In vivo metabolism of apolipoproteins A-IV and A-I associated with high density lipoprotein in normolipidemic subjects

Claude L. Malmendier,[1,*] Jean-François Lontie,[*] Laurent Lagrost,[†] Claude Delcroix,[*] D. Y. Dubois,[*] and Philippe Gambert[†]

Fondation de Recherche sur l'Athérosclérose,[*] Brussels, Belgium, and Laboratoire de Biochimie Médicale,[†] Faculté de Médecine, Dijon, France

Abstract The kinetics of apolipoprotein A-IV associated with high density lipoproteins (HDL) of plasma from fasting human subjects was followed for 15 days in five healthy normolipidemic volunteers. Purified apoA-IV and apoA-I were radioiodinated, respectively, with ^{125}I and ^{131}I, incubated in vitro with normal HDL, isolated at density 1.250 g/ml, and finally reinjected intravenously as HDL-^{125}I-labeled apoA-IV and HDL-^{131}I-labeled apoA-I. Blood samples were withdrawn at regular intervals for 15 days, and 24-h urine samples were collected. More than 93% (93.5 ± 0.9%) of apoA-IV was recovered in apoA-I-containing lipoprotein particles after affinity chromatography on an anti-apoA-I column and 69.7 ± 4.8% was bound to apoA-II in apoA-I:A-II particles separated on an anti-apoA-II column. ^{125}I-labeled apoA-IV showed a much faster decay than ^{131}I-labeled apoA-I for the first 5 days and thereafter the curves became parallel. Urinary/plasma ratios (U/P) for the ^{125}I-labeled apoA-IV were much higher than those for ^{131}I-labeled apoA-I for the first days, but the U/P curves became parallel for the last 7 days, suggesting heterogeneity of apoA-IV metabolism. A heterogeneous multicompartmental model was constructed to describe the metabolism of lipoprotein particles containing apoA-IV and apoA-I and to calculate the kinetic parameters, fitting simultaneously all plasma and urine data for both tracers. Fractional catabolic rates (FCR) and production rates (PR) of both apolipoproteins were also calculated from the model. This study demonstrates that apoA-IV is present in at least two populations of HDL with very different turnover rates. The FCR of apoA-IV was four times greater than that of apoA-I, whereas PR was about four times smaller. —**Malmendier, C. L., J-F. Lontie, L. Lagrost, C. Delcroix, D. Y. Dubois, and P. Gambert**. In vivo metabolism of apolipoproteins A-IV and A-I associated with high density lipoprotein in normolipidemic subjects. *J. Lipid Res.* 1991. **32:** 801–808.

Supplementary key words FPLC gel filtration • compartmental model • affinity chromatography

ApoA-IV, a protein of 46,000 daltons, is of potential physiological interest. It has been implicated in LCAT activation (1), in the HDL conversion process (2, 3), and in reverse cholesterol transport (4). As secreted partly in the small intestine into chylomicrons, apoA-IV is also postulated to play an important role in triglyceride transport (5). Despite these postulated major functions in lipoprotein metabolism, its in vivo metabolism in humans has been studied in a limited number of subjects after injection in different forms (5, 6). Triglyceride-rich lipoproteins (TRL: chylomicrons and intestinal VLDL) may be the major source of apoA-IV in HDL by transfer from their surface during lipolysis (7). This dissociation of apoA-IV from TRL surface may be a primary consequence of an exchange with apoC transferred from HDL (8). Only recently apoA-IV has been shown to be mainly associated with HDL in fasting serum (9). This justifies the use of apoA-IV associated with HDL in the present in vivo study. In this respect, apoA-IV linked to HDL appeared to be a good marker for studying apoA-IV metabolism in vivo. As apoA-I is the major component of HDL fraction, the study was designed to compare HDL-apoA-IV metabolism to that of HDL-apoA-I.

MATERIALS AND METHODS

Isolation and purification of apolipoproteins

Human apoA-I was isolated from delipidated HDL and fractionated by column chromatography. The purity was confirmed by polyacrylamide gel electrophoresis, amino acid composition, and combined immunodiffusion/im-

Abbreviations: apo, apolipoprotein; LCAT, lecithin:cholesterol acyltransferase; HDL, high density lipoproteins; EDTA, ethylenediamine tetraacetic acid; TG, triglycerides; TC, total cholesterol; PL, phospholipids; LDL-C, LDL-cholesterol; TRL, triglyceride-rich lipoproteins; ELISA, enzyme-linked immunosorbent assay; FPLC, fast protein liquid chromatography; FCR, fractional catabolic rate; PR, production rate; PV, plasma volume; LFF, lipoprotein-free fraction; LDF, lipoprotein-deficient fraction; VHDL, very high density lipoproteins.

[1]To whom reprint requests should be addressed at: Fondation de Recherche sur l'Athérosclérose, Fac. Médecine, Bât D., 2 rue Evers, 1000 Brussels, Belgium.

munoelectrophoresis (10). ApoA-IV was extracted from human serum using a lipid emulsion (Intralipid) and purified by preparative electrophoresis as previously described (11).

Labeling of apolipoproteins A-I and A-IV

ApoA-I and apoA-IV were labeled, respectively, with ^{131}I and ^{125}I by a modification of the iodine monochloride method (12). Briefly, the apolipoproteins were iodinated with an efficiency of 50% with incorporation of approximately 0.5 mole of iodine per mole of apolipoprotein. Unbound iodine was removed by dialysis.

HDL purification

High density lipoproteins were isolated from 70 ml of normal plasma by ultracentrifugation at d 1.250 g/ml, followed by chromatography on Bio-Gel A-5m agarose column (2.6 × 100 cm) (13).

Reassembly of labeled apoproteins with HDL

One mg of apoA-IV (500 μCi) and 1 mg apoA-I (500 μCi) were incubated separately with HDL for 60 min at 37°C (14). The material was ultracentrifuged at d 1.250 g/ml and then dialyzed for 24 h against 0.15 M NaCl, 0.01% EDTA, and 0.01% merthiolate, pH 7.4. The sample was sterilized by filtration through a 0.22-μm Millipore filter. Each preparation was tested for pyrogens prior to injection.

Subjects

Five normal volunteers (four male and one female) between the ages of 21 and 30 years participated in the study. **Table 1** gives the clinical data of the subjects. All had normal thyroid, cardiovascular, liver, renal, and endocrine functions and received no drugs known to alter lipid metabolism. They were placed on an isocaloric diet containing 15% protein, 45% carbohydrate, and 40% fat (P/S 0.5) and 300 mg cholesterol per day for 10 days before beginning the metabolic studies. Three days before injection, the subjects were started on potassium iodide (500 mg/day) in divided doses. The study protocol was approved by the ethical committee of the Faculty of Medicine and informed consent was obtained from each volunteer.

Metabolic studies

After an overnight fast, the five subjects were injected intravenously with 40 μCi of ^{125}I-labeled apoA-IV and 40 μCi of ^{131}I-labeled apoA-I. The study was performed for 16 days. Blood samples were drawn into tubes containing 0.1% EDTA at 10 and 30 min and at 1, 2, 4, 8 h, and then every morning for up to 16 days after an overnight fast. Urine collections were made from 0 to 4, 4 to 8, and 8 to 24 h after the injection and thereafter for each 24-h period for 15 days, in jars containing a preservative (15). The radioactivities of ^{131}I and ^{125}I in plasma and lipoprotein subfractions were quantitated in a gamma counter (Beckman 5500).

Lipid, lipoprotein, and apolipoprotein analyses

Plasma triglyceride (TG), cholesterol (TC), and phospholipid (PL) analyses were performed by the methods of the Lipid Research Clinics (16). HDL-cholesterol was determined in plasma after precipitation with phosphotungstic acid and magnesium chloride (17), and LDL-cholesterol (LDL-C) was calculated from the formula of Friedewald, Levy, and Fredrickson (18). Apolipoproteins A-I and A-II (19), B(20), C-II and C-III (21, 22) in the plasma and in the affinity column chromatography fractions were determined by sandwich ELISA. Apolipoprotein A-IV was measured by competition ELISA (10).

Gel permeation chromatography

One hundred microliters of plasma was fractionated by gel permeation chromatography (23) in an FPLC system (Pharmacia) using a 30-cm Superose 12-HR column with an eluting buffer of 0.15 M NaCl, 0.01% EDTA, pH 7.4, as previously described (11). The flow rate was 12 ml/h and 0.2-ml fractions were collected.

Affinity chromatography

Purified polyclonal anti-apoA-I and anti-apoA-II antibodies were covalently bound to CNBr-activated Sepharose 4B according to the manufacturer's instructions (Pharmacia, Uppsala, Sweden). Plasma (250 μl) was applied to this column and eluted with 0.1 M borate, 0.5 M NaCl, 0.1% EDTA, pH 8.0. The retained fractions were desorbed with 3 M sodium thiocyanate and the column

TABLE 1. Clinical data

Subject	Sex	Age	Weight	Height	TC	TG	HDL-C	PL	LDL-C	A-I	A-II	B	C-II	C-III	A-IV
		yr	*kg*	*cm*	*mg/dl (means ± SD for n = 5)*										
BO	F	28	63	165	170 ± 4	72 ± 20	48 ± 5	177 ± 9	108 ± 8	155 ± 7	38 ± 2	68 ± 7	3.5 ± 0.7	6.7 ± 1.2	10.4 ± 2.1
JM	M	30	58	169	212 ± 9	82 ± 11	53 ± 4	206 ± 12	143 ± 8	172 ± 7	44 ± 2	88 ± 4	3.3 ± 0.4	9.5 ± 0.9	9.9 ± 1.7
VW	M	21	64	182	136 ± 7	73 ± 24	50 ± 3	169 ± 10	71 ± 6	158 ± 7	36 ± 1	47 ± 3	1.7 ± 0.2	6.7 ± 0.9	9.1 ± 1.3
BU	M	23	81	182	116 ± 6	44 ± 8	38 ± 3	131 ± 7	69 ± 5	126 ± 9	29 ± 3	46 ± 8	1.6 ± 0.2	4.8 ± 0.5	6.7 ± 1.2
DE	M	25	61	175	142 ± 6	84 ± 14	48 ± 2	176 ± 5	75 ± 8	182 ± 7	45 ± 2	46 ± 6	3.3 ± 0.6	8.7 ± 0.9	10.1 ± 2.9

was reequilibrated with 0.1 M borate, 0.1 M NaCl, 0.1% EDTA, pH 8.0, (10 column volumes) (24). The cleaning procedure as recommended by Pharmacia was performed every 10 runs. ^{125}I and ^{131}I were counted in the unretained and retained fractions.

Compartmental model

A compartmental model was constructed to fit simultaneously plasma radioactivity decay curves and urinary excretion curves of both tracers using the SAAM program (25). The plasma volume was determined by dividing the total quantity of radioactivity injected by the radioactivity per unit volume determined in samples obtained 10 min after injection of the tracers. Each compartment represented by a circle (see Fig. 4) is a homogeneous, distinct pool of material. Arrows designate material flowing in or out of compartments. Each arrow is associated with a fractional rate of flow of material (rate constant L).

RESULTS

Binding of labeled apoA-IV to HDL

Labeled apoA-IV (93.5 ± 0.9%) was bound to apoA-I-containing particles in the fresh samples separated by affinity chromatography on an anti-apoA-I column within less than 5 h after sampling. When separated by affinity chromatography on an anti-apoA-II column, 69.7 ± 4.8% of labeled apoA-IV appeared to be bound to apoA-II-containing particles (LP A-I:A-II particles) in normal human subjects. Aging of the sample (more than 24 h) induced only a small decrease (less than 8%) in the percentage of apoA-IV bound to particles containing apoA-I without apoA-II.

Changes in apoA-IV plasma distribution

Gel permeation chromatography illustrated in **Fig. 1A and B** shows the following. *1*) Labeled apoA-I is distri-

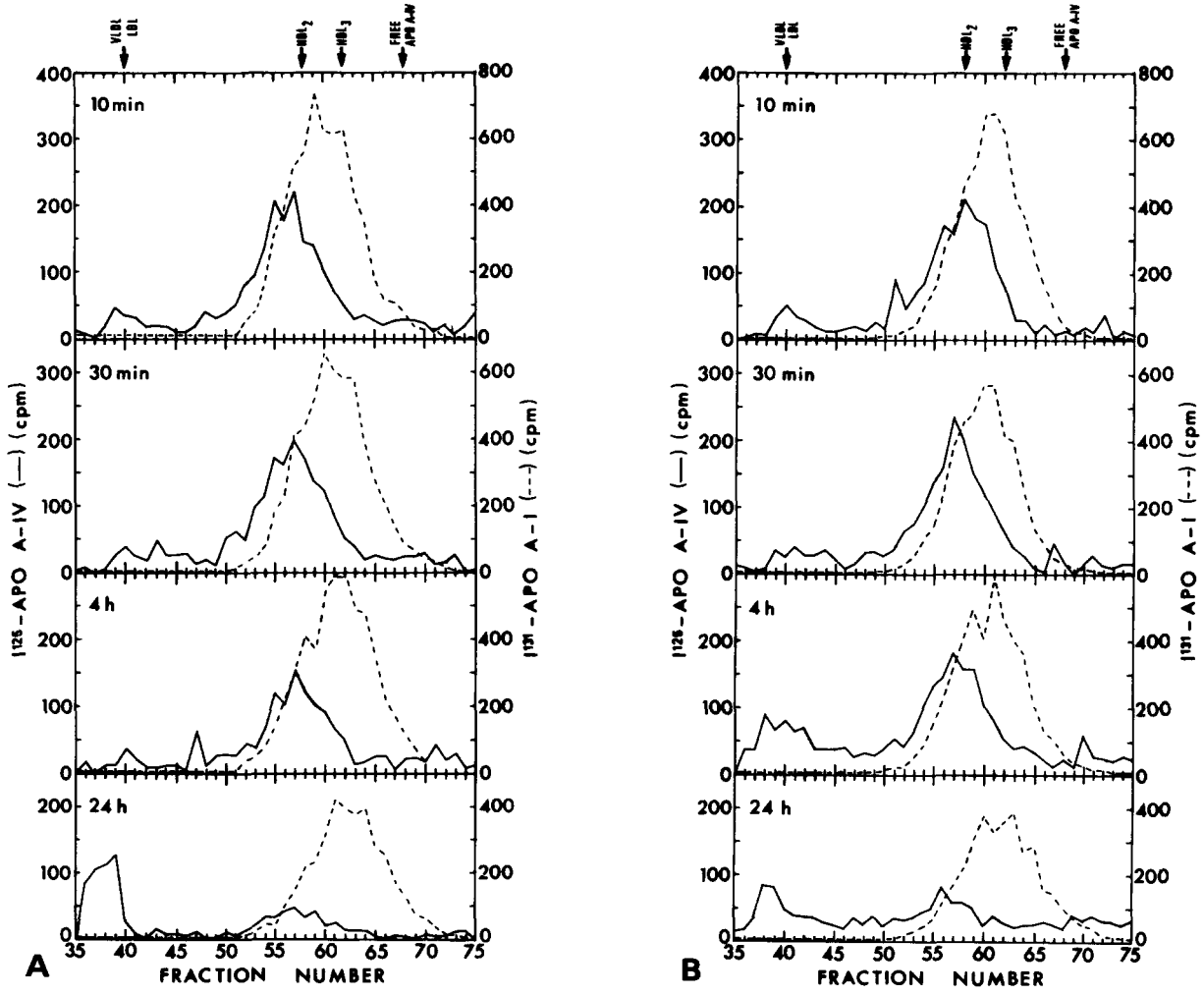

Fig. 1. Changes in the profiles of ^{125}I-labeled apoA-IV and ^{131}I-labeled apoA-I radioactivity in plasma of two normal subjects (BO, A, left and JM, B, right). Elution profiles at 10 and 30 min and 4 and 24 after intravenous injection. One hundred μl of total plasma was fractionated by gel chromatography (FPLC, Pharmacia) on a Superose 12-HR column. The flow rate was 12 ml/h and 0.2-ml fractions were collected. Radioactivities of ^{125}I (——) and ^{131}I (----) were measured simultaneously in each eluted fraction. The arrows indicate the elution of VLDL, LDL, HDL_2, HDL_3, and lipoprotein-free fractions.

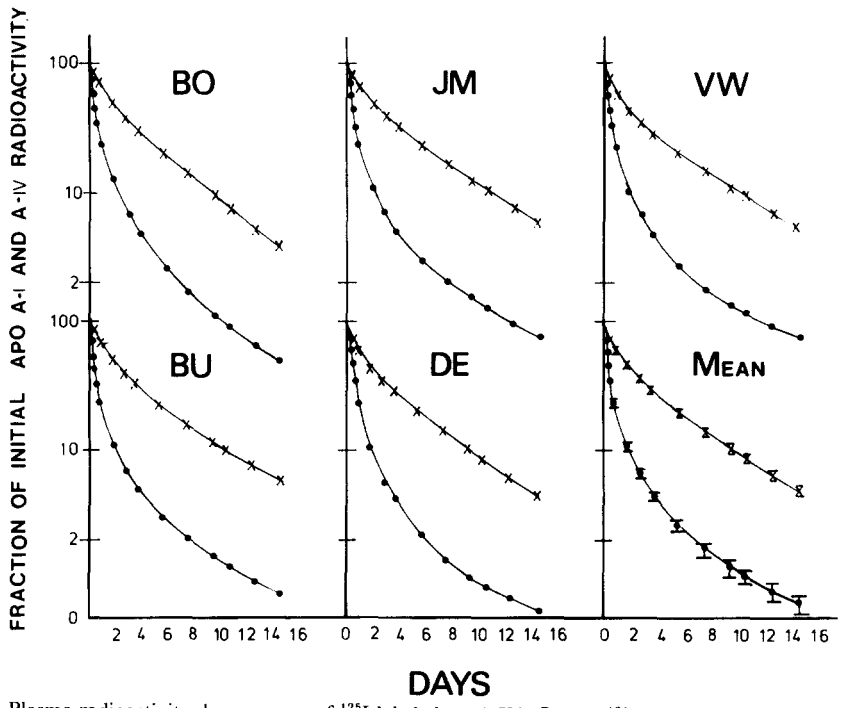

Fig. 2. Plasma radioactivity decay curves of ^{125}I-labeled apoA-IV (●) and ^{131}I-labeled apoA-I (x) in five normal volunteers. The curves at bottom right are the mean plasma percentages ± SD. The radioactivity was normalized to 1 at the first time point.

buted in HDL_2- and HDL_3-containing fractions whereas labeled apoA-IV appears mainly in large HDL_2 particles. *2*) The radioactivity in the free apoA-IV-containing fraction is very low (less than 3%). *3*) The radioactivity of apoA-IV from 10 min to 24 h after injection decreases much faster than radioactivity of apoA-I.

ApoA-IV and A-I metabolism

Plasma radioactivity decay curves are shown in **Fig. 2** and urine/plasma radioactivity ratios (U/P) in **Fig. 3**. Initially, the decrease of ^{125}I-labeled apoA-IV radioactivity was much faster than that of ^{131}I-labeled apoA-I radioac-

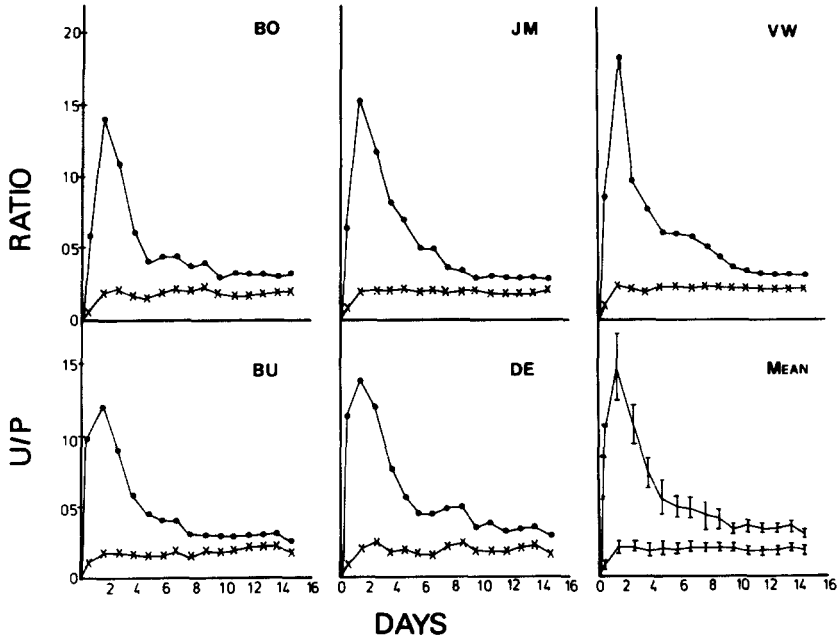

Fig. 3. Urine/plasma radioactivity ratio (U/P) curves for apolipoproteins A-I (x) and A-IV (●). The curves at bottom right are the mean U/P ratios ± SD.

tivity, but from approximately day 6 both curves became parallel. The U/P ratio of apoA-IV was much higher than that of apoA-I, revealing a peak greater than 1.0 at day 2 compared to about 0.25 for apoA-I, but from day 8 to day 16, the U/P ratio curve for apoA-IV paralleled that of apoA-I.

Model construction

Fig. 2 shows that the terminal slopes of plasma radioactivity curves of apoA-I and apoA-IV were parallel from approximately day 7 to day 16, suggesting that part of apoA-IV may have a common metabolic fate with that of apoA-I.

Using the SAAM program (25) and nomenclature (26), the compartmental model shown in **Fig. 4** was constructed as follows. First, ^{131}I-labeled apoA-I plasma and urinary data were fitted simultaneously to a variation of the model proposed by Zech et al. (27) when the turnover of the body iodide pool (BI) was fixed to 2/day. All adjustable parameters and initial conditions were determined assigning maximal fractional standard deviation (FSD) of 0.05 for plasma and 0.20 for urine data. This apoA-I subsystem (Fig. 4A) is already heterogeneous with two plasma pools, compartments 1 and 2, the sum of which, denoted by the triangle, is total plasma apoA-I radioactivity. The arrows into the two compartments represent sites of injection of label. This fit allows calculation of FCR and PR for apoA-I.

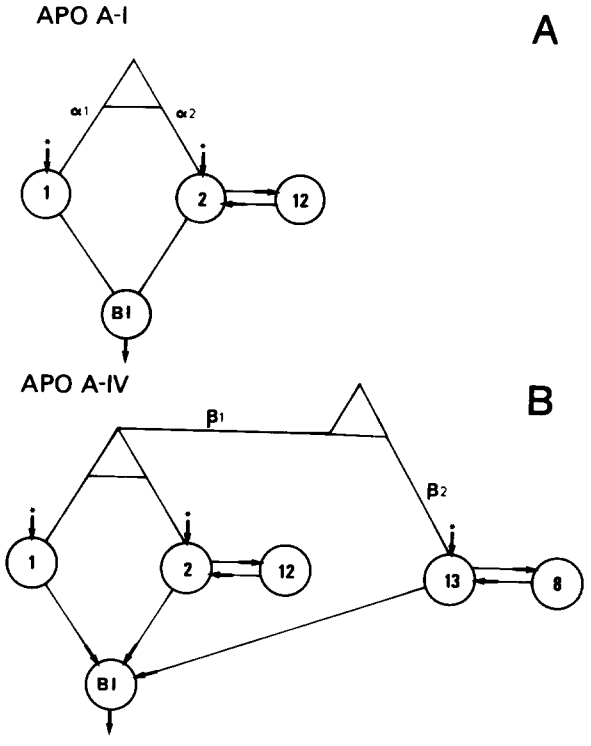

Fig. 4. A: Multicompartmental model used to describe apoA-I metabolism. In this heterogeneous model, compartments 1 and 2 represent plasma; compartment 12 is extraplasmic. Compartment labeled BI represents the body iodide pool turning over at a rate of 2/day. Arrows with an asterisk indicate introduction of labeled material into plasma (α_1 and α_2) in the apoA-I subsystem. B: Multicompartmental model used to describe apoA-IV metabolism. Compartments 1, 2, and 13 represent plasma; compartments 12 and 8 are extraplasmic. ApoA-IV consists of a fraction (β_1) that follows the apoA-I kinetic behavior, and a fraction (β_2) specific for apoA-IV.

Second, imposing the complete apoA-I subsystem with all rate constants fixed, the ^{125}I-labeled A-IV plasma and urinary data were fitted simultaneously with the same constraints as for apoA-I subsystem. The apoA-IV model was constructed as illustrated in Fig. 4B. ApoA-IV radioactivity is indicated by the triangle at the right. The fraction that has the same kinetics as apoA-I is indicated by the line joining this triangle with the triangle representing apoA-I. To fit the data it was necessary to add a plasma compartment 13 that exchanges with compartment 8.

Table 2 gives the rate constants calculated from the model shown in Fig. 4A and B. The individual variations between the five subjects are very small for the major parameters ($L_{I,1}$, $L_{I,2}$, and $L_{I,13}$). $L_{I,13}$ (for apoA-IV) is about eightfold higher than either $L_{I,1}$ or $L_{I,2}$. About 13% of apoA-IV (β_1) followed the fate of the apoA-I subsystem (54% of this percentage or 7.3% for one pathway, and 46% or 6.1% for the other pathway) and 87% (β_2) that of the other specific pathway for apoA-IV.

Table 3 gives the kinetic parameters (FCR and PR) of HDL apolipoproteins A-I and A-IV. $FCR_{A\text{-}I}$ is not the arithmetic sum of $L_{I,1}$ and $L_{I,2}$ but a combination of different proportions (α_1 and α_2) of the two particles. The calculation of $FCR_{apo\ A\text{-}I}$ is obtained using the formula:

$$FCR_{A-I} = 1/[\alpha_1/L_{I,1} + \alpha_2/L_{I,2}] \qquad Eq.\ 1)$$

The same is true for FCR of apoA-IV which is a combination of a fraction (β_1) following the fate of apoA-I and a fraction (β_2) specific for apoA-IV.

$$FCR_{A-IV} = 1/[\beta_2/L_{I,13} + \beta_1/FCR_{A-I}] \qquad Eq.\ 2)$$

FCR of apoA-IV is more than fourfold that of apoA-I. Production rates are calculated as follows:

$$PR_{A-I} = FCR_{A-I} \times M \qquad Eq.\ 3)$$

and

$$PR_{A-IV} = FCR_{A\text{-}I} \times M \times (\beta_1) + L_{I,13} \times M \times \beta_2 \qquad Eq.\ 4)$$

where M = PV × concentration per ml. Production rate of apoA-I is about 4 times that of apoA-IV.

TABLE 2. Rate constants (in units day^{-1}) of apolipoprotein A-I and A-IV metabolism calculated from the model illustrated in Fig. 4

Subject	$L_{1,1}$	$L_{1,2}$	$L_{2,12}$	$L_{12,2}$	$L_{1,13}$	$L_{13,8}$	$L_{8,13}$	ApoA-I $\alpha_1{}^a$	ApoA-I $\alpha_2{}^a$	ApoA-IV $\beta_1{}^b$	ApoA-IV $\beta_2{}^b$
BO	0.187 (8)	0.134 (20)	0.007 (63)	0.692 (22)	1.387 (4)	0.431 (13)	0.170 (18)	56.1 (8)	43.9 (10)	13.0	87.0 (3)
JM	0.170 (3)	0.170 (13)	0.039 (26)	0.559 (11)	1.413 (4)	0.747 (13)	0.378 (18)	59.0 (5)	41.0 (7)	13.4	86.6 (60)
VW	0.153 (3)	0.231 (22)	0.0007 (90)	1.011 (2)	1.591 (4)	0.400 (15)	0.165 (20)	51.6 (3)	48.4 (3)	13.4	86.6 (3)
BU	0.145 (6)	0.144 (28)	0.0007 (95)	0.707 (17)	1.242 (3)	0.317 (17)	0.109 (20)	48.6 (6)	51.4 (6)	14.8	85.2 (3)
DE	0.177 (3)	0.154 (22)	0.016 (9)	1.215 (14)	1.409 (4)	0.511 (13)	0.207 (16)	56.9 (3)	43.1 (4)	12.3	87.7 (3)
Mean ± SD	0.166 ± 0.017	0.167 ± 0.038	0.0127 ± 0.016	0.837 ± 0.269	1.408 ± 0.124	0.481 ± 0.164	0.206 ± 0.064	54.4 ± 4.2	45.6 ± 4.2	13.4 ± 0.9	86.6 ± 0.9

The values between brackets are percentages of errors.
[a]Percentage of the sum of the total apoA-I pool.
[b]Percentage of the sum of the total apoA-IV pool.

DISCUSSION

Inasmuch as apoA-IV has been recently demonstrated to be associated mainly with HDL particles labeled apoA-IV was injected as an HDL-associated apolipoprotein to compare its metabolism with that of HDL-apoA-I, a classical marker of HDL metabolism.

Our results showed that apoA-IV radioactivity located in HDL in the tracer material remains associated in vivo to HDL as demonstrated by FPLC gel filtration chromatography. FPLC showed that even 24 h after injection there was no significant amount of free apoA-IV radioactivity. Furthermore, affinity chromatography showed that in freshly treated plasma samples apoA-IV was almost completely (more than 90%) bound to apoA-I-containing particles and 70% in LPA-I:A-II particles. The persistence of radioactivity in HDL is in good agreement with the results previously reported (6) and confirms the previously shown distribution of apoA-IV in HDL (9). The fact that radiolabeled apoA-IV when injected in association with HDL remains with the originally injected fraction (5, 6) validates the use of HDL/A-IV as a major marker of apoA-IV metabolism and justifies the usefulness of a careful comparison with the metabolism of apoA-I.

As we know that the apoA-I radioactivity decay curve is described by three exponentials, the duration of the experiment was fixed to 16 days, allowing time to determine with precision the third and slowest component of the decay curve. Kinetic parameters calculated for apoA-I/HDL are in the same order of magnitude as those previously reported (10, 27). The parallelism of the terminal slope of plasma decay curves of both apoA-I and apoA-IV led us to test a model describing a partial common metabolism between these two apolipoproteins. Moreover, the existence of a high and broad early peak in the apoA-IV U/P curve compared to that of apoA-I indicated heterogeneity of apoA-IV metabolism. The proposed model was constructed by simultaneously fitting the plasma and urinary data with a high confidence level (95–96% for the FCR). The estimation of metabolic parameters for apoA-IV necessitates the application of model equations that combine the participation of at least three distinct populations of particles in the HDL density range with very different turnover rates. From these three populations, two (respectively, 7.3 and 6.1%) are apoA-I-containing whereas 86.6% are not. The results obtained by FPLC and affinity chromatography, which show that more than 90% of apoA-IV radioactivity was associated with HDL-apoA-I, imply that the "apparently" indepen-

TABLE 3. Kinetic parameters of apolipoproteins A-I and A-IV metabolism

Subject	Apolipoprotein A-I FCR	Apolipoprotein A-I PR	Apolipoprotein A-IV FCR	Apolipoprotein A-IV PR
BO	0.159	10.18	0.692	2.97
JM	0.170	12.07	0.713	2.91
VW	0.183	11.94	0.739	2.78
BU	0.144	7.49	0.584	1.62
DE	0.166	13.98	0.779	3.25
Mean ± SD	0.164 ± 0.014	11.13 ± 2.44	0.701 ± 0.073	2.71 ± 0.63

Fractional catabolic rates (FCR) are given in pools/day and production rates (PR) in mg/kg · day.

dent HDL-apoA-IV population contains a small amount of apoA-I. Because of the low apoA-IV to apoA-I molar ratio in the plasma, this amount must represent a small percentage of apoA-I radioactivity kinetically indistinguishable from the bulk of apoA-I radioactivity.

Inasmuch as apoA-IV is considered as an apolipoprotein (a major one of chylomicrons) (7), and it is not contested that even in lipoprotein-deficient fractions (LDF) or plasma lipoprotein-free fractions (LFF) apoA-IV exists in discrete lipid-protein complexes (5, 6, 28), and more than 90% is bound to apoA-I, implying that it exists in total fasting plasma in the form of a lipoprotein particle, LDF or LFF should not be considered as lipoprotein-free. In fact, lipid-free apoA-IV does not seem to exist physiologically. The HDL fraction used in the present experiments contained lipoproteins floating at d 1.25 g/ml, i.e., HDL ($d < 1.21$ g/ml) and VHDL ($1.21 < d < 1.25$ g/ml). By ultracentrifugation, apoA-IV is stripped off from this HDL + VHDL fraction by the combined effects of high ionic strength and high sheering forces (29). Neither FPLC nor affinity chromatography indicated the existence of a lipid-free fraction in vivo. Our kinetics concerned only the sum of HDL-A-IV and LDF-A-IV, with TRL-A-IV being excluded since no return of radioactivity could be detected from these latter pools.

When we compare parameter values to those of previous in vivo studies, there are essentially no large discrepancies. *a*) HDL-apoA-IV (6) had an FCR of 0.621 pools/day. That this value is slightly lower than the mean value (0.701) for the A-IV FCR calculated from the formula described in the "Model construction" section may be explained by the procedure used in the separation (column chromatography), the "so-called" HDL-A-IV (6) already containing a significant (but not all) amount of LFF-apoA-IV. *b*) LFF-A-IV (6) had an FCR of 1.81 pools/day, a value close to the FCR (1.41) of our apoA-IV independent pathway (β_2). The difference is attributable to the less precise mode of calculation used by Ghiselli et al. (6)—integration of the area under the curve was by a graphic procedure and not by computer fitting—and to the shorter duration of their kinetic experiment (4 instead of 16 days).

Thus far five isoproteins have been described for apoA-IV, the most common being A-IV-1 (30). We do not know if there are differences in catabolism between these variants as apoA-IV has not been phenotyped in the present study. However, considering the large predominance of apoA-IV-1 in the population and the very small individual variations in the five subjects studied, the experimental results probably reflect the metabolism of this isoprotein.

Two hypotheses may thus explain the high turnover rate of apoA-IV-containing particles compared to apoA-I. *a*) The rapid catabolism could be mediated by a highly selective and efficient binding and uptake mechanism similar to that described in rat hepatic tissue (31–33); as suggested by Ghiselli, Crump, and Gotto (31) apoA-IV could be catabolized without the whole HDL particle being degraded. *b*) Since the recent work of Bisgaier, Siebenkas, and Williams (34) does not support a direct role for apoA-IV or apoA-I in mediating the uptake of HDL by the liver, the high metabolic rate of apoA-IV-containing particles could be due mainly to structural features, possibly indirectly linked to the presence of apoA-IV.

In conclusion, this study demonstrates that the major part of apo-A-IV is localized in two populations of HDL particles with very different turnover rates. The faster catabolism of the major part of apoA-IV could emphasize the importance of the postulated role of apoA-IV in reverse cholesterol transport.

SAAM/CONSAM computer programs were kindly provided by the US PHS/NIH/DRR-NHBLI-NCI joint development project.

Manuscript received 6 September 1990 and in revised form 26 December 1990.

REFERENCES

1. Steinmetz, A., and G. Utermann. 1985. Activation of lecithin:cholesterol acyltransferase by human apolipoprotein A-IV. *J. Biol. Chem.* **260:** 2258–2264.
2. Barter, P. J., O. V. Rajaram, L. B. F. Chang, K. A. Rye, P. Gambert, L. Lagrost, C. Ehnholm, and N. H. Fidge. 1988. Isolation of a high density lipoprotein conversion factor from human plasma. A possible role of apolipoprotein A-IV as its activator. *Biochem. J.* **254:** 179–184.
3. 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.
4. 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 an ether analog of phosphatidylcholine. *Biochim. Biophys. Acta.* **878:** 7–13.
5. 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.
6. 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.
7. Green, P. H. R., R. M. Glickman, J. W. Riley, and E. Quinet. 1980. Human apolipoprotein A-IV. Intestinal origin and distribution in plasma. *J. Clin. Invest.* **65:** 911–919.
8. Weinberg, R. B., and M. S. Spector. 1985. Human apolipoprotein A-IV: displacement from the surface of triglyceride-rich particles by HDL_2-associated C-apoproteins. *J. Lipid Res.* **26:** 26–37.
9. 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.

10. Malmendier, C. L., C. Delcroix, and J. P. Ameryckx. 1983. In vivo metabolism of human apoprotein A-I-phospholipid complexes. Comparison with human high density lipoprotein-apoprotein A-I metabolism. *Clin. Chim. Acta.* **131:** 201–210.
11. 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.
12. Shepherd, J., A. M. Gotto, O. D. Taunton, M. J. Caslake, and E. Farish. 1977. The in vitro interaction of human apolipoprotein A-I and high density lipoproteins. *Biochim. Biophys. Acta.* **489:** 486–501.
13. Bedford, D. K., J. Shepherd, and H. G. Morgan. 1976. Radioimmunoassay for human plasma apolipoprotein B. *Clin. Chim. Acta.* **70:** 267–276.
14. Shepherd, J., J. R. Patsch, C. J. Packard, A. M. Gotto, and O. D. Taunton. 1978. Dynamic properties of human high density lipoprotein apoproteins. *J. Lipid Res.* **19:** 383–389.
15. Steinfeld, J. L., R. R. Paton, A. L. Flick, R. A. Milch, and F. E. Beach. 1957. Distribution and degradation of human serum albumin labeled with ^{131}I by different techniques. *Ann. N. Y. Acad. Sci.* **70:** 109–121.
16. Lipid Research Clinics Program. 1974. Manual of Laboratory Operations. Lipid and Lipoprotein Analysis. Department of HEW Publications. NIH No 75-628, Vol. 1.
17. Lopes-Virella, M. F., P. Stone, S. Ellis, and J. A. Colwell. 1977. Cholesterol determination in high-density lipoproteins separated by three different methods. *Clin. Chem.* **23:** 882–884.
18. Friedewald, W. T., R. I. Levy, and D. S. Fredrickson. 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.* **18:** 499–502.
19. Dubois, D. Y., F. Cantraine, and C. L. Malmendier. 1986. Comparison of different sandwich enzyme immunoassays for the quantitation of human apolipoproteins A-I and A-II. *J. Immunol. Methods.* **96:** 115–120.
20. Fruchart, J. C., C. Fievet, and P. Puchois. 1985. Apolipoproteins. *In* Methods of Enzymatic Analysis. Vol. VIII. H. U. Bergmeyer, editor. Verlag Chemie, Weinheim. 126–138.
21. Malmendier, C. L., J-F. Lontie, C. Delcroix, D. Y. Dubois, T. Magot, and L. DeRoy. 1989. Apolipoproteins C-II and C-III metabolism in hypertriglyceridemic patients. Effect of a drastic triglyceride reduction by combined diet restriction and fenofibrate administration. *Atherosclerosis.* **77:** 139–149.
22. Malmendier, C. L., J-F. Lontie, G. A. Grutman, and C. Delcroix. 1988. Metabolism of apolipoprotein C-III in normolipemic human subjects. *Atherosclerosis.* **69:** 51–59.
23. Ha, Y. C., and P. J. Barter. 1985. Rapid separation of plasma lipoproteins by gel permeation chromatography on agarose gel Superose 6B. *J. Chromatogr.* **341:** 154–159.
24. McConathy, W. J., E. Koren, H. Wieland, E. M. Campos, D. M. Lee, H. I. Kloer, and P. Alaupovic. 1985. Evaluation of immunoaffinity chromatography for isolating human lipoproteins containing apolipoprotein B. *J. Chromatogr.* **342:** 47–66.
25. Berman, M., and M. F. Weiss. 1978. SAAM Manual. DHEW Publication No (NIH) 78-180.
26. Berman, M. 1979. Kinetic analysis of turnover data. *Prog. Biochem. Pharmacol.* **15:** 67–108.
27. Zech, L. A., E. J. Schaefer, T. J. Bronzert, R. L. Aamodt, and H. B. Brewer. 1983. Metabolism of human apolipoproteins A-I and A-II: compartmental models. *J. Lipid Res.* **24:** 60–71.
28. 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.
29. Kunitake, S. T., and J. P. Kane. 1982. Factors affecting the integrity of high density lipoproteins in the ultracentrifuge. *J. Lipid Res.* **23:** 936–940.
30. Lohse, P., M. R. Kindt, D. J. Rader, and H. B. Brewer, Jr. 1990. Human plasma apolipoproteins A-IV-O and A-IV-3. Molecular basis for two rare variants of apolipoprotein A-IV-1. *J. Biol. Chem.* **265:** 12734–12739.
31. 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.
32. 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.
33. Mitchel, Y. B., V. A. Rifici, and H. A. Eder. 1987. Characterization of the specific binding of rat apolipoprotein E-deficiency HDL to rat hepatic plasma membranes. *Biochim. Biophys. Acta.* **917:** 324–332.
34. Bisgaier, C. L., M. V. Siebenkas, and K. J. Williams. 1989. Effects of apolipoproteins A-IV and A-I on the uptake of phospholipid liposomes by hepatocytes. *J. Biol. Chem.* **264:** 862–866.