Evaluation of apoA-I kinetics in humans using simultaneous endogenous stable isotope and exogenous radiotracer methods

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Abstract Apolipoprotein A-I is the major apolipoprotein constituent of high density lipoproteins (HDL). Methods used to investigate in vivo kinetics of apoA-I include exogenous labeling with radioiodine and endogenous labeling with stable isotopically labeled amino acids. We report here a direct comparison of these methods to determine the in vivo kinetics of apoA-I in four normal subjects. Purified apoA-I was labeled with ^{125}I, reassociated with autologous plasma, and injected into study subjects. At the same time, [$^{13}C_6$]phenylalanine was administered as a primed constant infusion for up to 14 hours. The kinetic parameters of apoA-I were determined from the ^{125}I-labeled apoA-I plasma curves. For the analysis of data from stable isotope studies, very low density lipoprotein (VLDL) apoB-100, VLDL apoB-48, and total apoA-I were isolated by ultracentrifugation and subsequent preparative NaDodSO$_4$-PAGE, hydrolyzed, and derivatized. The tracer/tracee ratio was determined by gas chromatography–mass spectrometry. Monoexponential function analysis was used to determine the tracer/tracee curves of VLDL apoB-100 and VLDL apoB-48, and total apoA-I. The mean plateau tracer/tracee ratio of VLDL apoB-100 (primarily liver-derived) was 5.19%, whereas that of VLDL apoB-48 (intestinally derived) was only 3.74%. Using the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool enrichment for apoA-I, the mean apoA-I residence time (RT) was 5.14 ± 0.41 days, compared with 4.80 ± 0.30 days for the exogenous labeling method. The apoA-I RTs using these two methods were highly correlated (r=0.874). We also used several different assumptions about the relative contribution of the liver and the intestine to the total plasma apoA-I pool and compared the kinetic parameters obtained with each of these assumptions to those obtained with the exogenous radiotracer method. The assumption that the liver contributed 90% of the total apoA-I pool resulted in the closest agreement between methods (RT 4.85 ± 0.35 days by stable isotope). In addition, the mean RT of VLDL apoB-48 was 3.9 hours, significantly longer than that of VLDL apoB-100 of 1.9 hours. These data indicate that endogenous labeling of apoA-I by primed constant infusion using the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool tracer/tracee ratio for apoA-I provides kinetic parameters that are highly comparable with those obtained by the exogenous labeling method.—**Ikewaki, K., D. J. Rader, J. R. Schaefer, T. Fairwell, L. A. Zech, and H. B. Brewer, Jr.** Evaluation of apoA-I kinetics in humans using simultaneous endogenous stable isotope and exogenous radiotracer methods. *J. Lipid Res.* 1993. **34:** 2207–2215.

Supplementary key words radiotracer • stable isotopes • high density lipoproteins

Epidemiologic studies have shown that HDL cholesterol (HDL-C) levels are inversely correlated with the incidence of coronary heart disease (CHD) (1, 2). ApoA-I is the major protein constituent of HDL. Plasma apoA-I levels are strongly correlated with HDL-C levels and inversely correlated with CHD risk (3). In vivo kinetic studies in humans have been used to gain insights into factors that regulate plasma levels of apoA-I. Traditionally, these studies have been performed using exogenous labeling with radioiodine. In some studies, HDL were isolated and radioiodinated, whereas in other studies apoA-I was preparatively purified, and radiolabeled. Vega et al. (4) and Schaefer et al. (5) have reported that apoA-I kinetic parameters determined simultaneously by labeling of whole HDL or purified apoA-I were comparable.

Human apolipoprotein metabolism has also been investigated using endogenous labeling methods. Fisher et al. (6) reported studies of apoB metabolism using bolus administration of radiolabeled leucine. Cryer et al. (7) first described the use of a primed constant infusion of stable

Abbreviations: SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; GC–MS, gas chromatography–mass spectrometry; HDL, high density lipoproteins; IE, isotopic enrichment; FCR, fractional catabolic rate; FSR, fractional synthetic rate; RT, residence time; PR, production rate; VLDL, very low density lipoproteins; BMI, body mass index.

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isotopically labeled glycine to assess VLDL apoB-100 metabolism. Cohn et al. (8) and Lichtenstein et al. (9) used primed constant infusion of stable isotopically labeled leucine to endogenously label apoB-100 and apoA-I in order to investigate apoA-I kinetics. In determining apoA-I kinetic parameters, they used the isotopic enrichment of VLDL apoB-100 as the estimate of the isotopic enrichment of the hepatic intracellular leucine precursor pool, based on two assumptions: *1)* that under the study conditions (fed state), the enrichment levels of hepatic and intestinal tissues were similar; and *2)* that the majority of apoA-I in humans is derived from the liver. The fractional synthetic rates of apoA-I by this method were found to be between 0.18 and 0.22 day^{-1}, which were consistent with the previously reported data by radiotracer labeling method. Lichtenstein et al. (10) subsequently showed that in the fed state the intestinally derived protein VLDL apoB-48 reached a lower plateau enrichment level than that of the liver-derived protein such as VLDL apoB-100. ApoA-I is synthesized by the intestine (11–14) as well as the liver, and the estimation of the relative apoA-I mass derived from the intestine ranges from 22% to 77% of the total apoA-I pool (15, 16). Because of these observations, there has been disagreement about the use of the VLDL apoB-100 plateau isotopic enrichment as the estimate of the apoA-I precursor pool enrichment in constant infusion endogenous labeling studies.

The present study was undertaken primarily to address the question whether the VLDL apoB-100 plateau isotopic enrichment can be used as an appropriate estimate of the apoA-I precursor pool isotopic enrichment by directly comparing the apoA-I kinetic parameters obtained using stable isotope and radiotracer methods in the same individuals. We found that the use of the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the apoA-I precursor pool enrichment resulted in the kinetic parameters highly comparable with those obtained using radiolabeled apoA-I in the same individuals.

METHODS

Study subjects

Four young female normolipidemic volunteers were investigated in this study. None of the study subjects had abnormal fasting glucose levels or evidence of thyroid, liver, or renal dysfunction and none were taking medications. Subjects gave informed written consent to the study protocols which were approved by the Clinical Research Subpanel of the National Heart, Lung, and Blood Institute. All subjects had normal plasma total cholesterol, triglyceride, HDL-C, and apoA-I levels, which were determined five times (0, 1, 4, 7, 14 days) during the metabolic study.

Isolation and iodination of apolipoproteins

ApoA-I was isolated from normal HDL by using gel permeation chromatography and ion exchange chromatography as previously described (17) and stored at −20°C. Lyophilized apoA-I was redissolved in a buffer of 6 M guanidine-HCl and 1 M glycine (pH 8.5), and iodinated with ^{125}I by a modification of the iodine monochloride method (18). Iodination efficiency was 15–40% with incorporation of approximately 0.5 mole iodine per mole of protein. Iodinated apoA-I was reassociated with autologous plasma and immediately dialyzed at 4°C for 12 h against four changes of PBS containing 0.01% EDTA (19). The samples were sterile-filtered through a 0.22-μm Millipore filter and tested for pyrogens and sterility prior to injection.

Study protocol

Three days prior to the study, subjects were placed on an isoweight diet containing 47% carbohydrate, 37% fat, 16% protein, 200 mg of cholesterol per 1000 kcal, and a polyunsaturated to saturated fat ratio of 0.3. Meals were given three times per day and the diet was continued during the metabolic study. One day prior to the study, the subjects were started on potassium iodide (900 mg) in divided doses and this was continued throughout the study period. After a 12-h fast, the subjects were injected with up to 50 μCi of ^{125}I-labeled apoA-I. [$^{13}C_6$]phenylalanine (ring-$^{13}C_6$ 99%, Cambridge Isotope Laboratories, Woburn, MA) was simultaneously administered as a priming bolus of 600 μg/kg, immediately followed by a constant infusion of 12 μg/kg per min over a period of 12–14 h. During the infusion, meals were served in equal small portions every 2 h. Blood samples were obtained 10 min after the injection and then at 1, 2, 3, 4, 5, 6, 8, 10, 12, 14, 18, 24, and 36 h, daily through day 5, and day 7, 9, 11, 14. Urine was collected continuously throughout the study.

Blood samples (20 ml) were drawn into the tubes containing EDTA at a final concentration of 0.1%. The blood was kept on ice and the plasma was immediately separated by centrifugation at 2300 rpm for 30 min at 4°C. Sodium azide and aprotinin were added to the plasma at final concentrations of 0.05% and 200 KIU/ml, respectively. Radioactivity in plasma, urine, and isolated lipoprotein fractions was quantitated in a Packard Cobra gamma counter (Packard Instrument Co., Downers Grove, IL).

Isolation of lipoproteins and apolipoproteins

Total plasma lipoproteins were isolated on a TL-100 ultracentrifuge (Beckman Instruments, Palo Alto, CA) using a TLA-100.3 rotor at 100,000 rpm for 10 h after 1 ml of plasma was adjusted at the density of 1.25 g/ml with solid KBr; 97.3 ± 1.8% of total plasma apoA-I was recovered from the supernatant. VLDL was isolated from 5 ml plasma by ultracentrifugation using a 40.3 rotor at 39,000 rpm for 20 h as previously described (20).

Lipoproteins were dialyzed against 10 mM ammonium bicarbonate, lyophilized, and delipidated. VLDL apoB-100 and total apoA-I were isolated by preparative gradient $NaDoSO_4$-PAGE (5–15%) (21).

Determination of isotopic enrichment

Samples were prepared for gas chromatograph–mass spectrometric (GC–MS) analysis as reported previously (21). Briefly, apolipoprotein bands were cut from gels and dried overnight (90°C), then hydrolyzed in 6 N HCl (Ultrapure grade, J. T. Baker, Inc., Phillipsburg, NJ) at 110°C for 24 h. The protein hydrolysates were lyophilized in a Speed-Vac evaporator (Savant Instrument, Inc., Farmingdale, NY). Free amino acids were purified from plasma or protein hydrolysates by cation exchange chromatography (AG-50W-X8, Bio-Rad Laboratories, Richmond, CA), then derivatized to the N-heptafluorobutyryl isobutyl esters, and analyzed by GC–MS on a Finnigan MAT 4500 (Finnigan MAT, San Jose, CA) in the chemical ionization mode, using isobutane as the reagent gas. Selective ion monitoring at 418 *m/z* for unlabeled phenylalanine and 424 *m/z* for [$^{13}C_6$]phenylalanine was used to determine the isotope ratio. Each sample was analyzed at least 3 times. Enrichment was calculated from the isotope ratio using the method of Cobelli, Toffolo, and Foster (22). Enrichment was then converted to tracer/tracee ratios (22) by using the formula: tracer/tracee ratio = $e(t)/(e_I - e(t))$, where $e(t)$ is the enrichment of each sample at time t, and e_I is the enrichment of the infusate, which was 99% in this study.

Analysis of kinetic data

Stable isotope studies. A monoexponential function was fitted to the tracer/tracee ratio curves of VLDL apoB-100, VLDL apoB-48, and total apoA-I using SAAM30. The function was defined as $A(t) = A_p(1-e^{(-k(t-d))})$, where $A(t)$ is the tracer/tracee ratio at time t, A_p is the tracer/tracee ratio of precursor pool for the apolipoprotein of interest, d is the delay time, and k is the fractional synthetic rate (FSR). In estimating the tracer/tracee ratio of the precursor pool for apoA-I synthesis, we used various assumptions about the relative contribution of the intestine (0, 10, 20, 30, 40, 50%) to the total plasma apoA-I pool. For example, use of the VLDL apoB-100 plateau tracer/tracee ratio is based on an assumption that all apoA-I is liver-derived, whereas the use of the mean value of the VLDL apoB-100 and VLDL apoB-48 plateau tracer/tracee ratios is based on an assumption that the liver and the intestine contribute equally to the total plasma apoA-I pool (50%/50%).

Radiotracer studies. Plasma radioactivity decay curves were constructed as fraction of injected dose using the 10-min plasma counts as the initial time, and were fitted to biexponential functions using the SAAM30 program (23). The residence time (RT) was obtained from the area under the plasma decay curve. The fractional catabolic rate (FCR) is the reciprocal of the RT and is equal to the FSR at a steady state.

Analytical methods

Plasma total cholesterol and triglyceride levels were determined by automated enzymatic techniques on an Abbott VPSS analyzer (Abbott Labs, North Chicago, IL). HDL cholesterol was measured by dextran sulfate precipitation (24). Plasma apoA-I concentrations were quantitated using an immunoturbidometric assay (Boehringer-Mannheim, Mannheim, Germany).

RESULTS

The sex, age, body mass index (BMI), and plasma lipid and apolipoprotein values of the study subjects are listed in **Table 1.** All the lipid and apolipoprotein values were normal and were stable throughout the study period, indicating that the studies were performed under steady state conditions.

The tracer/tracee curves of plasma free phenylalanine, VLDL apoB-100, and apoB-48 in a representative subject are shown in **Fig. 1.** The tracer/tracee ratio of plasma free plasma phenylalanine was relatively constant throughout the infusion period. The tracer/tracee ratio of VLDL apoB-100 increased rapidly and reached plateau during the infusion period; the mean VLDL apoB-100 plateau was 5.19 ± 0.49%, which was 90% that of the mean plasma free plasma phenylalanine (5.76 ± 0.39%). In

TABLE 1. Characteristics of study subjects

Subject	Age	BMI	TC	TG	HDL-C	ApoA-I
	yr	*kg/m²*		*mg/dl*		
1	21	19.3 ± 0.2	176 ± 9	61 ± 14	53 ± 3	128 ± 8
2	22	22.0 ± 0.1	185 ± 12	96 ± 19	53 ± 3	138 ± 6
3	21	21.9 ± 0.2	144 ± 6	62 ± 12	49 ± 2	131 ± 4
4	20	19.3 ± 0.1	163 ± 10	40 ± 5	66 ± 2	140 ± 7
Mean ± SD	21 ± 1	20.6 ± 1.3	167 ± 15	65 ± 20	55 ± 6	134 ± 5

BMI and lipids and apolipoproteins values are expressed as the mean ± SD based on the five determinations during the study period. BMI, body mass index; TC, total cholesterol; TG, triglyceride.

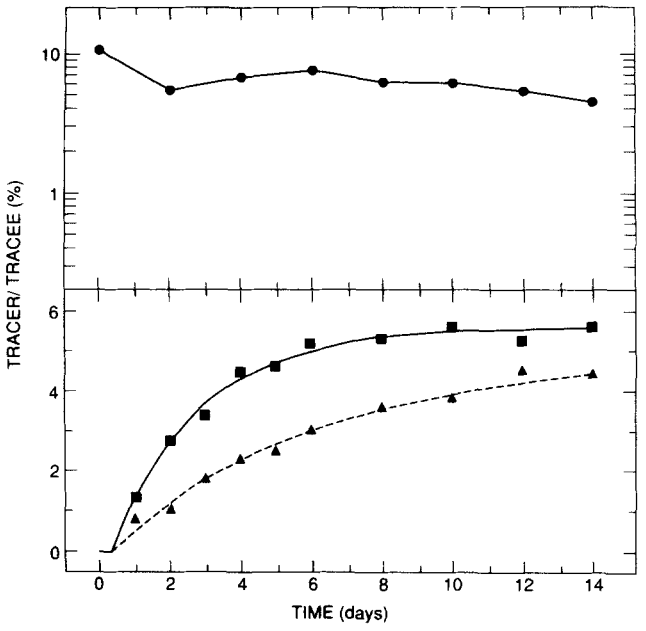

Fig. 1. Tracer/tracee ratio of plasma free phenylalanine (top), VLDL apoB-100 (bottom, solid squares with solid line), and VLDL apoB-48 (bottom, solid triangles with dotted line) in a representative subject (#1). Tracer/trace ratios of VLDL apoB-100 and apoB-48 were fitted by the monoexponential function.

contrast, the tracer/tracee ratio of VLDL apoB-48 increased more slowly to a lower mean plateau level of 3.74 ± 0.80% (68% of free plasma phenylalanine). The residence times of VLDL apoB-100 and apoB-48 are summarized in **Table 2.** The mean residence time of apoB-100 was 1.92 ± 0.40 h, which was significantly shorter than that of VLDL apoB-48 of 3.88 ± 1.38 h (P = 0.046).

The tracer/tracee curves of apoA-I in all four subjects are shown in **Fig. 2.** The tracer/tracee curves were nearly linear during the infusion period and were fitted to a monoexponential function. By endogenous labeling, the mean apoA-I RT determined by using the VLDL apoB-100 plateau tracer/tracee ratio as an estimate of the apoA-I precursor pool enrichment was 5.14 ± 0.41 days.

The plasma decay curves of ^{125}I-labeled apoA-I in all study subjects are shown in **Fig. 3.** The plasma decay curves were fitted to a biexponential function. The mean residence time of apoA-I was 4.80 ± 0.30 days and the mean fractional catabolic rate was 0.209 ± 0.013 day^{-1}, in agreement with the mean urine/plasma radioactivity ratio of 0.208 ± 0.017.

The residence times (RT) of apoA-I using stable isotope and radiotracer methods are summarized in **Table 3.** The residence times determined by these two independent methods were highly comparable, indicating that the use of the VLDL apoB-100 plateau tracer/tracee ratio to estimate apoA-I precursor pool enrichment is a reasonable method to determine apoA-I kinetic parameters.

The apoA-I RTs were also determined based on different assumptions about the apoA-I precursor pool enrichment. The VLDL apoB-100 plateau tracer/tracee ratio was used as the highest estimate of the precursor pool enrichment and the mean of the VLDL apoB-100 and apoB-48 plateau tracer/tracee ratios was used as the lowest estimate of the apoA-I precursor pool enrichment. The apoA-I RTs were significantly lower than the radiotracer values when the intestine was assumed to contribute 20% or more of the total plasma apoA-I pool. An assumption of an equal contribution of the liver and intestine (50% from each organ) resulted in a much lower apoA-I mean RT (3.63 ± 0.62 days, P=0.04). The assumption that resulted in the best agreement with radiotracer data was that liver contributed 90% of the total apoA-I pool (mean RT 4.85 ± 0.34 days, P=0.29).

DISCUSSION

The in vivo kinetics of apoA-I have been investigated primarily by using exogenous radiolabeling techniques. Starting in the late 1970s, several investigators described a method in which HDL was isolated, radioiodinated, and injected into subjects; then plasma apoA-I decay curves were constructed by isolating apoA-I (25–30). In

TABLE 2. Plateau tracer/tracee ratios of labeled phenylalanine and residence times of VLDL apoB-100 and VLDL apoB-48 in study subjects

	Plateau Tracer/Tracee Ratio[a]		Residence Time	
Subject	VLDL ApoB-100	VLDL ApoB-48	VLDL ApoB-100	VLDL ApoB-48
	%		*h*	
1	5.55	4.95	2.50	6.23
2	4.51	3.54	2.19	3.40
3	5.74	3.74	1.56	3.09
4	4.96	2.71	1.53	2.79
Mean ± SD	5.19 ± 0.49[b]	3.74 ± 0.80	1.92 ± 0.40[b]	3.88 ± 1.38

[a] Plateau tracer/tracee levels were determined by monoexponential analysis.
[b] Significantly different (P < 0.05) from VLDL apoB-48.

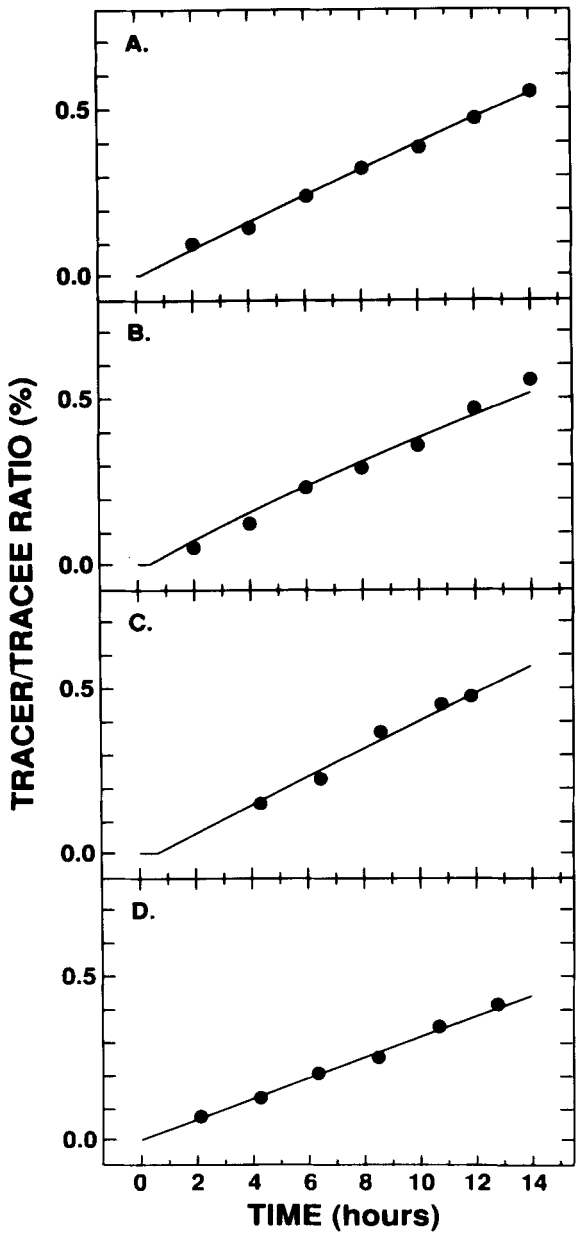

Fig. 2. Tracer/tracee ratio of total apoA-I in study subjects: A) subject #1; B) subject #2; C) subject #3; D) subject #4. Tracer/tracee ratios were fitted by the monoexponential function using VLDL apoB-100 plateau tracer/tracee ratio as the estimate of the precursor pool enrichment for apoA-I.

other reports, apoA-I was purified, then radiolabeled and injected with or without reassociation with lipoproteins (4, 5, 31, 32). ApoA-I kinetic parameters were obtained by fitting plasma decay curves using either computer-assisted multiexponential analysis (SAAM) (23) or the Matthews two-pool model (33). These studies have provided important insights into the regulation of apoA-I metabolism in humans. Nevertheless, there remain theoretical concerns related to the possible modification of apolipoproteins during the isolation and radioiodination (34).

An alternative approach for the investigation of apolipoprotein metabolism in humans is endogenous labeling with labeled amino acids. Radiolabeled amino acids were administered as a bolus injection to investigate apoB-containing lipoprotein metabolism (6, 35–37). More recently, stable isotopically labeled amino acids have been used to study apolipoprotein metabolism (7–10, 21, 38–42). Endogenous labeling has the theoretical advantage that there is no possibility of altering the nature of the apolipoprotein through isolation and labeling. It also permits the simultaneous investigation of multiple apolipoproteins in the same individuals. However, the kinetic analysis of endogenous labeling studies is more complex than that of exogenous radiotracer studies. As a way

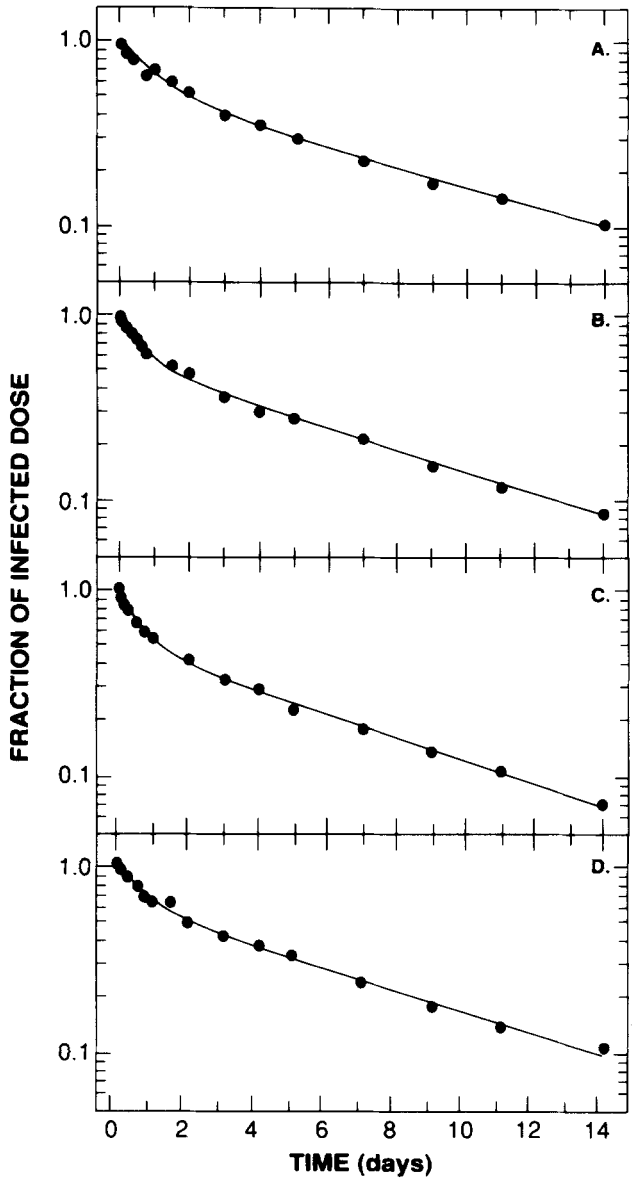

Fig. 3. Whole plasma decay curves of ^{125}I-labeled apoA-I in study subjects: A) subject #1; B) subject #2; C) subject #3; D) subject #4. Data were fitted by the biexponential function.

TABLE 3. Residence times of apoA-I by exogenous radiotracer and endogenous stable isotope labeling methods with different assumptions about the relative contribution of the liver and the intestine to the plasma total apoA-I pool

Subject	Radiotracer	Stable Isotope Relative Contribution to the Plasma Total ApoA-I Pool					
Liver	100	90	80	70	60	50
Intestine	0	10	20	30	40	50
				days			
1	5.10	5.23	5.12	5.01	4.89	4.77	4.65
2	4.42	4.61	4.41	4.21	4.00	3.80	3.59
3	4.59	4.98	4.62	4.26	3.90	3.62	3.18
4	5.08	5.75	5.24	4.70	4.16	3.55	3.08
Mean ± SD	4.80 ± 0.30	5.14 ± 0.41	4.85 ± 0.34	4.54 ± 0.33[a]	4.24 ± 0.39[a]	3.94 ± 0.49[a]	3.63 ± 0.62[a]

[a]Significantly different ($P < 0.05$) from the residence times obtained by radiotracer studies by paired t-test.

of obtaining apolipoprotein kinetic parameters without a compartmental model, Cryer et al. (7) used a primed constant infusion of [^{15}N]glycine and calculated the FSR of the VLDL apoB-100 by dividing the slope of the enrichment curve by the enrichment of urinary hippurate as the estimate of the enrichment of hepatic glycine precursor pool. Cohn et al. (8) first reported HDL apoA-I kinetics using an endogenous labeling technique. The VLDL apoB-100 plateau enrichment was used as the estimate for the precursor pool enrichment for apoA-I based on the assumption either that the majority of apoA-I in humans is derived from the liver or that the enrichment of hepatic and intestinal tissues is similar. Using this approach, they obtained mean apoA-I FSRs of 0.18 day^{-1} in the fasting state and 0.22 day^{-1} in the fed state in normal subjects, consistent with previously reported radiotracer data (4, 5, 43). Similar apoA-I kinetic parameters were found by Lichtenstein et al. (9) and Schaefer et al. (21) using different amino acids.

However, theoretical concerns have been raised about this analytical method for apoA-I kinetics. It has been questioned whether the VLDL apoB-100 plateau enrichment represents an adequate estimate of the precursor pool enrichment for apoA-I synthesis (8–10). ApoA-I has been reported to be synthesized by the intestine as well as the liver (12–14, 44). The plateau enrichment of the intestinal protein VLDL apoB-48 was considerably lower than that of the hepatic protein VLDL apoB-100 in the fed state (10), suggesting that the precursor pool enrichment may be different between the intestine and the liver. Thus, the true precursor pool enrichment for apoA-I may be determined by the relative contribution of each organ to the total plasma apoA-I pool.

The present study was undertaken to determine whether the VLDL apoB-100 plateau enrichment is a practical estimate of the precursor pool enrichment for apoA-I by comparing this method to a well-established exogenous radiotracer method for apoA-I kinetics. We simultaneously investigated apoA-I metabolism by both exogenous radiotracer and endogenous stable isotope labeling methods and then directly compared the kinetic parameters obtained with each method. Our results demonstrate that the apoA-I kinetic parameters using the VLDL apoB-100 plateau tracer/tracee ratio as the estimate of apoA-I precursor pool enrichment were highly comparable to those obtained with the radiotracer. The apoA-I RTs obtained by the two methods were highly correlated ($r = 0.87$), indicating that this endogenous labeling technique can detect differences in apoA-I kinetics among individuals similar to using exogenous radiotracers.

For the endogenous labeling studies, we used several different assumptions about the relative contribution of the liver and the intestine to the total plasma apoA-I pool and compared the kinetic parameters obtained with each of these assumptions to those obtained using the exogenous radiotracer. The kinetic parameters were significantly different when the intestine was assumed to contribute more than 20% of total apoA-I pool. We have previously conducted a primed constant infusion study in a patient with severe hypoalphalipoproteinemia and found that apoA-I reached a plateau tracer/tracee level of 3.0%, similar to that of VLDL apoB-100 of 3.3% (45). This further supports the use of the VLDL apoB-100 plateau tracer/tracee ratio as a reasonable estimate of apoA-I precursor pool tracer/tracee ratio.

We have recently investigated the apoA-I kinetics using an identical protocol with VLDL apoB-100 as the estimate of apoA-I precursor pool tracer/tracee ratio in four patients with extremes of plasma apoA-I levels, three less than 50 mg/dl and one greater than 210 mg/dl (46, 47). **Fig. 4** shows the correlation of apoA-I residence times between radiotracer and stable isotope labeling methods among the current study subjects as well as these other four patients. We found that the correlation observed in the subjects with normal plasma apoA-I levels is true in patients with very low and very high apoA-I concentrations as well, resulting in a very strong overall correlation ($r^2 = 0.98$, $P < 0.001$). These results suggest applicability of primed constant infusion endogenous apoA-I labeling over a wide range of plasma apoA-I levels. However, the

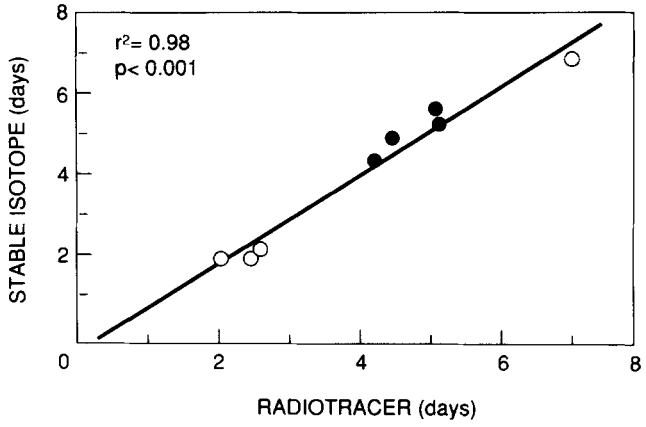

Fig. 4. Correlation of apoA-I residence times between radiotracer and stable isotope methods in four current normal subjects (closed circles), three hypoalphalipoproteinemic subjects (open circles), and one hyperalphalipoproteinemic subject (open circle).

relative contribution of the liver and the intestine could be altered in some subjects, particularly those with liver or intestinal disease, and in these cases the use of the VLDL apoB-100 plateau tracer/tracee ratio may not be reliable.

In the present study, kinetic parameters were determined in the exogenous radiotracer studies using a biexponential function, whereas a monoexponential function was used in the endogenous stable isotope studies. Both methods have been frequently used and are considered to be acceptable methods of analysis. The theoretical argument could be made that because a biexponential analysis was used for the radiotracer studies, a similar analysis should be applied to the stable isotope studies. However, it is highly unlikely that there exists a substantial difference in apoA-I kinetic parameters between monoexponential and biexponential function analysis of stable isotope data as apoA-I tracer/tracee curves were nearly linear during the infusion period.

Although the purpose of this study was not to determine the relative contribution of the liver and the intestine to the plasma apoA-I pool in humans, the assumption that resulted in the closest correlation with the radiotracer values was that 90% of total apoA-I pool was liver-derived (Table 3). There have been few studies in which the contribution of intestine to the plasma apoA-I pool was estimated in humans. Anderson et al. (15) estimated in patients with thoracic duct drainage that 22–77% of daily total apoA-I synthesis took place in the intestine. Green et al. (16) estimated that about 50% of apoA-I originated in the intestine in chyluric patients. However, in these studies, it is likely that the contribution of the intestine was overestimated because there is substantial filtration of apoA-I from plasma to lymph. Furthermore, subjects in these studies may have had altered intestinal apoA-I synthesis. Our results suggest that more than half of plasma apoA-I derives from the liver.

In this study, we confirmed the findings by Lichtenstein and co-workers (10) that VLDL apoB-100 turnover is similar to or slightly faster than VLDL apoB-48 turnover, in contrast to some radiotracer data (48, 49). Although the labeled amino acid they used was different from ours, the study protocol (fed state) and data analysis (monoexponential function) were similar. In this study, we found that the VLDL apoB-48 plateau tracer/tracee ratio was 28% lower than that of VLDL apoB-100, compared with 45% less observed by Lichtenstein et al. (10). The mean residence time of VLDL apoB-100 (1.92 ± 0.40 h) was significantly shorter than that of VLDL apoB-48 (3.88 ± 1.38 h) in our study. The mean residence times of VLDL apoB-100 and apoB-48 in the study by Lichtenstein et al. (10) were somewhat longer (mean 4.98 and 6.84 h, respectively). This may be partially explained by the use of different amino acids, the relatively higher mean fasting plasma TG levels in their study subjects, or the lack of a delay time in their monoexponential model.

In summary, endogenous labeling of apoA-I using a primed constant infusion of a stable isotopically labeled amino acid results in apoA-I kinetic parameters that are very similar to those obtained using radioiodinated apoA-I as a simultaneous tracer in the same individuals when the VLDL apoB-100 plateau tracer/tracee ratio is used as the estimate of the precursor pool enrichment for the total apoA-I pool. Strong positive correlation between the kinetic parameters obtained by the two methods was observed among subjects with a wide range of plasma apoA-I concentrations. Thus, the use of the VLDL apoB-100 plateau tracer/tracee ratio as an estimate of the precursor pool enrichment for apoA-I synthesis appears to be a reasonable method for determining the apoA-I turnover rate. The simultaneous comparison of these two independent methods helps to validate both methods as useful tools in the investigation of in vivo apoA-I metabolism in humans.

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