a homodimer (43); small dense lipoprotein complexes containing apoA-IV have also been described (44).

In the present study, gel filtration of plasma 10 min after injection demonstrated that most of the ^{125}I-labeled apoA-IV was associated with HDL, in agreement with a previous report in which the lipoproteins were separated according to size using gel filtration chromatography (21). Some of the ^{125}I-labeled apoA-IV was associated with TRL; the two hypertriglyceridemic patients had substantially more apoA-IV associated with TRL than the controls. At the 10-min time point, very little ^{125}I-labeled apoA-IV was found in the LDF. By 3 h, however, ^{125}I-labeled apoA-IV was detectable in the LDF and by 6 h the apoA-IV specific activity in the LDF was virtually equal to that in TRL, suggesting a transfer of labeled apoA-IV from TRL to the LDF. The specific activity decay curves

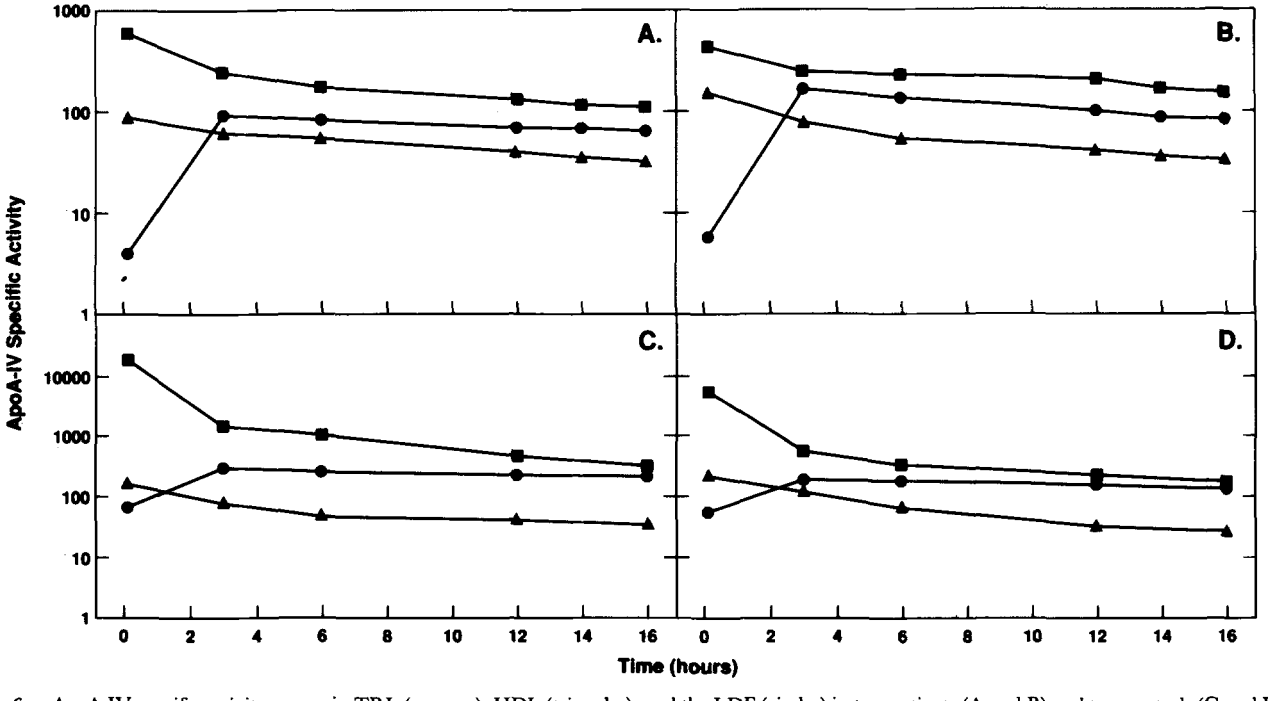

Fig. 6. ApoA-IV specific activity curves in TRL (squares), HDL (triangles), and the LDF (circles) in two patients (A and B) and two controls (C and D).

TABLE 5. Rate constants (L) and intercepts (α) of the three exponentials of apoA-IV in the hypertriglyceridemic subjects and the controls (mean ± SD)

	Hypertriglyceridemic Patients	Controls
Rate constants (day^{-1})		
L1 =	7.63 ± 0.72	8.70 ± 1.51
L2 =	0.78 ± 0.05	1.37 ± 0.24
L3 =	0.22 ± 0.01	0.25 ± 0.09
Intercepts (fraction of injected dose)		
$\alpha 1$ =	0.507 ± 0.021	0.648 ± 0.047
$\alpha 2$ =	0.414 ± 0.004	0.328 ± 0.038
$\alpha 3$ =	0.064 ± 0.002	0.023 ± 0.009

of labeled apoA-IV in the lipoprotein fractions demonstrated that the delayed catabolism of apoA-IV in hypertriglyceridemia was primarily due to delayed catabolism of TRL-associated apoA-IV. In contrast, the catabolism of apoA-IV associated with HDL and the LDF was not different between the patients and the controls, suggesting that the metabolism of apoA-IV in HDL and LDF is not influenced by the presence of high triglycerides. The data obtained from the apoA-IV specific activity curves of each lipoprotein fraction (TRL, HDL, LDF) are confirmed by the analysis of the fractional rate constants of the three exponentials (L1, L2, L3) used to fit the ^{125}I-labeled apoA-IV plasma decay curve. The main difference is reduced rate constant L2 in hypertriglyceridemic patients, indicating slower turnover in this compartment, which may represent the TRL. We cannot assign a physiologic role to each kinetic compartment, at this time. However, we speculate that the faster compartment (L1) may represent free apoA-IV, that the intermediate compartment (L2) may represent apoA-IV associated with triglyceride-rich lipoproteins, and that the slower compartment (L3) may represent HDL-associated A-IV. If this were the case, the delayed apoA-IV turnover in the hypertriglyceridemic patients would be related to the TRL compartment.

The metabolic fate of apoA-IV is not especially closely linked to that of apoA-I. Malmendier et al. (41) reported that only 13% of HDL-associated apoA-IV follows the metabolic fate of A-I. In our study, while apoA-IV catabolism was delayed in the hypertriglyceridemic patients, the catabolic rate of apoA-I was slightly faster in the patients than in the controls. Therefore, hypertriglyceridemia has opposite effects on the rates of apoA-IV and apoA-I catabolism.

The present study provides evidence that hypertriglyceridemia results in elevated plasma apoA-IV levels due to delayed catabolism of apoA-IV, primarily that associated with triglyceride-rich lipoproteins. Our two patients had type V hyperlipoproteinemia, which is characterized by overproduction of VLDL triglycerides as well as defective clearance of triglyceride-rich lipoproteins (45–47). While the catabolism of apoA-IV in these type V subjects was delayed, there was no increase in apoA-IV production, suggesting that apoA-IV production may not be tightly linked to VLDL TG synthesis. The delayed catabolism of apoA-IV could be a direct result of delayed triglyceride-rich lipoprotein clearance. However, elevated levels of apoA-IV have also been reported in patients with hypertriglyceridemia due to an overproduction of VLDL with no defect in triglyceride-rich lipoprotein clearance such as non-insulin-dependent diabetics (22, 48) and subjects with chronic alcohol use (22). Therefore, delayed catabolism of apoA-IV is probably not the direct result of delayed triglyceride-rich lipoprotein catabolism but rather due to elevations in steady state fasting triglyceride levels regardless of the etiology. The mechanism of this effect remains uncertain, but our data suggest that the high levels of triglyceride-rich lipoproteins retain apoA-IV within the TRL compartment, from which it is catabolized at a slower rate than from the LDF.

The consequences of elevated levels of apoA-IV in hypertriglyceridemic patients are unknown. So far, no data are available on the relationship between plasma apoA-IV levels and the incidence of atherosclerosis. Furthermore, the exact physiological roles of apoA-IV are still unknown. As apoA-IV has been shown to promote CETP activity indirectly (16), patients with elevated apoA-IV levels could be suspected to have increased CETP activity. CETP may be regarded as a potentially atherogenic factor, as decreased plasma CETP activity is associated with a low atherogenic potential and, conversely, elevated CETP activity is associated with a high atherogenic potential (49). Although it has not yet been shown, one may hypothesize that increased apoA-IV level could indicate risk for atherosclerosis. However, this has not been proved and remains speculative. On the other hand, one may argue that because apoA-IV has some similarities with apoA-I, it could be protective for atherosclerosis. The consequences of elevated apoA-IV levels in humans will become less obscure when the precise physiologic role of apoA-IV is known.

Limitations of the study

The mean age of the two hypertriglyceridemic patients was not identical to the mean age of the controls; however, as no correlation was found between age and plasma apoA-IV level in the 15 hypertriglyceridemic patients and in the 50 controls, we think that our results are not confounded by an age effect.

The apoA-IV phenotype was A-IV 1/0 in one hypertriglyceridemic patient and A-IV 1/1 in the other. Very little is known about the consequences of the apoA-IV 0 isoprotein on lipid metabolism. The only published report concerning the apoA-IV 1/0 phenotype describes five subjects who showed no statistically significant differences in

plasma cholesterol, triglyceride, apoA-I, apoA-IV, apoB, and apoE levels when compared with apoA-IV 1/1 phenotypes (50). No data are available on the kinetics of the apoA-IV 0 isoprotein. However, as the apoA-IV kinetic data are very similar in both hypertriglyceridemic patients, we think that the apoA-IV phenotype difference between the two patients has not significantly influenced the results of the present study.

For an endogenous stable isotope study, the isotopic enrichment plateau value of a rapidly secreted protein is commonly used to estimate the precursor pool enrichment (32, 33). For example, the isotopic enrichment of VLDL apoB-100 at plateau is a useful and interesting way to estimate the precursor pool enrichment of proteins synthesized by the liver as VLDL apoB-100 is (32, 33). However, apoA-IV is predominantly synthesized by the small intestine, which may have a different precursor pool enrichment than the liver. Therefore, we decided to use the isotopic enrichment of apoA-IV itself at plateau to estimate the precursor pool enrichment. The apoA-IV tracer/tracee ratios did not reach plateau within the infusion period in the two hypertriglyceridemic patients. However, in the controls, there was a strong relationship between the apoA-IV tracer/tracee ratios and the free plasma leucine tracer/tracee ratios, and therefore this fraction (0.48) was used to estimate the apoA-IV precursor tracer/tracee ratios in the two hypertriglyceridemic patients by multiplying it by the free plasma leucine tracer/tracee ratio. We anticipate that there is no reason to think that the apoA-IV precursor tracer/tracee ratio (isotopic enrichment of the protein synthetic amino acid precursor pool) could be different in hypertriglyceridemic patients from controls. Moreover, as kinetic data obtained from the radiotracer study and those obtained from the stable isotope study were very similar, we think that the method used to estimate the apoA-IV precursor tracer/tracee in the hypertriglyceridemic patients was appropriate.

The use of linear regression analysis to determine FSR of apolipoproteins from the stable isotope data is not the optimal way (40). If the monoexponential analysis had been possible, it would have been a preferred way to determine the apoA-IV FSR. In the present study, the kinetic data from the stable isotope study supported the data from the radiotracer study and were not used as the primary data. Moreover, the kinetic data obtained independently from the stable isotope and the radiotracer studies were very similar, indicating that linear regression analysis was not so wrong.

Our data clearly show delayed catabolism of total apoA-IV in two severe hypertriglyceridemic patients. However, these results cannot be extrapolated to all hypertriglyceridemic patients, particularly those with less severe hypertriglyceridemia.

One question, generated by the present study, is whether less severe hypertriglyceridemic patients also have delayed catabolism of apoA-IV. To answer this question, apoA-IV kinetic studies should be performed in patients with moderately elevated triglyceride levels. Moreover, to make sure that delayed catabolism of apoA-IV is directly related to hypertriglyceridemia itself and not to the cause of hypertriglyceridemia, different kinds of hypertriglyceridemia such as type IV HLP should be studied.

In summary, apoA-IV levels are increased in severe hypertriglyceridemic patients as a result of delayed apoA-IV catabolism. Increased levels of triglyceride-rich lipoproteins, probably regardless of the underlying etiology, may retain apoA-IV within the triglyceride-rich fraction and delay its catabolism, accounting for the increased apoA-IV levels seen in most hypertriglyceridemic states.

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REFERENCES

1. Weisgraber, K. H., T. P. Bersot, and R. W. Mahley. 1978. Isolation and characterization of an apoprotein from the d less than 1.006 lipoproteins of human and canine lymph homologous with the rat A-IV apoprotein. *Biochem. Biophys. Res. Commun.* **85:** 287–292.
2. Utermann, G., and U. Beisiegel. 1979. Apolipoprotein A-IV: a protein occurring in human mesenteric lymph chylomicrons and free in plasma. Isolation and quantification. *Eur. J. Biochem.* **99:** 333–343.
3. Green, P. H. R., R. M. Glickman, C. D. Saudek, C. B. Blum, and A. R. Tall. 1979. Human intestinal lipoproteins. Studies in chylemic subjects. *J. Clin. Invest.* **64:** 233–242.
4. Green, P. H., 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.
5. Savion, N., and A. Gamliel. 1988. Binding of apolipoprotein A-I and apolipoprotein A-IV to cultured bovine aortic endothelial cells. *Arteriosclerosis.* **8:** 178–186.
6. Stein, O., Y. Stein, M. Lefevre, and P. S. Roheim. 1986. The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from another analog of phosphatidylcholine. *Biochim. Biophys. Acta.* **878:** 7–13.
7. Steinmetz, A., R. Barbaras, N. Ghalim, V. Clavey, J. C. Fruchart, and G. Ailhaud. 1990. Human apolipoprotein A-IV binds to apolipoprotein A-I/A-II receptor sites and promotes cholesterol efflux from adipose cells. *J. Biol. Chem.* **265:** 7859–7863.
8. Steinmetz, A., and G. Utermann. 1985. Activation of leci-

thin:cholesterol acyltransferase by human apolipoprotein A-IV. *J. Biol. Chem.* **260:** 2258–2264.
9. Chen, C. H., and J. J. Albers. 1985. Activation of lecithin:cholesterol acyltransferase by apolipoproteins E-2, E-3, and A-IV isolated from human plasma. *Biochim. Biophys. Acta.* **836:** 279–285.
10. Barter, P. J., O. V. Rajaram, L. B. F. Chang, K. A. Rye, P. Gambert, L. Lagrost, C. Ehnholm, and N. M. Fidge. 1988. Isolation of a high density lipoprotein conversion factor from human plasma. A possible role of apolipoprotein A-IV as its activator. *Biochem. Biophys. Res. Commun.* **254:** 179–184.
11. Lagrost, L., P. Gambert, V. Dangremont, A. Athias, and C. Lallemant. 1990. Role of cholesteryl ester transfer protein (CETP) in the HDL conversion process as evidenced by using anti-CETP monoclonal antibodies. *J. Lipid Res.* **31:** 1569–1575.
12. Dvorin, E., N. L. Gorder, D. M. Benson, and A. M. Gotto, Jr. 1986. Apolipoprotein A-IV. A determinant for binding and uptake of high density lipoproteins by rat hepatocytes. *J. Biol. Chem.* **261:** 15714–15718.
13. Ghiselli, G., W. L. Crump III, and A. M. Gotto, Jr. 1986. Binding of apoA-IV phospholipid complexes to plasma membranes of rat liver. *Biochem. Biophys. Res. Commun.* **139:** 122–128.
14. Weinberg, R. B., and C. S. Patton. 1990. Binding of human apolipoprotein A-IV to human hepatocellular plasma membranes. *Biochim. Biophys. Acta.* **1044:** 255–261.
15. Goldberg, I. J., C. A. Scheraldi, L. X. Yacoub, U. Satena, and L. L. Bisgaier. 1990. Lipoprotein apoC-II activation of lipoprotein lipase. Modulation by apolipoprotein A-IV. *J. Biol. Chem.* **265:** 4266–4272.
16. Weinberg, R. B., J. A. Ibdah, and M. C. Phillips. 1992. Adsorption of apolipoprotein A-IV to phospholipid monolayers spread at the air/water interface. *J. Biol. Chem.* **267:** 8977–8983.
17. 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.
18. Bisgaier, C. L., O. P. Sachdev, E. S. Lee, K. J. Williams, C. B. Blum, and R. M. Glickman. 1987. Effect of lecithin:cholesterol acyltransferase on distribution of apolipoprotein A-IV among lipoproteins of human plasma. *J. Lipid Res.* **28:** 693–703.
19. Bisgaier, C. L., M. V. Siebenkas, C. B. Hesler, T. L. Swenson, C. B. Blum, Y. L. Marcel, R. W. Milne, R. M. Glickman, and A. R. Tall. 1989. Effect of a neutralizing monoclonal antibody to cholesteryl ester transfer protein on the redistribution of apolipoproteins A-IV and E among human lipoproteins. *J. Lipid Res.* **30:** 1025–1031.
20. Ghiselli, G., S. Krishnan, Y. Beigel, and A. M. Gotto, Jr. 1986. Plasma metabolism of apolipoprotein A-IV in humans. *J. Lipid Res.* **27:** 813–827.
21. Lagrost, L., P. Gambert, M. Boquillon, and C. Lallemant. 1989. Evidence for high density lipoproteins as the major apolipoprotein A-IV-containing fraction in normal human serum. *J. Lipid Res.* **30:** 1525–1534.
22. Lagrost, L., P. Gambert, S. Meunier, P. Morgado, J. Desgres, P. d'Athis, and C. Lallemant. 1989. Correlation between apolipoprotein A-IV and triglyceride concentrations in human sera. *J. Lipid Res.* **30:** 701–710.
23. Duverger, N. J., N. Ghalim, G. Ailhaud, A. Steinmetz, J. C. Fruchart, and G. Castro. 1993. Characterization of apolipoprotein A-IV-containing lipoprotein particles isolated from human plasma and interstitial fluid. *Arterioscler. Thromb.* **13:** 126–132.
24. Rader, D. J., J. R. Schaefer, P. Lohse, B. Verges, M. Kindt, L. A. Zech, A. Steinmetz, and H. B. Brewer, Jr. 1993. Rapid in vivo transport and catabolism of human apolipoprotein A-IV-1 and slower catabolism of the apoA-IV-2 isoprotein. *J. Clin. Invest.* **92:** 1009–1017.
25. 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.
26. Dieplinger, H., E. M. Lobentanz, P. Konig, H. Graf, C. Sandholzer, E. Matthys, M. Rosseneus, and G. Utermann. 1992. Plasma apolipoprotein A-IV metabolism in patients with chronic renal disease. *Eur. J. Clin. Invest.* **22:** 166–174.
27. Wolfe, R. R., editor. 1984. Gas chromatography–mass spectrometry methods. *In* Tracers in Metabolic Research: Radioisotope and Stable Isotope/Mass Spectrometry Methods. Alan R. Liss, New York. 261–271.
28. Schaefer, J. R., D. J. Rader, and H. B. Brewer, Jr. 1992. Investigation of lipoprotein kinetics using endogenous labeling with stable isotopes. *Curr. Opin. Lipidol.* **3:** 227–232.
29. Mac Kenzie, S. L., and D. Tenaschuk. 1974. Gas–liquid chromatography of N-heptafluorobutyryl isobutyl esters of aminoacids. *J. Chromatogr.* **97:** 19–24.
30. Cobelli, C., G. Toffolo, and D. M. Foster. 1992. Tracer-to-tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** E968–E975.
31. Berman, M., and M. Weiss. 1978. SAAM Manual. DHEW publ. No. (NIH) 78. National Institutes of Health, Bethesda, MD.
32. Cohn, J. S., D. A. Wagner, S. D. Cohn, J. S. Millar, and E. J. Schaeffer. 1990. Measurement of very low density lipoprotein apolipoprotein (apo) B-100 and high density lipoprotein apoA-I production in human subjects using deuterated leucine. Effect of fasting and feeding. *J. Clin. Invest.* **85:** 804–811.
33. Lichtenstein, A. H., J. S. Cohn, D. L. Hachey, J. S. Millar, J. M. Ordovas, and E. J. Schaefer. 1990. Comparison of deuterated leucine, valine and lysine in the measurement of human apolipoprotein A-I and B-100 kinetics. *J. Lipid Res.* **31:** 1693–1701.
34. Steinmetz, A., V. Clavey, N. Vu-Dac, H. Kaffarnic, and J. C. Fruchart. 1989. Purification of human apolipoprotein A-IV by fast protein liquid chromatography. *J. Chromatogr.* **487:** 154–160.
35. Brewer, H. B., Jr., T. Fairwell, A. Larue, A. Houser, and T. J. Bronzert. 1978. The amino acid sequence of human apoA-I, an apolipoprotein isolated from high density lipoproteins. *Biochem. Biophys. Res. Commun.* **80:** 623–630.
36. Rader, D. J., G. Castro, L. A. Zech, J. C. Fruchart, and H. B. Brewer, Jr. 1991. In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I, A-II. *J. Lipid Res.* **32:** 1849–1859.
37. Rosseneu, M., G. Michiels, W. de Keersgieter, J. Bury, J. P. de Slypere, H. Dieplinger, and G. Utermann. 1988. Quantification of human apolipoprotein A-IV by "sandwich" type enzyme-linked immunosorbent assay. *Clin. Chem.* **34:** 739–743.
38. Warnick, G. R., M. C. Cheung, and J. J. Albers. 1979. Comparison of current methods for high density lipoprotein cholesterol quantitation. *Clin. Chem.* **25:** 596–604.
39. Menzel, H. J., E. Boerwinkle, S. Schrangl-will, and G. Utermann. 1988. Human apolipoprotein A-IV polymorphism frequency and effect on lipid and lipoprotein levels. *Hum. Genet.* **79:** 368–372.
40. Foster, D. M., P. H. R. Barrett, G. Toffolo, W. F. Beltz, and C. Cobelli. 1993. Estimating the fractional synthetic rate of

plasma apolipoproteins and lipids from stable isotope data. *J. Lipid Res.* **34:** 2193–2205.
41. Malmendier, C. L., J. F. Lontie, L. Lagrost, C. Delcroix, D. Y. Dubois, and P. Gambert. 1991. In vivo metabolism of apolipoproteins A-IV and A-I associated with high density lipoprotein in normolipidemic subjects. *J. Lipid Res.* **32:** 801–808.
42. Ohta, 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.
43. 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.
44. Ohta, T., N. H. Fidge, and P. J. Nestel. 1984. Characterization of apolipoprotein A-IV complexes and A-IV isoforms in human lymph and plasma lipoproteins. *J. Biol. Chem.* **259:** 14888–14893.
45. Janus, E. D., A. M. Nicoll, P. R. Turner, P. Magill, and B. Lewis. 1980. Kinetic bases of the primary hyperlipidaemias: studies of apolipoprotein B turnover in genetically defined subjects. *Eur. J. Clin. Invest.* **10:** 161–172.
46. Kesaniemi, Y. A., and S. M. Grundy. 1984. Dual defect in metabolism of very low density lipoprotein triglycerides. Patients with type 5 hyperlipoproteinemia. *J. Am. Med. Assoc.* **251:** 2542–2527.
47. Sane, T., and E. A. Nikkilä. 1988. Very low density lipoprotein triglyceride metabolism in relatives of hypertriglyceridemic probands. *Arteriosclerosis.* **8:** 217–226.
48. Miyata, Y., S. Koga, and H. Ibayashi. 1986. Alterations in plasma levels of apolipoprotein A-IV in various clinical entities. *Gastroenterol. Jpn.* **21:** 479–485.
49. Tall, A. R. 1993. Plasma cholesteryl ester transfer protein. *J. Lipid Res.* **34:** 1255–1274.
50. De Knijff, P., M. Rosseneu, U. Beisiegel, W. De Keersgieter, R. R. Frants, and L. M. Havekes. 1988. Apolipoprotein A-IV polymorphism and its effect on plasma lipid and apolipoprotein concentrations. *J. Lipid Res.* **29:** 1621–1627.