In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I,A-II[1]

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Abstract Apolipoprotein (apo) A-I is the major protein in high density lipoproteins (HDL) and is found in two major subclasses of lipoproteins, those containing apolipoprotein A-II (termed LpA-I,A-II) and those without apoA-II (termed LpA-I). The in vivo kinetics of apoA-I on LpA-I and LpA-I,A-II were investigated in normolipidemic human subjects. In the first series of studies, radiolabeled apoA-I and apoA-II were reassociated with autologous plasma lipoproteins and injected into normal subjects. LpA-I and LpA-I,A-II were isolated from plasma at selected time points by immunoaffinity chromatography. By 24 h after injection, only 52.8 ± 1.0% of the apoA-I in LpA-I remained, whereas 66.9 ± 2.7% of apoA-I in LpA-I,A-II remained ($P < 0.01$). In the second series of studies, purified apoA-I was labeled with either ^{131}I or ^{125}I and reassociated with autologous plasma. Isolated LpA-I and LpA-I,A-II particles differentially labeled with ^{131}I-labeled apoA-I and ^{125}I-labeled apoA-I, respectively, were simultaneously injected into study subjects. The plasma residence time of apoA-I injected on LpA-I (mean 4.39 days) was substantially shorter than that of apoA-I injected on LpA-I,A-II (mean 5.17 days), with a mean difference in residence times of 0.79 ± 0.08 days ($P < 0.001$). These data demonstrate that apoA-I injected on LpA-I is catabolized more rapidly than apoA-I injected on LpA-I,A-II. The results are consistent with the concept that LpA-I and LpA-I,A-II have divergent metabolic pathways.—**Rader, D. J., G. Castro, L. A. Zech, J-C. Fruchart, and H. B. Brewer, Jr.** In vivo metabolism of apolipoprotein A-I on high density lipoprotein particles LpA-I and LpA-I, A-II. *J. Lipid Res.* 1991. **32:** 1849–1859.

Supplementary key words cholesterol • kinetics • immunoaffinity • atherosclerosis

Epidemiologic studies have consistently demonstrated that the plasma high density lipoproteins (HDL) are inversely correlated with risk of coronary artery disease (CAD) (1, 2). HDL are highly heterogeneous with respect to the hydrated density, size, and composition of the particles. The two major density subfractions, HDL_2 and HDL_3, have been found to differ in their utility as epidemiologic markers, with the concentration of HDL_2 having a stronger inverse correlation with CAD risk than the concentration of HDL_3 (3). The major apolipoproteins in HDL are apoA-I and apoA-II. The plasma concentration of apoA-I has been shown to have a strong inverse correlation with risk of CAD (3–5), whereas an inverse correlation with apoA-II levels has not been consistently demonstrated (3). It has recently been shown by several laboratories that there are several subclasses of apoA-I-containing particles within HDL (6–12). The two major subclasses include particles that contain both apoA-I and apoA-II (termed LpA-I,A-II) and those that contain apoA-I but not apoA-II (termed LpA-I). Puchois et al. (13), using a differential ELISA to measure the plasma levels of LpA-I,A-II (14) in a cohort of patients with angiographically proven CAD compared with control subjects, found that the presence of CAD was inversely correlated with levels of LpA-I but not LpA-I,A-II. Barbaras et al. (15) demonstrated that incubation of OB1771 cells, a mouse preadipocyte cell line, with LpA-I resulted in marked efflux of cellular cholesterol. In contrast, LpA-I,A-II was ineffective in stimulating cholesterol removal and in fact was found to inhibit the ability of LpA-I to mediate efflux from the cells. A recent case-control study of lipid and lipoprotein parameters in adolescent children of parents with premature CAD compared with matched controls found that the plasma LpA-I level was the single best discriminating factor between cases and controls, whereas the LpA-I,A-II level had no discriminating value (Amouyel, P., D. Isorez, J. M. Bard, M. Goldman, G. Zylberberg, and J. C. Fruchart, unpublished results). This body of data has led to the hypothesis that LpA-I and LpA-I,A-II have different physiologic functions and that LpA-I, but

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; BMI, body mass index; HDL, high density lipoproteins; LpA-I, lipoproteins containing only apoA-I; LpA-I,A-II, lipoproteins containing apoA-I and apoA-II; CAD, coronary artery disease; RT, residence time; FCR, fractional catabolic rate; PBS, phosphate-buffered saline.

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not LpA-I,A-II, is a specific "anti-atherogenic" particle within HDL.

Several investigators have studied the plasma kinetics of apoA-I and apoA-II in humans (16–24). Most of these studies (18–24) have demonstrated a longer plasma residence time for apoA-II than for apoA-I, suggesting divergence of their in vivo metabolism. Furthermore, Zech et al. (25), using compartmental modeling of apoA-I and apoA-II kinetic data, predicted that there are at least two kinetic pools of plasma apoA-I, one of which has a significantly faster rate of catabolism than the other. In contrast, the apoA-II kinetics in these studies could be explained using a single plasma pool.

We hypothesized that the major apoA-I-containing particles LpA-I and LpA-I,A-II may represent kinetically different compartments of apoA-I. We therefore undertook an investigation of the in vivo metabolism of apoA-I on LpA-I and LpA-I,A-II in normolipidemic human subjects.

METHODS

Study subjects

The eight subjects studied were healthy young adults who were admitted as normal volunteers to the Clinical Center of the National Institutes of Health. Their sex, age, body mass index (BMI), and plasma lipid and apolipoprotein values are presented in **Table 1.** Values are the mean of five fasting determinations made while the subjects were on the metabolic diet. All subjects had cholesterol, triglyceride, HDL cholesterol, apoA-I, and apoA-II levels within the normal range for age and sex, and values remained in steady state throughout the course of the study. All subjects had normal fasting glucose levels and normal thyroid, liver, and renal function. They were on no medications and had no illnesses. All subjects gave informed consent and the study protocol was approved by the Clinical Research Subpanel of the National Heart, Lung, and Blood Institute.

General study protocol

Three days before the start of a study, subjects were placed on a controlled isoweight diet containing 42% of calories as carbohydrate, 42% as fat, and 16% as protein, with 200 mg cholesterol per 1000 Kcal, and a polyunsaturated to saturated fat ratio of 0.1 to 0.3. Meals were prepared by the Metabolic Kitchen and were served three times a day; the diet was continued for the duration of the study. Subjects were weighed daily and remained in steady state. One day prior to injection, the subjects were given potassium iodide at a dose of 900 mg per day in divided doses and this was continued for the duration of the study. After a 12-h fast, subjects were injected intravenously with the iodinated lipoproteins. Blood samples were obtained 10 min after injection and then at 1, 3, 6, 12, 24, and 36 h, and at 2, 4, 7, 10, and 14 days. Blood (20 ml) was drawn into tubes containing EDTA at a final concentration of 0.1%, immediately placed at 4°C, and spun at 2300 rpm for 30 min at 4°C. Sodium azide and aprotinin were added to plasma at a final concentration of 0.05% and 200 KIU/ml, respectively. Radioactivity in 5-ml plasma aliquots was quantitated in a Packard Autogamma 5260 gamma counter (Packard Instrument Co., Morton Grove, IL). Plasma decay 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 whole plasma decay curves by using a multiexponential computer curve-fitting technique (26). Fractional catabolic rates (FCR) are the reciprocals of the residence times.

Isolation and iodination of apolipoproteins

ApoA-I and apoA-II were isolated from the HDL of a healthy donor with normal lipid and apolipoprotein levels using gel permeation and ion exchange chromatography as previously described (27, 28) and stored at −20°C.

Purified apolipoproteins were iodinated by a modification of the iodine monochloride method as previously

TABLE 1. Characterization of study subjects

	Subject	Sex	Age	BMI	TG	TC	HDL-C	ApoA-I	ApoA-II
			yr	*kg/m²*			*mg/dl*		
Series I	1	F	24	29.2	141	152	44	126	51.8
	2	M	20	21.8	76	115	33	110	42.5
	3	M	22	22.7	127	167	34	134	34.9
Series II	1	F	20	26.6	56	179	64	160	38.7
	2	F	19	27.9	59	171	46	126	28.2
	3	M	20	23.3	56	153	53	141	36.1
	4	F	20	21.2	27	142	59	149	51.3
	5	F	22	24.4	110	173	38	107	37.3

reported (29). Lyophilized apoA-I and apoA-II were dissolved in a 6 M guanidine-HCl, 1 M glycine (pH 8.5) buffer, 5 mCi of $Na^{125}I$ or $Na^{131}I$ were added, and then iodine monochloride was added during vortexing of the sample. Approximately 0.5 ml iodine was incorporated per mole of apolipoprotein.

Isolation of lipoprotein particles by immunoaffinity chromatography

Monoclonal antibodies against apoA-I and apoA-II were prepared by intraperitoneal immunization of a Balb/C mouse with intact HDL_3 and characterized as previously reported (30). A mixture of three different monoclonal antibodies to apoA-I (A05, A17, and A30) was found to effectively recognize all plasma apoA-I. This mixture was covalently coupled to CNBr-activated Sepharose 4B (Pharmacia, Uppsala, Sweden) as instructed by the manufacturer (5 mg/g gel). A mixture of three different monoclonal antibodies to apoA-II (G03, G05, and G11) was found which effectively recognized all plasma apoA-II. This mixture was coupled to CNBr-Sepharose (5 mg/g gel) in an identical fashion. A polyclonal antiserum against apoB (Boehringer-Mannheim, Mannhein, Germany) was also coupled to CNBr-Sepharose (5 mg/g gel). The immunosorbents for apoA-I, apoA-II, and apoB were packed separately into columns (Kontes, Vineland, NJ) equilibrated with PBS containing 0.05% EDTA, pH 7.4. Columns were tested for specificity and capacity using SDS-PAGE, immunoblotting, and specific immunoassays for apoA-I and apoA-II as described below.

LpA-I,A-II and LpA-I were isolated according to the procedure described by Barkia et al. (31), who used the same mixtures of monoclonal antibodies to apoA-I and apoA-II. All isolations were performed at 4°C. Plasma samples were first chromatographed on an anti-apoB column equilibrated with the PBS/0.05% EDTA buffer at a flow rate of 10 ml/h. Lipoproteins that did not bind to the immunosorbent were eluted with 100 ml of the PBS/EDTA buffer. The plasma fraction not retained by the anti-apoB column was then chromatographed on an anti-apoA-II column at a flow rate of 10 ml/h. Lipoproteins that did not bind were removed with 100 ml of the PBS/EDTA buffer. The retained apoA-II-containing particles (designated LpA-I,A-II) were eluted from the column with 3 M NaSCN at a flow rate of 30 ml/h. The non-retained fraction from the anti-apoA-II column was chromatographed on an anti-apoA-I column at a flow rate of 10 ml/h. Unbound lipoproteins were removed with 100 ml PBS/EDTA. The retained apoA-I-containing particles (designated LpA-I) were eluted from the column with 3 M NaSCN at a rate of 30 ml/h. All non-retained and retained lipoproteins were collected in 5-ml fractions and immediately counted for radioactivity. Total recovery of radioactivity averaged 92 ± 4% of that applied to the columns and was similar for all three immunosorbents. Non-retained fractions were monitored carefully for evidence of overloading. When radiolabeled apoA-II was used (Series I), the non-retained fraction from the anti-apoA-II columns always contained less than 0.5% of the apoA-II tracer applied to the columns. Likewise, the non-retained fraction from the anti-apoA-I columns always contained less than 0.5% of the apoA-I tracer applied to the columns.

Study protocol: Series I

^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II were reassociated with separate aliquots of autologous plasma and dialyzed at 4°C against PBS containing 0.01% EDTA. Human serum albumin was added to a final concentration of 5% (wt/vol). Samples were sterile-filtered through a 0.22 μm Millipore filter and tested for pyrogens and sterility. Plasma samples containing ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II were simultaneously injected into three normal subjects. A 0.5-ml sample of plasma drawn at selected time points after injection was immediately subjected to sequential immunoaffinity chromatography to isolate the LpA-I,A-II and LpA-I particles as described above. The amount of ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II in each particle class was quantitated. There was no ^{131}I-labeled apoA-II found in the LpA-I particle. The radioactivity of each tracer in each particle at the initial 10-min time point was normalized to 1.0 and the fraction of radiolabeled apoA-I and apoA-II remaining in each subclass at each time point was determined. Kinetic parameters of apoA-I and apoA-II were obtained from the 14-day whole plasma decay curves as described above.

Study protocol: Series II

Two identical aliquots of purified apoA-I (100 μg each) were labeled separately with ^{125}I and ^{131}I. ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-I were reassociated with separate aliquots of autologous plasma (0.5 ml) and dialyzed at 4°C for 12 h against four changes of PBS containing 0.01% EDTA. LpA-I,A-II and LpA-I particles differentially labeled with ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-I, respectively, were then isolated at 4°C according to the scheme in **Fig. 1.** Plasma containing ^{125}I-labeled apoA-I was used to isolate LpA-I,A-II particles by fractionation over an anti-apoB affinity column to remove apoB-containing particles, then by chromatographing the non-retained fraction over an anti-apoA-II affinity column. The retained fraction from this column consisted of LpA-I,A-II particles selectively labeled with ^{125}I-labeled apoA-I. Plasma containing ^{131}I-labeled apoA-I was used to isolate LpA-I particles utilizing the same method, except that after chromatography over an anti-apoA-II column to remove all apoA-II-containing particles, the non-retained fraction was chromatographed on an anti-

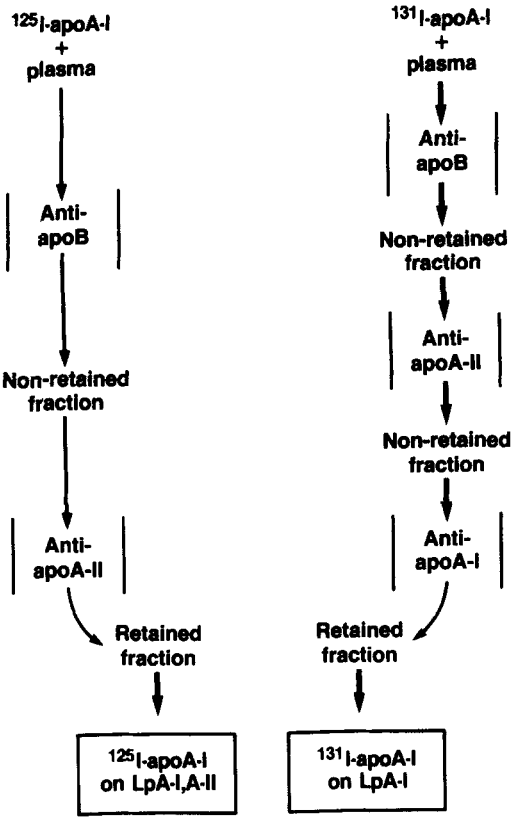

Fig. 1. Schematic diagram of the isolation of ^{131}I-labeled apoA-I-LpA-I and ^{125}I-labeled apoA-I-LpA-I,A-II used in the kinetic studies, Series II.

apoA-I affinity column. The retained fraction from this column contained LpA-I particles selectively labeled with ^{131}I-labeled apoA-I. After elution from their respective columns, both lipoprotein particles were immediately and extensively dialyzed against the PBS/EDTA buffer at 4°C to remove all NaSCN. Human serum albumin was added to a final concentration of 5% (wt/vol). Samples were sterile-filtered through a 0.22 μm Millipore filter and tested for pyrogens and sterility. Labeled particles were used for kinetic studies within 24 h of isolation. Specific activities of the radiolabeled apoA-I in injected samples at time of injection were similar for both particles and ranged from 9.7×10^5 to 4.3×10^6 dpm/μg apoA-I. ^{125}I-labeled apoA-I-LpA-I,A-II (50 μCi) and ^{131}I-labeled apoA-I-LpA-I (25 μCi) were simultaneously injected into the study subjects and blood samples were drawn as described above. Plasma (0.5 ml) from selected time points was fractionated by sequential immunoaffinity chromatography to isolate LpA-I and LpA-I,A-II as described above. Kinetic parameters of apoA-I injected on LpA-I and LpA-I,A-II were obtained from the 14-day whole plasma decay curves as described above.

Ultracentrifugation procedures

VLDL, LDL, HDL_2, and HDL_3 were isolated from 5 ml plasma by sequential ultracentrifugation (32). The radioactivity in both top and bottom fractions after each centrifugation was determined, and the ratio of top/(top + bottom) was used to determine the total radioactivity in each density fraction at each time point.

Analytical methods

Agarose gel electrophoresis was performed using preformed 0.6% agarose plates (Helena Laboratories, Beaumont, TX). Plasma cholesterol and triglycerides were quantitated by automated enzymatic techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol was determined in plasma after dextran sulfate precipitation (33). Plasma apoA-I and apoA-II concentrations were quantitated by ELISA (34, 35). Concentration of plasma apoA-I in LpA-I was measured by differential electroimmunoassay using the method described by Parra et al. (36) with plates prepared in an identical fashion; values are expressed as the apoA-I concentration in LpA-I. The apoA-I concentration in LpA-I,A-II was obtained by subtracting this value from the total plasma apoA-I concentration. Statistical comparisons were made using the Student's t test (Series I) and the paired Student's t test (Series II).

RESULTS

Evaluation of radiolabeled apolipoproteins and lipoproteins

Radiolabeled apoA-I and apoA-II migrated as single bands to the expected positions on SDS-PAGE as assessed by autoradiography of the dried gels. The apoA-I and apoA-II content of preparatively isolated radiolabeled LpA-I and LpA-I,A-II was determined by ELISA, SDS-PAGE, and Western blotting with monospecific antisera. LpA-I contained apoA-I but no detectable apoA-II, whereas LpA-I,A-II contained both apoA-I and apoA-II, in an approximate apoA-I/apoA-II molar ratio of 1.3. Autoradiograms of the nitrocellulose membrane confirmed that the radioactive label was associated exclusively with the apoA-I in both types of lipoprotein particles. Both radiolabeled LpA-I and LpA-I,A-II exhibited alpha mobility on agarose electrophoresis.

Kinetic studies: Series I

The kinetic parameters of radiolabeled apoA-I and apoA-II derived from the full 14-day plasma curves are presented in **Table 2.** In all three subjects, the FCR of apoA-I was substantially faster than that of apoA-II, consistent with previous reports. The turnover of radiolabeled apoA-I and apoA-II within LpA-I and LpA-I,A-II is illustrated in **Fig. 2.** The curves are shown through only 4 days because the radioactivity in isolated particle subfractions by day 7 was too low for reliable quantitation. The turnover rate of ^{125}I-labeled apoA-I in LpA-I was

TABLE 2. Kinetic parameters of apoA-I and apoA-II (Series I)

Subject	ApoA-I		ApoA-II	
	RT	FCR	RT	FCR
	days	d^{-1}	*days*	d^{-1}
1	4.21	0.238	4.87	0.205
2	4.19	0.239	5.17	0.194
3	4.02	0.249	4.95	0.202
Mean ± SEM	4.14 ± 0.06	0.242 ± 0.004	5.00 ± 0.09	0.200 ± 0.003

significantly faster than that of ^{125}I-labeled apoA-I in LpA-I,A-II. At 24 h after injection, an average of 52.8 ± 1.0% of the ^{125}I-labeled apoA-I remained in LpA-I, compared with 66.9 ± 2.7% of the labeled apoA-I remaining in LpA-I,A-II ($P < 0.01$). The turnover rate of ^{131}I-labeled apoA-II in LpA-I,A-II was similar to that of ^{125}I-labeled apoA-I in the same particle, with 65.9 ± 0.2% of the labeled apoA-II remaining at 24 h.

Kinetic studies: Series II

In the second series of studies, isolated LpA-I labeled with ^{131}I-labeled apoA-I and isolated LpA-I,A-II labeled with ^{125}I-labeled apoA-I were simultaneously injected into normal subjects. The plasma curves for all five subjects are illustrated in **Fig. 3.** The residence times of apoA-I injected on LpA-I and LpA-I,A-II were determined directly from the full 14-day plasma decay curves of ^{131}I-labeled apoA-I and ^{125}I-labeled apoA-I, respectively. The kinetic parameters are presented in **Table 3.** In all subjects, the FCR of apoA-I injected on LpA-I was faster than that of apoA-I injected on LpA-I,A-II. The mean difference in residence times of 0.79 ± 0.08 days is highly statistically significant ($P < 0.001$).

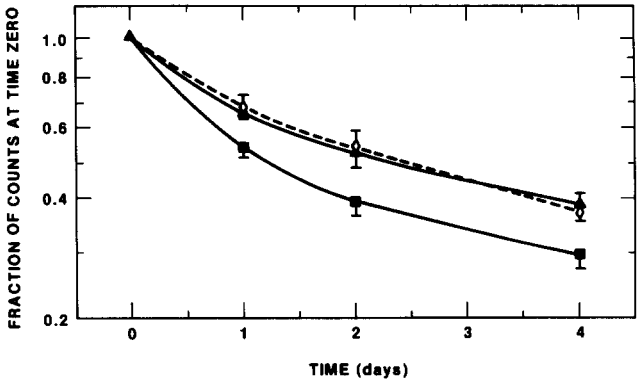

Fig. 2. Metabolism of injected ^{125}I-labeled apoA-I and ^{131}I-labeled apoA-II in LpA-I and LpA-I,A-II (series I) through 4 days. ApoA-I in LpA-I (closed squares); apoA-I in LpA-I,A-II (closed triangles); apoA-II in LpA-I,A-II (open triangles). Each point represents the arithmetic mean ± SD of values from the three normal subjects.

The urine/plasma radioactivity ratios for the same five subjects are illustrated in **Fig. 4.** They demonstrate that within the first 2 days, the apoA-I injected on LpA-I was degraded at a faster rate than the apoA-I injected on LpA-I,A-II. Thereafter the urine/plasma ratios became equal, consistent with the observation that the whole plasma curves became parallel after 2 days.

At selected time points after injection, LpA-I and LpA-I,A-II were isolated from plasma and the radioactivity was quantitated. The lipoprotein particle kinetic

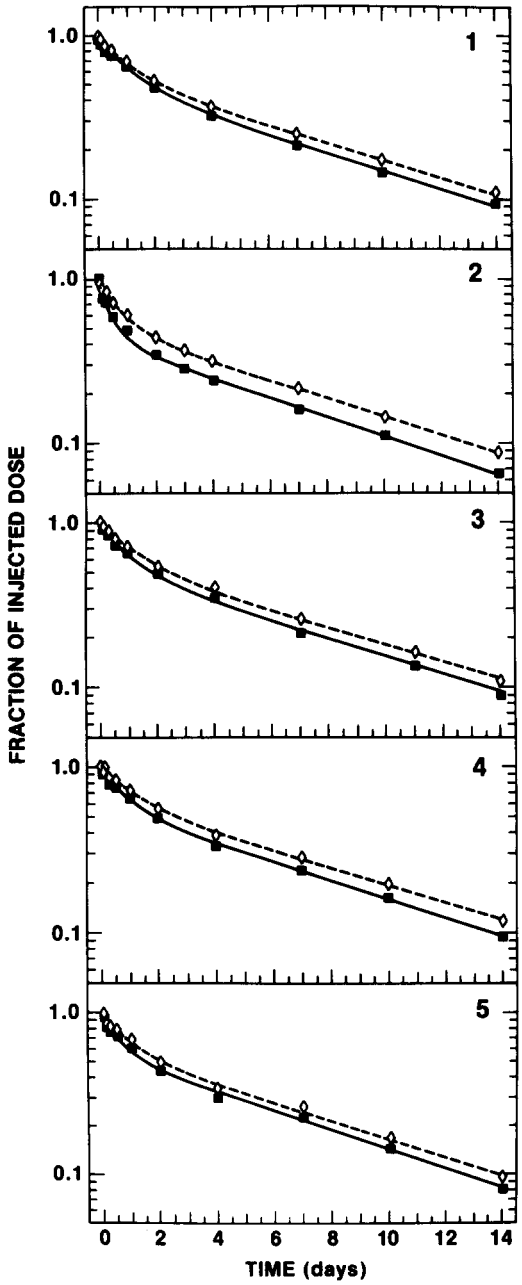

Fig. 3. Whole plasma radioactivity decay curves of ^{131}I-labeled apoA-I injected on LpA-I (closed squares) and ^{125}I-labeled apoA-I injected on LpA-I,A-II (open diamonds) in five normal subjects (Series II).

TABLE 3. Kinetic parameters of apoA-I injected on LpA-I and LpA-I,A-II (Series II)

Subject	Total ApoA-I Conc	LpA-I			LpA-I,A-II			Difference[a]	
		Conc	RT	FCR	Conc	RT	FCR	RT	FCR
	mg/dl	*mg/dl*	*days*	d^{-1}	*mg/dl*	*days*	d^{-1}	*days*	d^{-1}
1	160	59	4.63	0.216	101	5.24	0.191	0.61	− 0.025
2	126	45	3.41	0.294	81	4.42	0.226	0.01	− 0.068
3	141	45	4.75	0.211	96	5.50	0.182	0.75	− 0.029
4	149	56	4.91	0.208	93	5.73	0.175	0.92	− 0.033
5	107	36	4.33	0.230	71	4.97	0.201	0.64	− 0.029
Mean	137	48	4.41	0.232	88	5.17	0.195	0.79[b]	− 0.037[c]
± SEM	± 9	± 4	± 0.26	± 0.016	± 5	± 0.23	± 0.009	± 0.08	± 0.008

[a](LpA-I,A-II) − (LpA-I).
[b]Significantly different by paired *t*-test, $P < 0.001$.
[c]Significantly different by paired *t*-test, $P < 0.01$.

curves through the first 4 days are illustrated in **Fig. 5.** When ^{131}I-labeled apoA-I was injected on LpA-I, approximately one-third of the labeled apoA-I became rapidly associated with LpA-I,A-II within the first 10 min, generating ^{131}I-labeled apoA-I-LpA-I,A-II particles. Similarly, when ^{125}I-labeled apoA-I was injected on LpA-I,A-II, approximately one-third of the labeled apoA-I associated rapidly with LpA-I, generating ^{125}I-labeled apoA-I-LpA-I particles. The kinetic curves of the injected ^{131}I-labeled apoA-I-LpA-I and the in vivo generated ^{125}I-labeled apoA-I-LpA-I (Fig. 5, top panel) had identical slopes, as did the kinetic curves for the injected ^{125}I-labeled apoA-I-LpA-I,A-II and the in vivo generated ^{131}I-labeled apoA-I-LpA-I,A-II (Fig. 5, bottom panel). These results indicate that the apoA-I on lipoprotein particles created by apolipoprotein exchange and/or particle conversion in vivo had identical kinetic behavior to the apoA-I on the corresponding lipoprotein particles that were isolated in vitro and injected.

The turnover of apoA-I injected on LpA-I and LpA-I,A-II in the density fractions HDL_2 and HDL_3 was determined in three of the subjects. The kinetic curves of lipoproteins separated by density fractionation through the first 4 days are shown in **Fig. 6.** Upon injection of ^{131}I-labeled apoA-I-LpA-I, radiolabeled apoA-I was initially distributed relatively evenly between HDL_2 (40.2 ± 1.1%) and HDL_3 (42.8 ± 3.8%). There was rapid loss of ^{131}I-labeled apoA-I from HDL_3 with a corresponding increase in ^{131}I-labeled apoA-I in HDL_2, suggesting a precursor-product relationship (Fig. 6, top panel). Upon injection of ^{125}I-labeled apoA-I-LpA-I,A-II, radiolabeled apoA-I was initially distributed to a greater extent in HDL_3 (49.0 ± 6.9%) than in HDL_2 (40.2 ± 4.0%). As we observed with radiolabeled LpA-I, there was a rapid decrease of ^{125}I-labeled apoA-I from HDL_3 with a corresponding increase in HDL_2 ^{125}I-labeled apoA-I, again suggesting precursor-product kinetics (Fig. 6, bottom panel).

DISCUSSION

The principal objective of this study was to determine whether the apoA-I on LpA-I represents a kinetically different pool than the apoA-I on LpA-I,A-II. As discussed in the introduction, these two types of particles may have different physiologic functions, with LpA-I being a potentially more "anti-atherogenic" particle within HDL. Furthermore, the presence of apoA-II in apoA-I-containing particles influences their ability to bind in vitro to specific cell-surface receptors. Fong et al. (37) reported that incubation of HDL_3 with increasing amounts of apoA-II decreased both the maximum binding capacity of the particles to adipocyte plasma membranes as well as the uptake of HDL cholesteryl ester by intact adipocytes. De Crom et al. (38) demonstrated in binding studies to porcine liver membranes that LpA-I had a higher K_d and greater maximal binding capacity than LpA-I,A-II. More recently, Kilsdonk, van Gent, and van Tol (39) showed that LpA-I binds to HepG2 cells with greater affinity than does LpA-I,A-II. Thus, there is increasing evidence that these two types of apoA-I-containing particles interact differently with specific cell-surface receptors.

Earlier studies suggested that apoA-I and apoA-II may have different in vivo metabolism. Atmeh, Shepherd, and Packard (40) reported that the plasma apoA-I/apoA-II ratio could be modulated pharmacologically, with nicotonic acid raising and probucol lowering the ratio. Furthermore, several investigators have found that the FCR of apoA-I is faster than that of apoA-II, with differences ranging from 10 to 40% (18–24). Zech et al. (25) developed a compartmental model for apoA-I and apoA-II using human in vivo kinetic data. It consisted of two plasma compartments for apoA-I that decayed at different rates and one plasma compartment for apoA-II that decayed at a rate similar to the slower of the two apoA-I

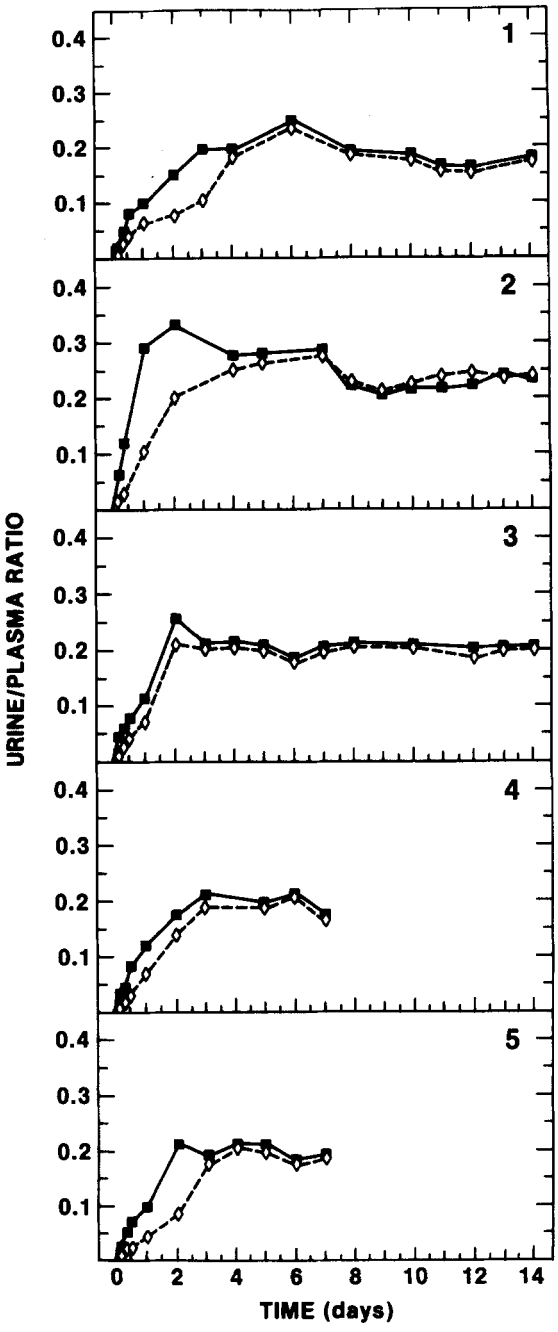

Fig. 4. Urine/plasma radioactivity ratios for ^{131}I-labeled apoA-I injected on LpA-I (closed squares) and ^{125}I-labeled apoA-I injected on LpA-I,A-II (open diamonds) in five normal subjects (Series II). Subjects #4 and #5 collected urine for only 7 days.

compartments. These combined results suggest that the catabolic pathways of apoA-I and apoA-II are not identical and may in fact be differentially regulated. We hypothesized that the two pools of apoA-I on LpA-I and LpA-I,A-II particles are kinetically heterogeneous. The studies described in this report were designed to test this hypothesis.

We chose to radiolabel purified apolipoproteins and reassociate them with plasma before isolating specific lipoprotein particles, rather than radiolabeling intact isolated particles. We felt that this approach would allow us to follow the radiotracer within different lipoprotein particle subclasses more informatively, in that a given label is present only on a single apolipoprotein. Recent kinetic studies by Vega et al. (41) have provided evidence that isolated radioiodinated apoA-I has kinetic behavior similar to apoA-I radiolabeled as part of intact HDL. In using this approach, only potentially exchangeable apoA-I pools on the lipoprotein particles have been labeled with the radioiodinated apoA-I. If there are any truly nonexchangeable pools of apoA-I on these particles, they have not been adequately labeled by this method.

Exchange of apolipoproteins among lipoprotein particles and interconversion of particles within the plasma compartment pose significant potential obstacles to the investigation of the in vivo kinetics of HDL particles. In the studies presented here, a rapid equilibration of the radiolabeled apoA-I between LpA-I and LpA-I,A-II would have obliterated any possibility of detecting a kinetic difference between the apoA-I injected on these two lipoprotein particles. The fact that we were able to detect a consistent kinetic difference implies that at least a portion of the apoA-I exchanges between particles at a slower rate than the catabolic rate of a population of apoA-I-containing

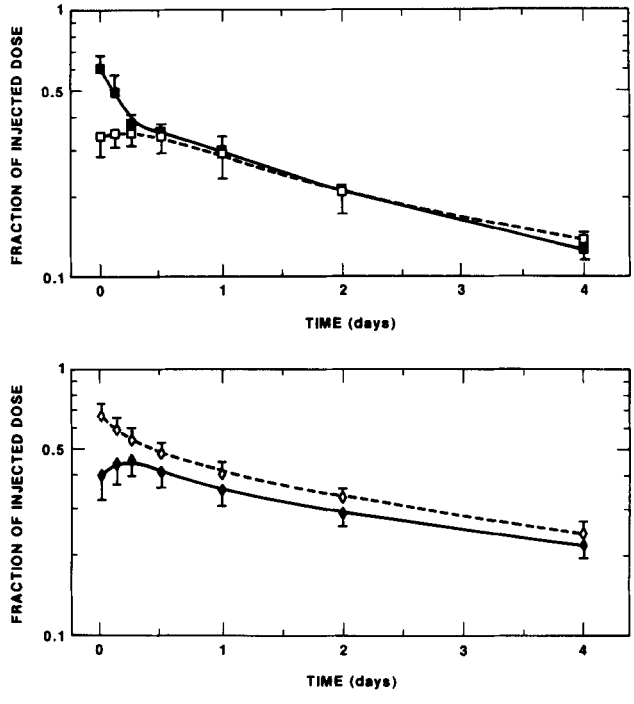

Fig. 5. Turnover of apoA-I injected on LpA-I or LpA-I,A-II (Series II): comparison of radiolabeled apoA-I on injected and in vivo generated particles through 4 days. Top panel: radiolabeled apoA-I reisolated from plasma on LpA-I after being injected on LpA-I (closed squares) or on LpA-I,A-II (open squares). Bottom panel: radiolabeled apoA-I reisolated from plasma on LpA-I,A-II after being injected on LpA-I,A-II (open diamonds) or on LpA-I (closed diamonds). Each point represents the arithmetic mean ± SD of the values in four normal subjects.

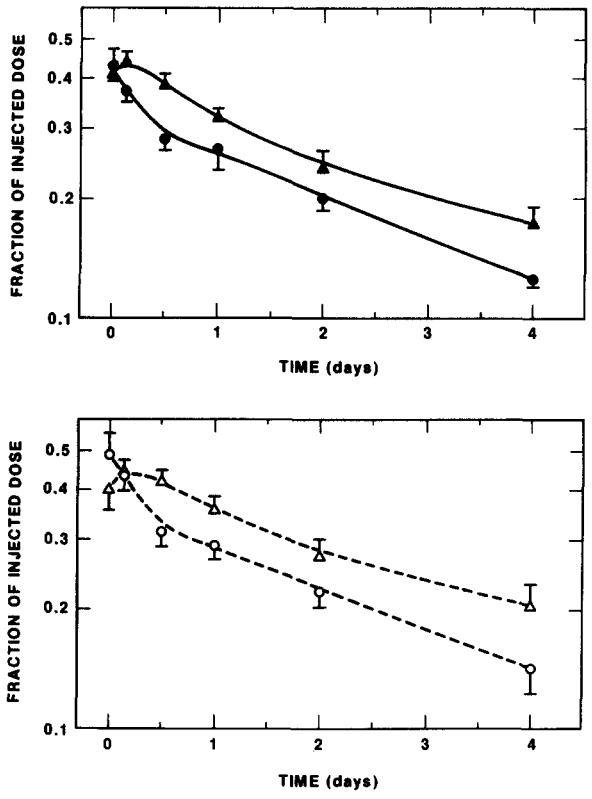

Fig. 6. Turnover of apoA-I injected on LpA-I or LpA-I,A-II (Series II) in HDL_2 and HDL_3 through 4 days. Top panel: ^{131}I-labeled apoA-I injected on LpA-I and reisolated in HDL_3 (closed circles) and HDL_2 (closed triangles). Bottom panel: ^{125}I-labeled apoA-I injected on LpA-I,A-II and reisolated in HDL_3 (open circles) and HDL_2 (open triangles). Each point represents the arithmetic mean ± SD of the values in three normal subjects.

particles. In other words, the time frame required for complete equilibration of the injected apoA-I tracers in vivo allows a "window of time" in which the differential kinetics of apoA-I entering the system on LpA-I and LpA-I,A-II can be observed. This is consistent with the findings of Shepherd et al. (42), who suggested that there may be a poorly exchangeable pool of apoA-I. Furthermore, Ibdah et al. (43) recently demonstrated that a significant proportion of purified apoA-I incorporated into phospholipid vesicles in vitro is in a pool that transfers to other vesicles slowly on the order of hours to days.

In the first series of studies, the whole plasma kinetic parameters of radiolabeled apoA-I and apoA-II that were reassociated with autologous plasma before injection are comparable to these reported in previous studies in which radiolabeled apoA-I and apoA-II were directly injected (18, 22–24, 41). The turnover of ^{125}I-labeled apoA-I in LpA-I was substantially faster than that of ^{125}I-labeled apoA-I in LpA-I,A-II. Furthermore, the turnover rates of apoA-I and apoA-II in LpA-I,A-II were very similar, suggesting that their metabolic pathways may be linked. This series of studies demonstrates the metabolic heterogeneity of apoA-I in LpA-I and LpA-I,A-II and establishes that differential kinetics can be detected in vivo despite exchange of apolipoproteins among lipoprotein particles. In this first series of studies, however, it is impossible to differentiate between different rates of lipoprotein particle removal from plasma and conversion of particles within the plasma compartment. The more rapid turnover of apoA-I in LpA-I compared with LpA-I,A-II could be due to more rapid removal of LpA-I particles from plasma, to a net exchange or conversion of apoA-I in LpA-I to LpA-I,A-II, or to a combination of both processes.

Therefore, the second series of studies was designed to directly assess the whole plasma fractional catabolic rate of apoA-I injected on LpA-I compared with that of apoA-I injected on LpA-I,A-II. These two types of particles, differentially labeled only in the apoA-I moiety, were simultaneously injected into normal subjects. The total 14-day plasma radioactivity curves and the kinetic parameters derived from them demonstrate that the fractional catabolic rate of apoA-I injected on LpA-I is significantly faster than that of apoA-I injected on LpA-I,A-II. The urine/plasma radioactivity ratios show that the faster plasma catabolism of apoA-I injected on LpA-I is not simply due to differential extravascular exchange, but rather leads to degradation. These results suggest that the statistical likelihood of apoA-I being irreversibly removed from the plasma compartment and degraded is dependent, at least in part, on whether it is present on a lipoprotein particle which also contains apoA-II.

Because of apolipoprotein exchange and/or particle interconversion, the kinetic parameters in Table 3 should not be considered kinetic parameters of the LpA-I and LpA-I,A-II particles themselves. In fact, the total apoA-I plasma curves in Series II actually represent the summation of the turnover of both the apoA-I remaining on the injected particle as well as the apoA-I that exchanges to the other particle in vivo. The ^{131}I-labeled apoA-I on LpA-I,A-II generated from the injected ^{131}I-labeled apoA-I-LpA-I by the exchange of labeled apoA-I turns over more slowly than the ^{131}I-labeled apoA-I remaining on LpA-I. Likewise, the ^{125}I-labeled apoA-I on LpA-I generated from the injected ^{125}I-labeled apoA-I-LpA-I,A-II is catabolized more rapidly than the ^{125}I-labeled apoA-I remaining on LpA-I,A-II. Hence, exchange of the radiolabeled apoA-I brings both plasma curves closer to each other; immediate equilibration of the tracers after injection would result in identical plasma curves. The fact that the whole plasma curves become parallel and the urine/plasma ratios become equal after 2 days is probably because the two radiolabeled apoA-I tracers have finally come into full equilibration between LpA-I and LpA-I,A-II. Therefore, the physiologic difference in fractional catabolic rates between the LpA-I and LpA-I,A-II particles themselves may be even greater than the difference derived from the analysis of the total apoA-I plasma curves

in this study. Comprehensive modelling of the data generated from these studies may help to determine the amount and rate of particle interconversion as well as the catabolic rates of the particles themselves.

The higher plasma levels of apoA-I in LpA-I,A-II than in LpA-I seen in most normal individuals are likely due at least in part to the slower catabolism of apoA-I that enters the HDL compartment on LpA-I,A-II. Because of the exchange of apoA-I between particles, true production or transport rates of apoA-I into each particle class cannot be accurately determined. It therefore remains possible that differences in production rates of apoA-I into the particles may also influence the steady state distribution of apoA-I between the two particle types.

Analysis of HDL density subfractions isolated in the second series of studies revealed that apoA-I in HDL_3 has a substantially faster turnover rate than does the apoA-I in HDL_2, irrespective of whether the apoA-I was injected on LpA-I or LpA-I,A-II. There is also a significant increase in radioactivity in the HDL_2 fraction over the first several hours, suggesting a net exchange or conversion of apoA-I in HDL_3 to HDL_2. The relationship of HDL density subfractions to LpA-I and LpA-I,A-II metabolism remains to be fully elucidated. However, Patsch et al. (44) have demonstrated that in the postprandial state, LpA-I,A-II in HDL_2 are preferentially converted to HDL_3 compared with LpA-I in HDL_2. Furthermore, Mowri et al. (45) have recently reported that postprandial LpA-I,A-II in HDL_2 are a preferred substrate for hepatic lipase in vitro. Such preferential "recycling" of LpA-I,A-II from HDL_2 to HDL_3 via hepatic lipase may account, at least in part, for its prolonged plasma residence time compared with LpA-I.

In summary, these studies demonstrate that apoA-I that is injected on LpA-I has a substantially faster plasma catabolic rate than apoA-I injected on LpA-I,A-II in normolipidemic human subjects. We suggest that the presence of apoA-II on an apoA-I-containing HDL particle prolongs the plasma residence time of its associated apoA-I. This effect of apoA-II may be to produce a pool of more slowly turning over HDL particles which are unlikely to participate actively in cholesterol removal from tissues. Rather, the major purpose of these particles may be to deliver cholesterol to specialized organs such as adrenals and gonads (46). These results demonstrate that the in vivo metabolism of apoA-I on these two classes of apoA-I-containing particles is fundamentally divergent and may have important implications for the regulation of plasma apoA-I levels.

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REFERENCES

1. Miller, G. J., and N. E. Miller. 1975. Plasma high-density-lipoprotein concentration and development of ischaemic heart-disease. *Lancet.* **1:** 16–19.
2. Gordon, D. J., and B. M. Rifkind. 1989. High-density lipoprotein—the clinical implications of recent studies. *N. Engl. J. Med.* **321:** 1311–1316.
3. Miller, N. E. 1987. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. *Am. Heart J.* **113:** 589–597.
4. Brunzell, J. D., A. D. Sniderman, J. J. Albers, and P. O. Kwiterovich. 1984. Apoproteins B and A-I and coronary artery disease in humans. *Arteriosclerosis.* **4:** 79–83.
5. Maciejko, J. J., D. R. Holmes, B. A. Kottke, A. R. Zinsmeister, D. M. Dinh, and S. J. Mao. 1983. Apolipoprotein A-I as a marker of angiographically assessed coronary-artery disease. *N. Engl. J. Med.* **309:** 385–389.
6. Alaupovic, P., D. M. Lee, and W. J. McConathy. 1972. Studies on the composition and structure of plasma lipoproteins: distribution of lipoprotein families in major density classes of normal human plasma lipoproteins. *Biochim. Biophys. Acta.* **260:** 689–707.
7. Norfeldt, P. I., S. O. Olofsson, G. Fager, and G. Bondjers. 1981. Isolation and partial characterization of the lipoprotein families A and A-I from high-density lipoproteins of human serum. *Eur. J. Biochem.* **118:** 1–8.
8. Nestruck, A. C., P. D. Niedmann, H. Wieland, and D. Seidel. 1983. Chromatofocusing of human high density lipoproteins and isolation of lipoproteins A and A-I. *Biochim. Biophys. Acta.* **753:** 65–73.
9. Cheung, M. C., and J. J. Albers. 1984. Characterization of lipoprotein particles isolated by immunoaffinity chromatography. Particles containing A-I and A-II and particles containing A-I but no A-II. *J. Biol. Chem.* **259:** 12201–12209.
10. Ohta, T., S. Hattori, S. Nishiyama, and I. Matsuda. 1988. Studies on the lipid and apolipoprotein compositions of two species of apoA-I-containing lipoproteins in normolipidemic males and females. *J. Lipid Res.* **29:** 721–728.
11. James, R. W., D. Hochstrasser, J. D. Tissot, M. Funk, R. Appel, F. Barja, C. Pellegrini, A. F. Muller, and D. Pometta. 1988. Protein heterogeneity of lipoprotein particles containing apolipoprotein A-I without apolipoprotein A-II and apolipoprotein A-I with apolipoprotein A-II isolated from human plasma. *J. Lipid Res.* **29:** 1557–1571.
12. McVicar, J. P., S. T. Kunitake, R. L. Hamilton, and J. P. Kane. 1984. Characteristics of human lipoproteins isolated by selected-affinity immunosorption of apolipoprotein A-I. *Proc. Natl. Acad. Sci. USA.* **81:** 1356–1360.
13. Puchois, P., A. Kandoussi, P. Fievet, J. L. Fourrier, M. Bertrand, E. Koren, and J. C. Fruchart. 1987. Apolipoprotein A-I-containing lipoproteins in coronary artery disease. *Atherosclerosis.* **68:** 35–40.
14. Koren, E., P. Puchois, P. Alaupovic, J. Fesmire, A. Kandoussi, and J. C. Fruchart. 1987. Quantification of two different types of apolipoprotein A-I-containing lipoprotein particles in plasma by enzyme-linked differential-antibody immunosorbent assay. *Clin. Chem.* **33:** 38–43.
15. Barbaras, R., P. Puchois, J. C. Fruchart, and G. Ailhaud. 1987. Cholesterol efflux from cultured adipose cells is medi-

ated by LpA-I particles but not by LpA-I:A-II particles. *Biochem. Biophys. Res. Commun.* **142:** 63–69.
16. Blum, C. B., R. I. Levy, S. Eisenberg, M. Hall III, R. H. Goebel, and M. Berman. 1977. High density lipoprotein metabolism in man. *J. Clin. Invest.* **60:** 795–807.
17. Fidge, N., P. Nestel, T. Ishikawa, M. Reardon, and T. Billington. 1980. Turnover of apoproteins A-I and A-II of high density lipprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. *Metabolism.* **29:** 643–653.
18. Schaefer, E. J., L. A. Zech, L. L. Jenkins, T. J. Bronzert, E. A. Rubalcaba, F. T. Lindgren, R. L. Aamodt, and H. B. Brewer, Jr. 1982. Human apolipoprotein A-I and A-II metabolism. *J. Lipid Res.* **23:** 850–862.
19. Haffner, S. M., R. S. Kushwaha, D. M. Foster, D. Applebaum-Bowden, and W. R. Hazzard. 1983. Studies on the metabolic mechanism of reduced high density lipoproteins during anabolic steroid therapy. *Metabolism.* **32:** 413–420.
20. Magill, P., S. N. Rao, N. E. Miller, A. Nicoll, J. Brunzell, J. St. Hilaire, and B. Lewis. 1982. Relationships between the metabolism of high-density and very-low-density lipoproteins in man: studies of apolipoprotein kinetics and adipose tissue lipoprotein lipase activity. *Eur. J. Clin. Invest.* **12:** 113–120.
21. Saku, K., P. S. Gartside, B. A. Hynd, S. G. Mendoza, and M. L. Kashyap. 1985. Apolipoprotein A-I and A-II metabolism in patients with primary high-density lipoprotein deficiency associated with familial hypertriglyceridemia. *Metabolism.* **34:** 754–764.
22. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1989. Elevated high density lipoprotein cholesterol levels correlate with decreased apolipoprotein A-I and A-II fractional catabolic rate in women. *J. Clin. Invest.* **84:** 262–269.
23. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1990. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J. Clin. Invest.* **85:** 144–151.
24. Brinton, E. A., S. Eisenberg, and J. L. Breslow. 1991. Increased apoA-I and apoA-II fractional catabolic rate in patients with low high density lipoprotein-cholesterol levels with or without hypertriglyceridemia. *J. Clin. Invest.* **87:** 536–544.
25. Zech, L. A., E. J. Schaefer, T. J. Bronzert, R. L. Aamodt, and H. B. Brewer, Jr. 1983. Metabolism of human apolipoproteins A-I and A-II: compartmental models. *J. Lipid Res.* **24:** 60–71.
26. Berman, M., and M. Weiss. 1978. SAAM Manual. DHEW Publ. No. (NIH) 78. National Institutes of Health, Bethesda, MD.
27. Brewer, H. B., Jr., T. Fairwell, A. LaRue, R. Ronan, 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.
28. Brewer, H. B., Jr., S. E. Lux, R. Ronan, and K. M. John. 1972. Amino acid sequence of human apoLp-Gln-II (apoA-II), an apolipoprotein isolated from the high-density lipoprotein complex. *Proc. Natl. Acad. Sci. USA.* **69:** 1304–1308.
29. Gregg, R. E., L. A. Zech, E. J. Schaefer, and H. B. Brewer, Jr. 1984. Apolipoprotein E metabolism in normolipoproteinemic human subjects. *J. Lipid Res.* **25:** 1167–1176.
30. Betard, C., N. Vu-Dac, H. Mezdour, A. C. Nestruck, A. Leroy, and J. C. Fruchart. 1987. Standardization of an enzymometric assay for apolipoprotein A-I by using mixtures of monoclonal antibodies. *J. Clin. Chem. Clin. Biochem.* **25:** 893–899.
31. Barkia, A., R. Barbaras, N. Ghalim, P. Puchois, G. Ailhaud, and J. C. Fruchart. 1988. Effect of different apoA-I-containing lipoprotein particles on reverse cholesterol transport in fat cells. *Horm. Metab. Res. Suppl.* **19:** 10–12.
32. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
33. 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.
34. Bojanovski, M., R. E. Gregg, D. M. Wilson, and H. B. Brewer, Jr. 1988. Competitive enzyme-linked immunosorbent assay (ELISA) for the quantitation of apolipoprotein A-I using a monoclonal antibody. *Clin. Chim. Acta.* **178:** 159–169.
35. Stein, E. A., L. DePersio, A. J. Pesce, M. Kashyap, J. T. Kao, L. Srivastava, and C. McNerney. 1986. Enzyme-linked immunoabsorbant assay of apolipoprotein A-II in plasma, with use of a monoclonal antibody. *Clin. Chem.* **32:** 967–971.
36. Parra, H. J., H. Mezdour, N. Ghalim, J. M. Bard, and J. C. Fruchart. 1990. Differential electroimmunoassay of human LpA-I lipoprotein particles on ready-to-use plates. *Clin. Chem.* **36:** 1431–1435.
37. Fong, B. S., A. M. Salter, J. Jimenez, and A. Angel. 1987. The role of apolipoprotein A-I and apolipoprotein A-II in high-density lipoprotein binding to human adipocyte plasma membranes. *Biochim. Biophys. Acta.* **920:** 105–113.
38. De Crom, M. P., M. J. van Haperen, P. Puchois, J. C. Fruchart, T. van Gent, A. van Tol, and A. W. van der Kamp. 1989. Binding characteristics of high density lipoprotein subclasses to porcine liver, adrenal and skeletal muscle plasma membranes. *Int. J. Biochem.* **21:** 649–656.
39. Kilsdonk, E. P. C., T. van Gent, and A. van Tol. 1990. Characterization of human high-density lipoprotein subclasses LpA-I and LpA-I/A-II and binding to HepG2 cells. *Biochim. Biophys. Acta.* **1045:** 205–212.
40. Atmeh, R. F., J. Shepherd, and C. J. Packard. 1983. Subpopulations of apolipoprotein A-I in human high-density lipoproteins: their metabolic properties and response to drug therapy. *Biochim Biophys. Acta.* **751:** 175–188.
41. Vega, G. L., H. Gylling, A. V. Nichols, and S. M. Grundy. 1991. Evaluation of a method for study of kinetics of autologous apolipoprotein A-I. *J. Lipid Res.* **32:** 867–875.
42. Shepherd, J., A. M. Gotto, Jr., 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.
43. Ibdah, J. A., C. Smith, S. Lund-Katz, and M. C. Phillips. 1991. Effects of apolipoprotein structure on the kinetics of apolipoprotein transfer between phospholipid vesicles. *Biochim. Biophys. Acta.* **1081:** 220–228.
44. Patsch, J. R., S. Prasad, A. M. Gotto. Jr., and G. Bengtsson-Olivecrona. 1984. Postprandial lipemia. A key for the conversion of high density lipoprotein$_2$ into high density lipoprotein$_3$ by hepatic lipase. *J. Clin. Invest.* **74:** 2017–2023.

45. Mowri, H., W. Patsch, L. C. Smith, A. M. Gotto, Jr., and J. R. Patsch. 1990. Different reactivities of HDL_2 subfractions with hepatic lipase. *Circulation.* **82, Suppl.III:** 558 (abstract).
46. Glass, C., R. C. Pittman, D. B. Weinstein, and D. Steinberg. 1983. Dissociation of tissue uptake of cholesterol ester from that of apoprotein A-I of rat plasma high density lipoprotein: selective delivery of cholesterol ester to liver, adrenal, and gonad. *Proc. Natl. Acad. Sci. USA.* **80:** 5435–5439.