Evaluation of a method for study of kinetics of autologous apolipoprotein A-I

Gloria Lena Vega,*,† Helena Gylling,* Alex V. Nichols,†† and Scott M. Grundy*,†,§,**

Center for Human Nutrition,* Departments of Clinical Nutrition,† Internal Medicine,§ and Biochemistry,** University of Texas Southwestern Medical Center, Dallas, TX; and The Donner Laboratories,†† University of California, Berkeley, CA

Abstract Apolipoprotein A-I (apoA-I) participates in transport of plasma cholesterol. Concentrations of apoA-I depend on the balance between production and fractional clearance. To elucidate factors influencing apoA-I levels, accurate estimates of apoA-I turnover rates may be valuable. We describe a method for isolation of autologous apoA-I and its use in turnover studies. Free apoA-I was isolated from high density lipoproteins (HDL) by treatment with guanidine hydrochloride. This free apoA-I was radioiodinated with ^{131}I and injected into eleven subjects simultaneously with HDL labeled with ^{125}I. Plasma die-away curves of free apoA-I (^{131}I) and HDL apoA-I (^{125}I) were compared; fractional clearance rates averaged 0.256 ± 0.019 (SEM) and 0.254 ± 0.017 pools/day, respectively. Although slight differences between the two die-away curves were noted for some of the patients, the differences were relatively small; for the group as a whole, average fractional catabolic rates were not significantly different. Thus, by isolation of autologous apoA-I under the conditions described, free apoA-I seemingly provides a valid method for estimating apoA-I turnover. —**Vega, G. L., H. Gylling, A. V. Nichols, and S. M. Grundy.** Evaluation of a method for study of kinetics of autologous apolipoprotein A-I. *J. Lipid Res.* 1991. **32:** 867–875.

Supplementary key words turnover • lipoproteins • high density lipoprotein • radioiodination • guanidine hydrochloride

Apolipoprotein A-I (apoA-I) plays an important role in transport of cholesterol in plasma. It may, in part, mediate reverse cholesterol transport, the process whereby cholesterol is mobilized from extrahepatic tissues to the liver for excretion (1, 2). In support of this concept, high levels of plasma apoA-I are associated with low risk for atherosclerotic coronary heart disease (CHD), and vice versa (3–8). Consequently, factors determining the plasma concentrations of apoA-I could indirectly affect atherogenesis. To understand the mechanisms whereby these factors influence apoA-I levels, studies of apoA-I kinetics may be informative.

Relatively few investigations have been carried out on the metabolism of apoA-I in humans, and methodology for study of apoA-I kinetics needs further development and evaluation. One approach for estimating turnover rates of apoA-I has been to isolate and radioiodinate autologous HDL, to reinject it into subjects, and to follow isotopic decay curves for apoA-I isolated from plasma samples obtained over a period of several days. Another approach has been to label purified homologous apoA-I, to inject it into subjects, and to obtain isotope decay curves simply by measuring radioactivity in plasma. In the present study we report a method for isolation of autologous apoA-I and for using it in apoA-I turnover studies. To document the method's validity, we have compared isotope kinetic curves for purified autologous apoA-I with those obtained for apoA-I after labeling and reinjecting whole HDL.

METHODS

Patients

Eleven men participated as volunteers in this study; they were recruited from outpatients of the Veterans Administration Medical Centers, Dallas and Bohnam, Texas. Their ages, body mass indexes (BMI), plasma lipids, and lipoprotein cholesterol are presented in **Table 1**. These values were obtained as outpatients and were used for selection of patients into the study. Lipid values represent the mean of three determinations obtained on 3 consecutive days. The patients' average age was 55 ± 4 (SEM) years. BMIs averaged 26.2 ± 1.1 kg/m^2. Plasma total cholesterol upon entry ranged from 148 to 283 mg/dl, and total triglycerides from 66 to 946 mg/dl; HDL-cholesterol concentrations were between 23 and 60 mg/dl. These lipid measurements were made as described previously (9). All had normal function of liver, kidneys, and endocrine system. None of the patients had diabetes mellitus. Seven patients had a history of CHD, but none had

Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; HDL, high density lipoprotein; BMI, body mass index; FCR, fractional catabolic rate; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PC, phosphatidylcholine.

TABLE 1. Clinical characteristics of patients for selection into study

Patient	Age	Body Mass Index	Plasma Lipids		Lipoprotein Cholesterol		
			Cholesterol	Triglyceride	VLDL	LDL	HDL
	yr	*kg/m²*	*mg/dl*			*mg/dl*	
1	63	26.4	278	946	165	90	23
2	46	27.3	283	425	112	148	23
3	54	33.4	177	190	33	120	24
4	72	23.4	208	391	73	109	26
5	61	29.1	206	221	43	133	30
6	66	28.2	166	204	38	95	33
7	68	27.9	212	370	68	111	33
8	41	26.4	216	135	21	154	41
9	48	21.6	148	66	9	94	45
10	43	24.6	157	107	13	94	50
11	40	19.7	162	87	13	89	60
Mean	55	26.2	201	286	53	112	35
± SEM	± 4	± 1.1	± 14	± 76	± 15	± 7	± 4

sustained myocardial infarction for at least 6 months preceding the study. None of the patients had congestive heart failure, and none had ever taken hypolipidemic agents. Each patient gave written, informed consent to participate in a study that had been approved by the appropriate institutional review boards. The studies were conducted on the metabolic unit of the Veterans Administration Medical Center, Dallas, or at the General Clinical Research Center, Parkland Memorial Medical Center, Dallas, Texas.

Experimental design

The patients were started on a solid-food diet containing 40% of total calories as fat (18% saturated, 17% monounsaturated, and 5% polyunsaturated fatty acids), 45% as carbohydrate, and 15% as protein. The average intake of cholesterol was 400 mg/day. This diet was similar to the "typical" American diet, and it has been used previously in our lipoprotein turnover studies (10–15). Patients were instructed by a dietitian on the details of the study diet, and caloric intake of the diet was adjusted to maintain a constant body weight. The patients consumed this diet as outpatients for 3 weeks and then underwent a one-unit plasmapheresis, which was obtained for isolation of HDL and apoA-I. HDL was isolated by preparative ultracentrifugation, and from one aliquot, apoA-I was isolated, as will be described below. Whole HDL and isolated apoA-I were labeled with ^{125}I and ^{131}I, respectively, and both tracers were injected simultaneously into patients 6 days after plasmapheresis. For the first 4 to 7 days after injection patients remained in the metabolic ward, and they were fed the same diet from the metabolic kitchen. Thereafter, after reinforcement of diet instructions, some patients completed their studies as outpatients. Blood samples were taken frequently over a 14-day period. Fractional catabolic rate (FCR) for isolated apoA-I, henceforth called free apoA-I, was estimated from the die-away curve for ^{131}I in whole plasma. On an aliquot of each plasma sample, lipoproteins of density less than 1.21 g/ml were isolated by preparative ultracentrifugation, and apolipoproteins were separated by electrophoresis. Radioactivity in the apoA-I band was determined, and the die-away curve of ^{125}I-labeled apoA-I was constructed. Since most apoA-I in the lipoprotein fraction resided in HDL, ^{125}I-labeled apoA-I will be designated HDL apoA-I. The FCR for ^{125}I-labeled HDL apoA-I was determined from its die-away curve.

Preparation of tracers

High density lipoproteins (d 1.090–1.21 g/ml) from 100 ml plasma were isolated by sequential preparative ultracentrifugation by the method of Lindgren, Jensen, and Hatch (16). The lipoprotein was resuspended at its native density and subjected to ultracentrifugation in a TV-865B vertical rotor (Dupont-Sorvall, Wilmington, DE) for 2.5 h at 65,000 rpm to remove any contaminating proteins. An aliquot containing approximately 3 mg HDL protein was dialyzed against 150 mM NaCl, 0.01% disodium EDTA, pH 7.4, in preparation for labeling with ^{125}I.

Another aliquot of the HDL preparation containing approximately 9 mg HDL protein was dialyzed against 150 mM NaCl, 10 mM Tris-chloride, and 0.01% disodium EDTA, pH 8.0, and was used for purification of apoA-I by a modification of a procedure detailed previously by Nichols et al. (17). Briefly the method was as follows: 3 ml of dialyzed HDL (~3 mg/ml HDL protein) was transferred into a sterile, pyrogen-free vial and 1.146 mg guanidine hydrochloride was added to bring the solution to 4 M; this solution was incubated for 3 h at 37°C. After incubation, the mixture was dialyzed four times against a

dialysis solution containing 150 mM NaCl, 10 mM Tris-chloride, 0.01% EDTA, pH 8.0. A total of 21 liters of dialysis solution was used over a period of 20 h. The HDL solution was then transferred into a sterile 6-ml Ultraclear ultracentrifuge tube; the density was adjusted to 1.21 g/ml using solid NaBr, and the solution was overlayered with an equal volume of NaBr solution of similar density. The sample was subjected to ultracentrifugation at 50,000 rpm for 24 h in a 50.3 fixed-angle rotor (Beckman Instruments, Palo Alto, CA), at a temperature of 10°C. The top 4.5 ml was removed and discarded (except for an aliquot for analysis), and the bottom 1.5 ml was dialyzed extensively against 150 mM NaCl, 0.01% EDTA, pH 7.4. Protein concentration was determined by the method of Lowry et al. (18) in the d 1.21 g/ml supernatant and infranatant. Consistently, approximately 30% of total HDL protein was in the supernatant and 70% of HDL protein was in the infranatant. The protein of both fractions was assessed by electrophoresis (E) in sodium dodecyl sulfate (SDS) polyacrylamide gels (PAG) (19). In the supernatant, apoA-II was the predominant component, with only small amounts of apoA-I present. No other proteins or apolipoproteins could be detected in the supernatant. The infranatant appeared to contain pure apoA-I by SDS-PAGE; no apoA-II or other protein was detected. The infranatant also was tested for the presence of apoB and apoA-II, as well as apoA-I, by immunodiffusion (20) and electroimmunoassay (21). Neither of the former two apolipoproteins was detected; in the previous description of this technique (17), the infranatant was shown to contain no phospholipid or other lipids.

Approximately 1 mg of isolated apoA-I and HDL proteins were radiolabeled with ^{131}I and ^{125}I, respectively. A modification of the iodine monochloride method of McFarlane (22) as modified by Bilheimer, Eisenberg, and Levy (23) was used for iodination. Free iodine was removed from the preparations by dialysis against 150 mM NaCl, 10 mM Tris-chloride, 0.01% EDTA, pH 8.0. Each tracer preparation was characterized for percent distribution of counts in protein (trichloracetic acid (TCA)-precipitable counts), and in the case of HDL, lipid-associated counts. Both tracers, ^{125}I-labeled HDL and ^{131}I-labeled apoA-I, consistently had over 99% of counts precipitable with 15% TCA. For ^{125}I-labeled HDL, 97% of radioactivity was associated with HDL protein, and 3% was with HDL lipid. When the distribution of radioactivity in HDL proteins was estimated by SDS-PAGE in the eleven patients, 62 ± 2% (SEM) was in apoA-I and 38.0 ± 2% in apoA-II. For ^{131}I-labeled apoA-I, over 95% of radioactivity was associated with apoA-I on SDS-PAGE, and no other proteins could be detected.

An injection mixture consisting of tracer and 4% human serum albumin was prepared. Each injection mixture was passed through a pyrogen-free, 0.22-μm, filter. Preparations for injection were shown to be free of pyrogens by a Limulus assay. Twenty five to 30 μCi of each tracer was injected intravenously. Twenty three blood samples of 15 ml each were collected over a period of 14 days. On the day of injection, samples were drawn at 10, 20, 30, and 60 min, followed by sampling at 4, 8, 12, and 24 h. Thereafter, samples were collected every 12 h until day 4, and daily thereafter until day 14. All plasma samples were collected into tubes containing 0.005% chloramphenicol, 0.005% gentamycin sulfate, and 0.1% sodium azide.

Construction of isotope decay curves

The isotope decay curve for free apoA-I (^{131}I) was constructed by counting 4 ml of plasma at each time point; the percent injected dose was plotted as a function of time, using the 10-min sample as the zero time. The HDL apoA-I (^{125}I) curve was obtained as follows. Each plasma sample was adjusted to a density of 1.21 g/ml and subjected to ultracentrifugation in a 50.3 rotor (Beckman Instruments, Palo Alto, CA) at 39,000 rpm for 36 h. The top 2 ml was collected as supernatant, and the remainder was taken as the infranatant; both were counted to determine recoveries of radioactivity. The supernatant (lipoprotein fraction) was delipidated using the method of Scanu and Edelstein (24), and apolipoproteins were resolubilized in 5% SDS, 50 mM Tris-HCl, pH 8.6. The radioactivity in apoA-I was determined by a modification of the method of Fidge et al. (25), briefly as follows. The apolipoprotein mixture was subjected to 15% SDS-PAGE using a preparative cylindrical gel apparatus (1 cm internal diameter × 12 cm length; model 175, Bio-Rad Laboratories, Richmond, CA). After electrophoresis, the gels were stained and destained; the protein bands were cut, and all gel segments were counted for radioactivity. All radioactivity was corrected for decay and quenching and converted to disintegrations per minute (dpm). The distribution of dpm and percent recovery of dpm were calculated. Absolute dpm associated with apoA-I was determined by multiplying the fraction of radioactivity in the plasma lipoprotein fractions of density less than 1.21 gm/ml. To construct the plasma die-away curve for ^{125}I-labeled apoA-I, the radioactivity calculated for apoA-I at all time points was compared to that determined at the 10-min sample.

Analysis of isotope decay curves

Decay curves for both free apoA-I (^{131}I) and HDL apoA-I (^{125}I) were used to calculate FCRs. The curves were analyzed according to the two-pool model of Matthews (26). This model assumes the existence of an intravascular pool in dynamic equilibrium with an extravascular pool. According to this model, both new input and exit of apoA-I occur from the intravascular pool. The die-away curves for free apoA-I (^{131}I) and HDL apoA-I (^{125}I) were compared statistically as follows. For each

curve, 23 data points were collected over the 14-day period, and the resulting activity-time curves were modeled separately for the two types of apoA-I. A standard F-test was performed to determined whether the two curves were significantly different ($P<0.05$) (27, 28).

Analysis of HDL cholesterol and apoA-I levels

Lipoprotein-cholesterol levels were determined by Lipid Research Clinic procedures (29) except that enzymatic methods were used (30, 31). ApoA-I levels were estimated by electroimmunoassay (20, 21), as described previously (9).

RESULTS

The average fraction of radioactivity associated with lipoproteins of d < 1.21 g/ml isolated from plasma during the turnover study are shown for ^{125}I and ^{131}I in **Table 2**. Fractions of ^{125}I and ^{131}I represent radioactivities derived from HDL and free apoA-I, respectively, that floated at d < 1.21 g/ml. The average ^{125}I was 0.89 ± 0.01 (SEM), whereas that for ^{131}I was 0.90 ± 0.01. The remainder of radioactivity was in the 1.21 g/ml infranatant. The results indicated that in vivo the apoA-I (^{131}I) associated with plasma lipoproteins to the same extent as ^{125}I-labeled HDL apolipoproteins. In other words, after reinjection, free apoA-I (^{131}I) did not sediment with the d 1.21 g/ml infranatant to a greater extent than ^{125}I-labeled HDL proteins.

Kinetic parameters for HDL apoA-I (^{125}I) and free apoA-I (^{131}I) are presented in **Table 3** for eleven subjects. Their HDL-cholesterol and total apoA-I levels as determined during the turnover study ranged from 18 to 55 mg/dl and from 60 to 129 mg/dl, respectively. For the whole group, average FCRs for the two fractions were not statistically different by paired *t*-test. However, some small differences were found for some of the curves when each pair was compared statistically by the F-test. In four of the patients, the two curves were not statistically different by this test (**Fig. 1**). In six others (**Fig. 2**), small but significant differences were noted; in four of these (nos. 1, 2, 5, 8 and 9), the free apoA-I (^{131}I) curve decayed slightly faster than the HDL apoA-I (^{125}I) curve, whereas in the other patient (no. 3), the opposite occurred. It is apparent from these two figures that in most patients the

TABLE 2. Fraction of plasma radioactivity associated with plasma lipoproteins

Patient	d < 1.21 g/ml Lipoprotein Fraction	
	^{125}I[a]	^{131}I[b]
1	0.89 ± 0.01	0.89 ± 0.01
2	0.87 ± 0.01	0.87 ± 0.01
3	0.90 ± 0.01	0.91 ± 0.01
4	0.89 ± 0.01	0.89 ± 0.01
5	0.86 ± 0.01	0.87 ± 0.01
6	0.92 ± 0.01	0.95 ± 0.01
7	0.88 ± 0.01	0.89 ± 0.01
8	0.87 ± 0.01	0.87 ± 0.01
9	0.90 ± 0.01	0.92 ± 0.01
10	0.90 ± 0.01	0.92 ± 0.01
11	0.92 ± 0.01	0.93 ± 0.01
Mean ± SEM	0.89 ± 0.01	0.90 ± 0.01

[a] ^{125}I represents radioactivity derived from labeled HDL that floated at d < 1.21 g/ml.
[b] ^{131}I represents radioactivity derived from labeled isolated apoA-I that floated at d < 1.21 g/ml.

TABLE 3. Kinetic parameters of ^{131}I-labeled apoA-I and ^{125}I-labeled apoA-I

Patient	HDL Cholesterol[a]	ApoA-I Conc[a]	Fractional Catabolic Rate		
			^{131}I-Labeled ApoA-I	^{125}I-Labeled HDL ApoA-I	Difference
	mg/dl	*mg/dl*		*pools/day*	
1	18	60	0.296	0.281	0.015[b]
2	32	79	0.378	0.364	0.014[b]
3	35	68	0.255	0.279	−0.024[b]
4	26	85	0.236	0.228	0.008[c]
5	26	97	0.256	0.249	0.007[b]
6	26	97	0.248	0.265	−0.017[c]
7	23	75	0.287	0.289	−0.002[c]
8	36	100	0.311	0.299	0.012[b]
9	40	88	0.210	0.193	0.017[b]
10	45	116	0.201	0.199	0.002[a]
11	55	129	0.143	0.153	−0.010[c]
Mean ± SEM	33 ± 2	90 ± 6	0.256 ± 0.019	0.254 ± 0.017	0.002[d] ± 0.004

[a] These values represent the mean of five samples obtained every third day throughout the study.
[b] Significantly different by Fisher F-test.
[c] Not significantly different by Fisher F-test.
[d] Not significantly different by paired *t*-test.

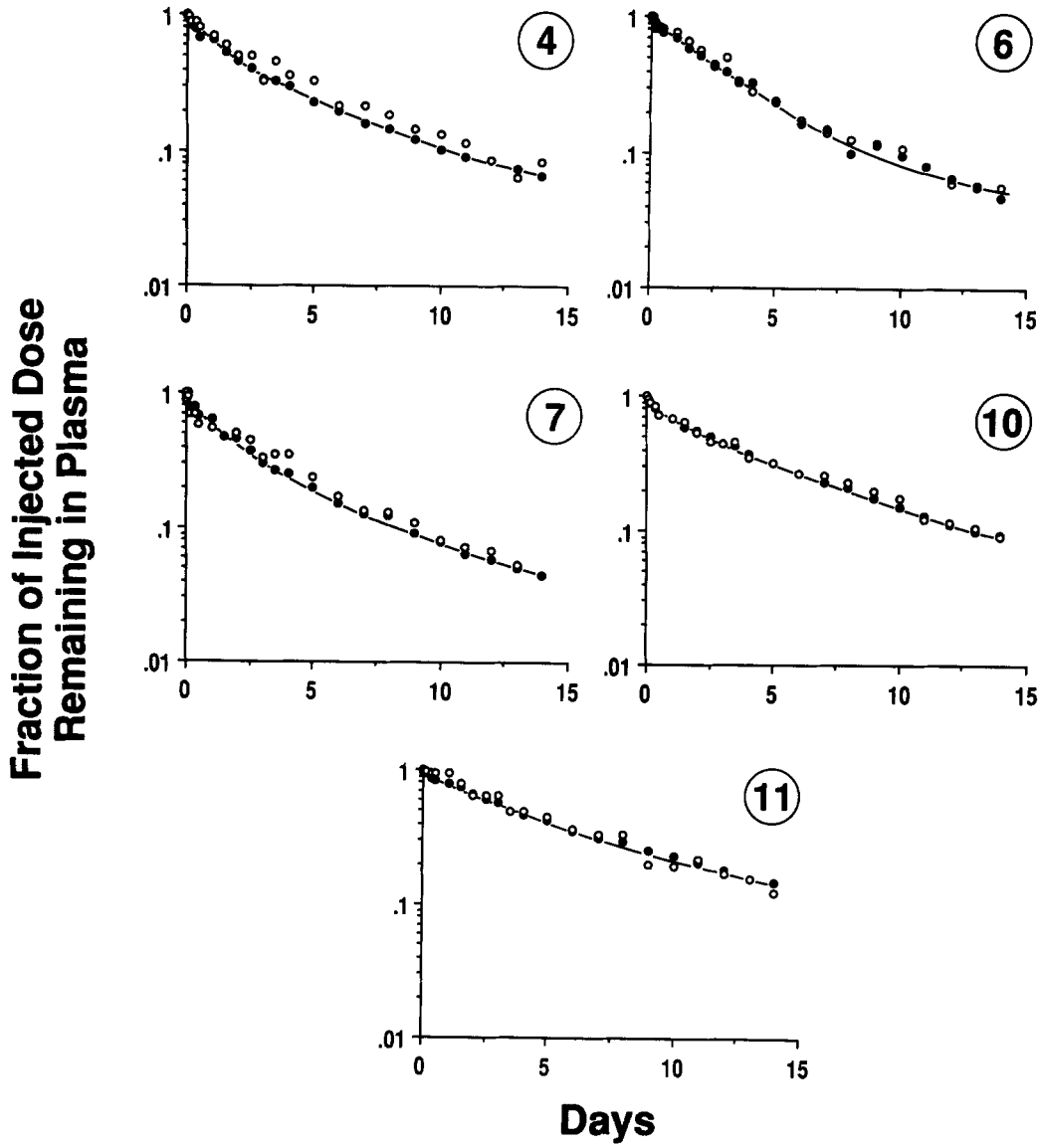

Fig. 1. Activity-time curves for free apoA-I (^{131}I) (●) and HDL apoA-I (^{125}I) (○) for five patients (nos. 4, 6, 7, 10, and 11). The curves are best-fit curves for free apoA-I (^{131}I). In these patients, FCRs estimated from the two sets of data points were not statistically significant by F-test. For those points where only an open circle (○) is shown, the closed (●) is always present but is hidden behind the open circle.

free apoA-I (^{131}I) curve was much smoother than the HDL apoA-I (^{125}I) curve. The greater scatter of the points in the latter curve undoubtedly was due to the complexity of the procedure required to isolate and measure ^{125}I-labeled apoA-I from the lipoproteins of each sample.

DISCUSSION

Previous investigators have used two general approaches for determining turnover rates of apoA-I in humans. Some workers have radiolabeled pure homologous A-I and have followed its die-away curve after intravenous injection. Others have radioiodinated and reinjected whole HDL particles and determined radioactivities in apoA-I isolated from lipoproteins in subsequent blood samples. Both methods have potential advantages and disadvantages, and these can be reviewed briefly as they relate to current findings.

In 1977, Blum et al. (32) described a method in which whole HDL was labeled with ^{125}I, and the rates of clearance of labeled apoA-I and apoA-II were followed after intravenous injection. The two apolipoproteins were separated by column chromatography on samples obtained at each time point, and from their die-away curves, respective clearance rates were determined. A similar technique was employed by Fidge et al. (25), except that

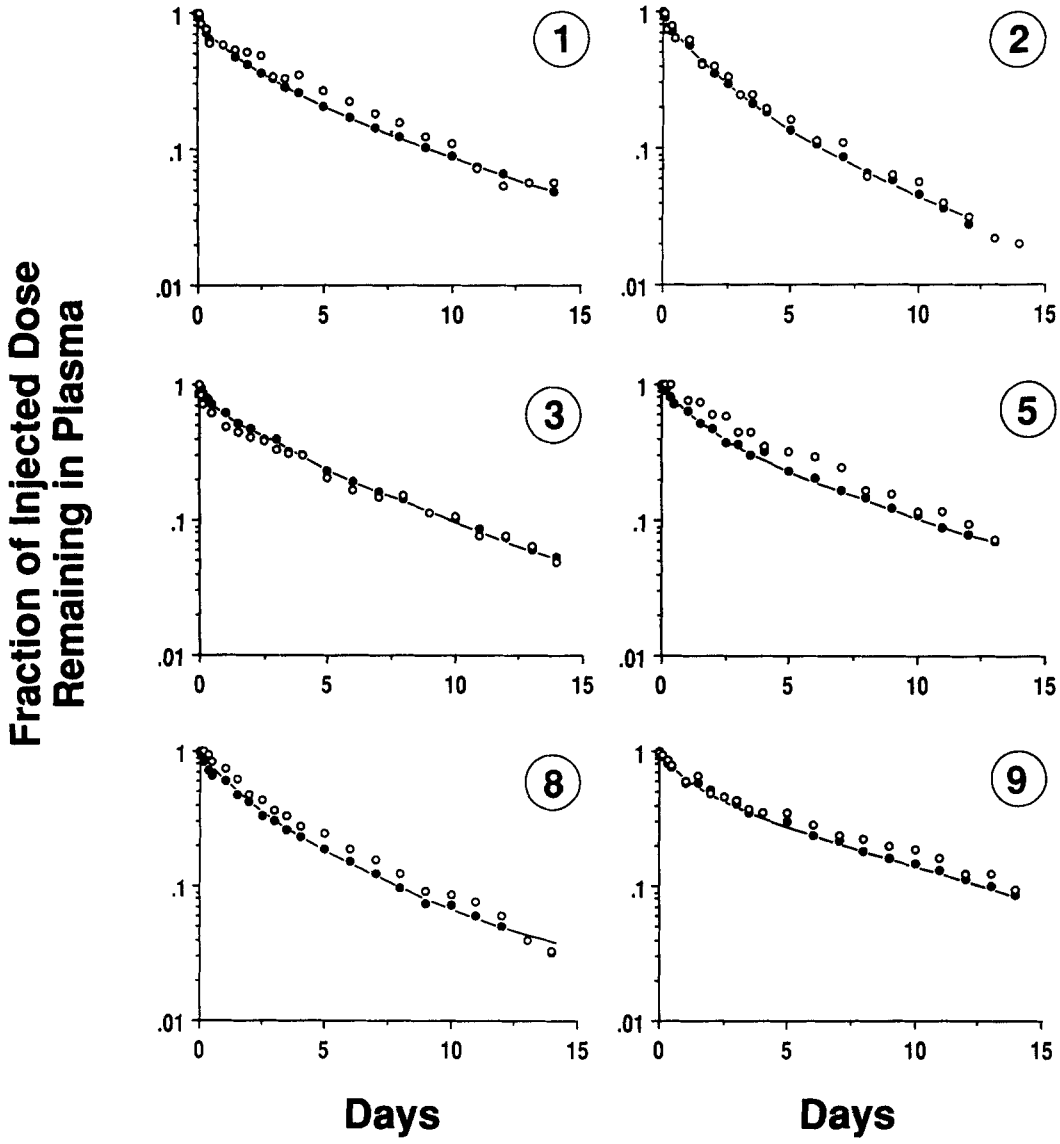

Fig. 2. Activity-time curves for free apoA-I (^{131}I) (●) and HDL apoA-I (^{125}I) (○) for six patients (nos. 1, 2, 3, 5, 8, and 9). The curves are best-fit curves for free apoA-I. In patients 1, 2, 5, 8, and 9, the free apoA-I (^{131}I) curve decayed slightly and significnantly faster than the HDL apoA-I curve, whereas in one patient (no. 3), the opposite occurred. For those points where only an open circle (○) is shown, the closed circle (●) is always present but is hidden behind the open circle.

HDL apolipoproteins were separated by preparative SDS-PAGE, bands containing apolipoproteins were eluted by SDS-Tris chloride buffer, and specific activities were determined. Similar procedures have been used subsequently by other workers for estimating turnover rates of apoA-I (33–39).

A second general approach was developed by other investigators. They isolated and radiolabeled pure apoA-I, and some form of this free apoA-I was reinjected into subjects for estimation of apoA-I turnover rates. They also compared these turnover rates with those obtained for apoA-I labeled on intact HDL, similar to the methods described by Blum et al. (32) and/or Fidge et al. (25). For free apoA-I, Shepherd et al. (40) complexed isolated, radioiodinated apoA-I with whole HDL particles by in vitro incubation and the resulting complex was injected; simultaneously, HDL that had been radioiodinated in situ was also injected. The turnover rate of complexed apoA-I was determined from the plasma decay curve; HDL apoA-I was also isolated, counted for radioactivity, and its decay curve was analyzed. In another study, Schaefer et al. (41) differentially radioiodinated whole HDL particles and purified apoA-I; they compared turnover rates of the free apoA-I with those of HDL apoA-I,

the latter isolated from HDL at multiple time points during the kinetic study. In this study, preincubation to complex free apoA-I with HDL was not used because the distribution of radioactivity of labeled apoA-I among plasma lipoprotein fractions during in vitro incubation was similar to that observed in vivo. Finally, Malmendier, Delcroix, and Ameryckx (42) compared turnover rates for radioiodinated apoA-I in three forms, namely, as free apoA-I, as apoA-I complexed with phosphatidylcholine (PC), and as apoA-I labeled in situ on whole HDL.

The results of these latter three studies are summarized in **Table 4**. In two subjects, Shepherd et al. (40) found that free apoA-I (preincubated with HDL) had higher FCRs than apoA-I labeled in situ with HDL (HDL apoA-I). They speculated that free apoA-I did not equilibrate completely with apoA-I contained in HDL; consequently, a portion of the free apoA-I was cleared at an increased rate. However, in two other subjects, Schaefer et al. (41) found identical turnover rates for free apoA-I and HDL apoA-I, and they concluded that rapid and complete equilibration between free apoA-I and HDL apoA-I does occur, even when pure, uncomplexed apoA-I is injected intravenously. On the other hand, in one subject, Malmendier et al. (42) found a much higher FCR for free, uncomplexed apoA-I than for HDL apoA-I. In contrast, when free apoA-I was complexed with PC in two other subjects, the FCR for free apoA-I was essentially identical to that for HDL apoA-I. Thus, results of these three studies in a relatively small number of subjects were inconclusive. That reinjection of free apoA-I consistently gives the same FCR as HDL apoA-I was by no means established by these comparisons.

TABLE 4. Comparison of apolipoprotein A-I kinetics from previous studies

Author	Subject	Fractional Catabolic Rate	
		HDL ApoA-I[a]	Free ApoA-I[b]
		pools/day	
Shepherd et al. (40)	M.Y.	0.24	0.37[c]
	D.E.	0.24	0.30[c]
Schaefer et al. (41)	4	0.146	0.154[d]
	11	0.269	0.267[d]
Malmendier et al. (42)	D.J.	0.307	0.735[d]
	C.R.	0.255	0.257[e]
	H.G.	0.275	0.288[e]

[a]HDL ApoA-I represents apoA-I that was labeled in situ on whole HDL particles.

[b]Free ApoA-I represents apoA-I that was isolated, purified, labeled, and reinjected as either uncomplexed apoprotein or preincubated with either HDL or phosphatidyl choline (see subsequent footnotes).

[c]Free apoA-I was preincubated with whole HDL before injection.

[d]Free apoA-I was injected as uncomplexed, soluble apolipoprotein.

[e]Free apoA-I was preincubated with phosphatidylcholine before injection.

Although the discrepancies among the results of these three studies (40–42) have not been resolved, a partial explanation may reside in the more recent study of Osborne et al. (43). These investigators examined the molecular and metabolic properties of radioiodinated apoA-I. They were able to separate radioiodinated apoA-I into two fractions. The first was indistinguishable in physical properties from unlabeled apoA-I; the second, in contrast, had less secondary structure than unlabeled apoA-I and was less immunoreactive. The authors designated the second fraction "incompetent" apoA-I, which presumably means that it was partially denatured. In vivo kinetic studies revealed that the second, "incompetent" fraction was catabolized more rapidly than the first, suggesting that the second fraction did not reassociate completely with lipoproteins. Results of a similar type have been reported by Patterson and Lee (44). It seems possible that radioiodination of free apoA-I would produce more denatured apoA-I than labeling apoA-I in situ in whole HDL; this is because the iodination of free apoA-I might modify tyrosine residues in a way to prevent the normal reassociation of apoA-I with HDL. If so, this could account for the discrepancies noted by Shepherd et al. (40) and Malmandier et al. (42), and postulated by Patterson and Lee (44). Since the radiolabeling of free apoA-I may partially denature apoA-I, the conditions of labeling may be critical to the accuracy, and even the apparent model, of the kinetic study. Regarding the model, as pointed out by Osborne et al. (43), "incompetent" apoA-I may in part account for the rapidly catabolized, intravascular pool of apoA-I reported by Zech et al. (45).

Recently, several investigations have used the injection of labeled, free apoA-I to initiate apoA-I turnover studies (46–48). Pure homologous apoA-I frequently has been used. Still, the identity of turnover rates of free apoA-I and HDL apoA-I, while perhaps assumed, lacks experimental verification (Table 4). One purpose of the present study was to compare turnover rates of free apoA-I and HDL apoA-I in a larger number of subjects than reported previously. The data obtained in eleven patients with varying levels of HDL clearly revealed that the two forms of apoA-I on the average have similar clearance rates (Table 3). Therefore, in our view, free apoA-I can be used as a valid marker for whole plasma apoA-I kinetics, provided it is isolated and labeled appropriately.

As shown in Fig. 2, free apoA-I is sometimes cleared at a slightly more rapid rate than HDL apoA-I. When this occurs, there are two possible explanations. First, labeling of free apoA-I may slightly damage the apolipoprotein, and the modified portion may be removed somewhat more rapidly from the circulation; this could give it a higher FCR than HDL apoA-I. Alternatively, in some patients, free apoA-I may reassociate with HDL less tightly than that already present on HDL during labeling, causing the former to be cleared a little more rapidly. If this

mechanism pertains, it is a matter of conjecture which of the two FCRs most closely reflects that of "physiologic" apoA-I. Regardless of reason, differences between the two curves, and hence FCRs, are small and probably are of little biological significance.

It is interesting to speculate why labeled, free apoA-I is a reliable tracer for whole plasma apoA-I, most of which is associated with lipoproteins. For this to be possible, exchange rates among different plasma pools of apoA-I (i.e., HDL subfractions, very low density lipoproteins, and the lipoprotein-free fraction) must greatly exceed turnover rates of apoA-I in most of these subfractions. Although the apoA-Is in the different subfractions almost certainly do not have identical turnover rates, the rapid exchange of apoA-I among these various subfractions seemingly creates a common "kinetic pool" of apoA-I that has a "turnover rate" independent of turnover rates in the different subfractions. This rapid exchange thus allows for free apoA-I to measure the turnover rate of the common "kinetic pool" of apoA-I just as well as that contained in HDL.

In this report a technique was utilized for isolation of autologous apoA-I for use in turnover studies. We suggest that this technique has several useful features. First, it allows for use of the patient's own apoA-I and thus avoids even the slightest danger of transferring any type of viral infection from the donor to the recipient. Although the danger of using homologous apoA-I seems to be small, cross infection is at least a theoretical possibility. Second, the method yields relatively large quantities of apoA-I, which should minimize overlabeling and denaturation of the apolipoprotein upon treatment with radioiodine. In previous studies that used homologous apoA-I, it generally was the practice to label relatively small quantities of apolipoprotein, which may increase the chance of denaturation; as shown by Osborne et al. (43), partial denaturation of apoA-I can alter its estimated turnover rate. And third, autologous labeled apoA-I should be metabolized identically to the patient's own apoA-I. If homologous apoA-I is used, it could have a primary structure different from that of autologous apoA-I; if so, homologous apoA-I could be metabolized differently, and thus might not accurately trace the catabolism of autologous apoA-I.

The authors express appreciation for their excellent technical assistance to Biman Pramanik, Carolyn Croy, Leslie Weiss, and Kathy Schutt. The assistance of Marjorie Whelan in clinical coordination also is acknowledged. The authors acknowledge the invaluable contributions of Elaine Gong to development of the technique for isolation of pure apoA-I. We also thank the clinical staffs of the Metabolic Unit at the Veterans Administration Medical Center and the General Clinical Research Center at Parkland Medical Center. Beverly Adams, program analyst, was helpful with the management of CLINFO. The clinical research was supported by the Veterans Administration, grants HL-29252 and MO-IRR00633 (NIH/DHS/DHHS), the Southwestern Medical Foundation, and the Moss Heart Foundation, Dallas, Texas. The method for isolating and purifying apoA-I was supported by Program Project HL-18574 (NIH/DHS/DHHS) and the Lawrence Berkley Laboratory (Department of Energy contract DR-AC03-76SF00098 to the University of California).

Manuscript received 4 October 1990 and in revised form 11 January 1991.

REFERENCES

1. Glomset, J. A. 1968. The plasma lecithin:cholesterol acyltransferase reaction. *J. Lipid Res.* **9:** 155–167.
2. Pittman, R. C., and D. Steinberg. 1984. Sites and mechanism of uptake and degradation of high density and low density lipoproteins. *J. Lipid Res.* **25:** 1577–1585.
3. Berg, K., A. Borresen, and G. Dahlen. 1976. Serum-high-density-lipoprotein and atherosclerotic hear-disease. *Lancet.* **1:** 499–501.
4. Albers, J. J., P. W. Wahl, V. G. Cabana, W. R. Hazzard, and J. J. Hoover. 1976. Quantitation of apolipoprotein A-I of human plasma high density lipoproteins. *Metabolism.* **25:** 633–644.
5. Albers, J. J., M. C. Cheung, and W. R. Hazzard. 1978. High-density lipoproteins in myocardial infarction survivors. *Metabolism.* **27:** 479–485.
6. Avogaro, P., G. Cazzolato, B. G. Bittolo, and G. B. Quinci. 1979. Are apolipoproteins better discriminators than lipids for atherosclerosis. *Lancet.* **1:** 901–903.
7. Maciejio, J. J., D. R. Holmes, B. A. Kottke, A. R. Zinsmeister, D. M. Dinh, and S. J. T. Mao. 1983. Apolipoprotein A-I as a marker of angiographically assessed coronary artery disease. *N. Engl. J. Med.* **309:** 385–389.
8. 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.
9. Vega, G. L., and S. M. Grundy. 1989. Comparison of lovastatin and gemfibrozil in normolipidemic patients with hypoalphalipoproteinemia. *JAMA.* **262:** 3148–3153.
10. Vega, G. L., W. Beltz, and S. M. Grundy. 1985. Low density lipoprotein metabolism in hypertriglyceridemic and normolipidemic patients with coronary heart disease. *J. Lipid Res.* **26:** 115–126.
11. Grundy, S. M., G. L. Vega, and D. W. Bilheimer. 1985. Influence of combined therapy with mevinolin and interruption of bile acid reabsorption on low density lipoproteins in heterozygous familial hypercholesterolemia. *Ann. Int. Med.* **103:** 339–343.
12. Grundy, S. M., G. L. Vega, and D. W. Bilheimer. 1985. Kinetic mechanisms determining variability in low density lipoprotein levels and their rise with age. *Arteriosclerosis.* **5:** 623–630.
13. Grundy, S. M., and G. L. Vega. 1985. Influence of mevinolin on metabolism of low density lipoproteins in primary moderate hypercholesterolemia. *J. Lipid Res.* **26:** 1464–1475.
14. Vega, G. L., and S. M. Grundy. 1988. Lovastatin therapy in nephrotic hyperlipidemia: effects on lipoprotein metabolism. *Kidney Int.* **33:** 1160–1168.
15. Vega, G. L., R. M. Krauss, and S. M. Grundy. 1990. Pravastatin therapy in primary moderate hypercholesterolemia: changes in metabolism of apolipoprotein B-containing lipoproteins. *J. Intern. Med.* **227:** 81–94.

16. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition and Metabolism. G. S. Nelson, editor. Wiley-Interscience, New York. 181–274.
17. Nichols, A. V., E. L. Gong, P. J. Blanche, T. M. Forte, and D. W. Anderson. 1976. Effects of guanidine hydrolchloride on human plasma high density lipoproteins. *Biochim. Biophys. Acta.* **446:** 226–239.
18. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.
19. Weber, K., and M. Osborn. 1975. Protein and sodium dodecyl sulfate: molecular weight determination on polyacrylamide gels and related procedures. *In* The Proteins. H. Neurah, and R. L. Hill, editors. Academic Press, New York. 179–223.
20. Ouchterlony, O. 1964. Immunological methods. A symposium organized by the Council for International Organization of Medical Sciences under the joint auspices of UNESCO and WHO. S. F. Ackroyd, editor. Blackwell Scientific Publication, Oxford. 55–78.
21. Laurell, C-B. 1972. Electroimmunoassay. *Scand. J. Clin. Lab. Invest.* **(Suppl) 124:** 21–37.
22. McFarlane, A. S. 1958. Efficient trace-labeling of proteins with iodine. *Nature.* **28:** 53–57.
23. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. The metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
24. Scanu, A. M., and C. D. Edelstein. 1971. Solubility in aqueous solutions of ethanol of small molecular weight peptides of the serum very low density and high density lipoproteins: relevance to the recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.
25. Fidge, N., P. Nestel, T. Ishikawa, M. Reardon, and T. Billington. 1980. Turnover of apoprotein A-I and A-II of high density lipoprotein and the relationship to other lipoproteins in normal and hyperlipidemic individuals. *Metabolism.* **29:** 643–653.
26. Matthews, C. M. E. 1957. The theory of tracer experiments with iodine 131-labeled plasma proteins. *Phys. Med. Biol.* **2:** 36–53.
27. Beck, J. V., and K. J. Arnold. 1977. Parameter estimation. *In* Engineering and Science. Wiley Publishers, New York. 242.
28. Fleiss, J. L. 1973. Statistical Methods for Rates and Proportions. John Wiley, New York. 115–117.
29. Lipid and Lipoprotein Analysis in Manuals of Laboratory Operations. 1974. Lipid Research Clinic Program. Department of Health, Education, and Welfare, Washington, D. C., Publication NIH/75-628.
30. Roeschlau, P., E. Bernt, and W. Gruber. 1974. Enzymatic determination of total cholesterol in serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 226–227.
31. McGowan, M. W., J. D. Artis, D. R. Strandbergh, and B. Zak. 1983. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin. Chem.* **29:** 538–542.
32. Blum, C. B., R. I. Levy, S. Eisenberg, M. Hall, R. H. Goebel, and M. Berman. 1977. High density lipoprotein metabolism in man. *J. Clin. Invest.* **60:** 795–807.
33. Rao, S. N., P. J. Magill, N. E. Miller, and B. Lewis. 1980. Plasma high-density lipoprotein metabolism in subjects with primary hypertriglyceridemia: altered metabolism of apolipoproteins A-I and A-II. *Clin. Sci.* **59:** 359–367.
34. Nestel, P. J., and T. Billington. 1981. Effect of probucol on low density lipoprotein removal and high density lipoprotein synthesis. *Atherosclerosis.* **38:** 203–209.
35. Parks, J. S., and L. L. Rudel. 1982. Different kinetic fates of apolipoproteins A-I and A-II from lymph chylomicra of nonhuman primates: effects of saturated versus polyunsaturated dietary fat. *J. Lipid Res.* **23:** 410–421.
36. Schaefer, E. J., D. M. Foster, L. A. Zech, F. T. Lindgren, H. B. Brewer, Jr., and R. I. Levy 1983. The effects of estrogen administration on plasma lipoprotein metabolism in premenopausal females. *J. Clin. Endocrinol. Metab.* **57:** 262–267.
37. 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.
38. Atmeh, R. F., J. M. Stewart, D. E. Boag, C. J. Packard, A. R. Lorimer, and J. Shepherd. 1983. The hypolipidemic action of probucol: a study of its effects on high and low density lipoproteins. *J. Lipid Res.* **24:** 588–595.
39. Ginsberg, H. N., N. A. Le, and H. S. Gilbert. 1986. Altered high density lipoprotein metabolism in patients with myeloproliferative disorder and hypercholesterolemia. *Metabolism.* **35:** 878–882.
40. Shepherd, J., C. J. Packard, A. M. Gotto, Jr., and O. D. Taunton. 1978. A comparison of two methods to investigate the metabolism of human apolipoproteins A-I and A-II. *J. Lipid Res.* **19:** 656–661.
41. 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.
42. 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.
43. Osborne, J. C., Jr., E. J. Schaefer, G. M. Powell, N. S. Lee, and L. A. Zech. 1984. Molecular properties of radioiodinated apolipoprotein A-I. *J. Biol. Chem.* **259:** 347–353.
44. Patterson, B. W., and A. M. Lee. 1986. Self-association and phospholipid binding properties of iodinated apolipoprotein A-I. *Biochemistry.* **25:** 4953–4957.
45. 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.
46. Bojanovski, D., R. E. Gregg, G. Ghiselli, E. J. Schaefer, J. A. Light, and H. B. Brewer, Jr. 1985. Human apolipoprotein A-I isoprotein metabolism: proapoA-I conversion to mature apoA-I. *J. Lipid Res.* **26:** 185–193.
47. 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.
48. 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.