Human apolipoprotein A-I kinetics within triglyceride-rich lipoproteins and high density lipoproteins

Wanda Vélez-Carrasco,* Alice H. Lichtenstein,* P. Hugh R. Barrett,† Zhiyong Sun,* Gregory G. Dolnikowski,* Francine K. Welty,* and Ernst J. Schaefer[1,*]

Lipid Metabolism Laboratory, Mass Spectrometry Laboratory,* Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University, Boston, MA 02111, and the Resource Facility for Kinetic Analysis, Center for Bioengineering,† University of Washington, Seattle, WA 98195

Abstract **Stable isotope methodology was used to determine the kinetic behavior of apolipoprotein (apo) A-I within the triglyceride-rich lipoprotein (TRL) fraction and to compare TRL apoA-I kinetics with that of apoA-I in high density lipoprotein (HDL) and TRL apoB-48. Eight subjects (5 males and 3 females) over the age of 40 were placed on a baseline average American diet and after 6 weeks received a primed-constant infusion of [5,5,5-$^2H_3$]-l-leucine for 15 h while consuming small hourly meals of identical composition. HDL and TRL apoA-I and TRL apoB-48 tracer/tracee enrichment curves were obtained by gas chromatography–mass spectrometry. Data were fitted to a compartmental model to determine the fractional secretion rates of apoA-I and apoB-48 within each lipoprotein fraction. Mean plasma apoA-I levels in TRL and HDL fractions were 0.204 ± 0.057 and 134 ± 15 mg/dl, respectively. The mean fractional catabolic rate (FCR) of TRL apoA-I was 0.250 ± 0.069 and HDL apoA-I was 0.239 ± 0.054 pools/day, with mean estimated residence times (RT) of 4.27 and 4.37 days, respectively. The mean TRL apoB-48 FCR was 5.2 ± 2.0 pools/day and the estimated mean RT was 5.1 ± 1.8 h. Our results indicate that apoA-I is catabolized at a slower rate than apoB-48 within TRL, and that apoA-I within TRL and HDL fractions are catabolized at similar rates.**—Vélez-Carrasco, W., A. H. Lichtenstein, P. H. R. Barrett, Z. Sun, G. G. Dolnikowski, F. K. Welty, and E. J. Schaefer. **Human apolipoprotein A-I kinetics within triglyceride-rich lipoproteins and high density lipoproteins.** ***J. Lipid Res.*** **1999.** 40: **1695–1700.**

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In mammals, both the small intestine and the liver are the major organs involved in apolipoprotein (apo)A-I biosynthesis (1–3). In fat-fed rats, the small intestine and the liver contribute to approximately 56% and 44% of the total apoA-I in plasma, respectively (4). Human liver and small intestinal biopsies obtained from fasted subjects showed significant amounts of apoA-I mRNA (5).

The direct contribution of the intestine to human plasma apoA-I has been difficult to determine in part because of the limitations in isolating nascent intestinal lipoproteins. In a study by Glickman et al. (6), intestinal biopsies were obtained in four subjects before and after a fat load. Small amounts of apoA-I were found in the fasting intestinal epithelial cells but there was a marked increase in apoA-I content after fat ingestion. Subsequently, Green et al. (7) isolated the triglyceride-rich lipoprotein (TRL) particles excreted in the urine of two subjects with chyluria and showed that the small intestine contributes to approximately 50% of the total amount of apoA-I synthesized daily in normal subjects. In a third study (8) the thoracic duct lymph was collected in five subjects undergoing lymph drainage. About one-third of lymph apoA-I was found in the TRL fraction. Ikewaki et al. (9) compared the apoA-I residence times obtained using exogenous radiotracers with those ones determined by stable isotopes and made several assumptions about the intestinal and hepatic contributions to plasma apoA-I. The authors reported that the best agreement between the two labeling methods was obtained when contributions of the liver and the intestine to plasma apoA-I were 90% and 10%, respectively.

The fate of intestinal apoA-I once chylomicrons are released into the circulation was examined by Schaefer, Jenkins, and Brewer (10) during incubations of chylomicrons with plasma and in vivo after an intravenous injection of radiolabeled chylomicrons. The authors demonstrated that most of the radiolabeled apoA-I was rapidly transferred to plasma high density lipoprotein (HDL). Furthermore, Green and colleagues (7) observed that the loss of apoA-I from chylomicrons was influenced by the concentration of HDL in plasma. The amount of apoA-I in chylomicrons decreased when the concentration of HDL was increased

Abbreviations: apo, apolipoprotein; TRL, triglyceride-rich lipoproteins; HDL, high density lipoproteins; HDL-C, HDL-cholesterol; LCAT, lecithin:cholesterol acyltransferase; TC, total cholesterol; HFBA, heptafluorobutyric anhydride; T/T, tracer/tracee; FSR, fractional secretion rate; FCR, fractional catabolic rate; SR, secretion rate; RT, residence time.

[1] To whom correspondence should be addressed.

(0.05 to 1.5 mg/ml) in the incubation mixture. The transfer of apoA-I to HDL was not altered by incubation of chylomicrons with plasma at 4°C or with an inhibitor of lecithin:cholesterol acyltransferase (LCAT) activity, suggesting that the loss of apoA-I from chylomicrons occurred spontaneously and did not depend on LCAT activity. Loss of apoA-I from chylomicrons incubated with lipoprotein lipase was observed in the absence of HDL particles (11). Most of the apoA-I radioactivity was found in the HDL density region.

In plasma, the majority of apoA-I is found in the HDL fraction (80–90%) but small amounts are present at the density fraction of $d > 1.21$ g/ml and in the TRL fraction (12). Whether apoA-I within TRL is associated with chylomicrons or very low density lipoproteins (VLDL) was examined by Cohn et al. (13) using an affinity column to separate apoB-100 TRL from apoB-48 TRL. The authors observed that only the TRL fraction containing apoB-48 also contained apoA-I, indicating that the intestine and not the liver produces TRL particles with apoA-I.

The purpose of this study was to examine the kinetic behavior of TRL apoA-I and compare it to HDL apoA-I and apoB-48 in plasma during constant feeding conditions using stable isotopes. Our specific aim was to investigate whether the apoA-I associated with TRL has the same or different kinetics as the apoA-I recovered in HDL.

METHODS

Subjects

Eight normolipidemic subjects underwent a complete physical examination and medical history prior to admission into the study. None of the volunteers had any evidence of hepatic, cardiac, renal, or endocrine dysfunction or a family history of these illnesses. The subjects did not smoke or consume alcohol regularly and were not taking medications known to alter plasma lipid levels. All females were postmenopausal. The Human Investigation Review Committee of the New England Medical Center and Tufts University approved the experimental protocol. All subjects gave informed consent to participate in the study.

Experimental design

The subjects were placed on a baseline average American diet for 6 weeks. The diet consisted of 49% carbohydrate, 15% protein, 35% fat (14% saturated, 14% monounsaturated, and 7% polyunsaturated fat) and 147 mg of cholesterol/1000 kcal. All food and drink was prepared and provided to the subjects by the Metabolic Research Unit of the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University. Energy intake was adjusted to keep body weight constant (± 1 kg) throughout the 6-week period.

After consumption of the average American diet, a primed-constant infusion of [5,5,5-$^2H_3$]-l-leucine was carried out for 15 h. After fasting for 12 h, subjects were fed hourly for 20 h starting at 6 am, and each meal consisted of 1/20th their daily caloric intake. Five hours after their first meal, subjects received an intravenous bolus dose (10 μmol/kg) followed by a constant infusion of [5,5,5-$^2H_3$]-l-leucine at 10 μmol/kg per h. After 15 h, the primed constant infusion and hourly feeding were terminated. Blood samples (20 ml) via a second intravenous line were collected at h 0, 1, 2, 3, 4, 6, 8, 10, 12, and 15 h.

Plasma lipid and lipoprotein determinations

Blood samples were collected in tubes containing 0.15% EDTA and were centrifuged at 2500 rpm for 20 min at 4°C to separate plasma. HDL-cholesterol (HDL-C) was measured in plasma after precipitation of apoB-containing lipoproteins with dextran sulfate–$MgCl_2$ (14). Triglyceride-rich lipoproteins (TRL, $d <$ 1.006 g/ml) and HDL (1.063–1.21g/ml) fractions were obtained by sequential density gradient ultracentrifugation (Beckman Instruments Inc, Palo Alto, CA) as previously described (15). Plasma lipids and the 1.006 g/ml supernatant fraction cholesterol were determined by enzymatic methods using an automated analyzer (Abbott Diagnostics Spectrum CCX analyzer) and Abbott enzymatic reagents (16). LDL-C was calculated as: total cholesterol (TC) − (1.006 g/ml supernatant cholesterol + HDL-C). All lipid and protein assays were performed in duplicate and the coefficients of variation within and between runs were 2 to 5%.

Quantitation and isolation of apolipoproteins

Plasma apoA-I concentration was measured by an immunoturbidimetric assay (17), using the Spectrum CCx analyzer (Abbott Diagnostics), with reagents and calibrators from Incstar Corp. (Stillwater, MN). The coefficients of variation were 4% between run and 3% within run. TRL apoA-I and total apoB were measured by ELISA using immunopurified polyclonal antibodies (18). The coefficient of variation for the apoA-I assay within and between runs was less than 7%. For the apoB determination, the coefficient of variation within runs was less than 5% and between runs was less than 10%. ApoA-I, apoB-100, and apoB-48 from the TRL fraction and apoA-I from the HDL fraction were isolated by SDS polyacrylamide gradient gel electrophoresis using a 4–22% and a 6–30% acrylamide linear gradient, respectively (19). Protein bands were identified by comparing their migration bands with those of known molecular weight proteins (low molecular weight proteins, Sigma Chemical Co. St Louis, MO). The gel bands were stained and scanned in two dimensions using a laser densitometer (LKB Instruments Inc., Paramus, NJ). The relative intensity of the apoB-48 and the apoB-100 bands was measured. The concentration of apoB-48 was then estimated from the total apoB TRL measurement obtained by ELISA.

Isotopic enrichment determinations

Polyacrylamide gel bands for apoA-I were cut and hydrolyzed with 12 N HCl at 110°C for 24 h (19). The amino acids were derivatized by adding propanol/acetyl chloride and heptafluorobutyric anhydride (HFBA) reagents. The derivatives were then extracted into ethyl acetate for analysis using a gas chromatograph–mass spectrometer (Hewlett-Packard Co., Model 5890/5988, Palo Alto, CA). The amino acid derivatives were ionized by methane negative chemical ionization and ion monitoring at m/z = 349 to 352 was used to determined the isotopic ratio (labeled/unlabeled leucine × 100) of each sample. Enrichment was calculated from the isotopic ratio and then converted to a tracer/tracee (T/T) ratio (20) according to the formula: $e(t)/e_{(I)}$-$e(t)$, where $e(t)$ is the enrichment at time t and $e_{(I)}$ represents the isotopic abundance of the infusate which for this study was 99.8%. As controls, gel slices containing no protein band were also processed.

Model assumptions

Fractional secretion rates (FSR) of TRL and HDL apoA-I were determined by fitting the tracer/tracee ratios to a multicompartment model using the SAAM II program. The model consisted of a total of 6 compartments (**Fig. 1**). Compartments 1 and 4 were the apolipoprotein precursor pools, defined by a forcing function representing the apoB-100 and apoB-48 enrichment plateaus, respectively (21). Compartments 2 and 5 were the intracellular delay compartments representing the time required for the

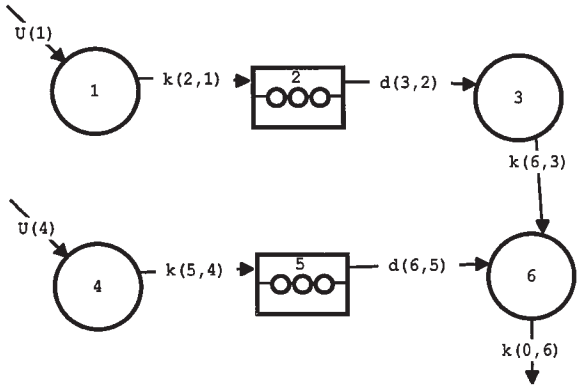

Fig. 1. ApoA-I compartmental model, where compartments 1 and 4 are precursor pools, compartment 4 is TRL, and compartment 6 is HDL.

synthesis and secretion of apoA-I from intestine and liver. The delay time was estimated at 30 mins

Compartments 3 and 6 were the tracer/tracee enrichment curves of apoA-I within TRL and HDL lipoproteins, respectively. There is a transfer of apoA-I from the TRL to the HDL compartment and a direct catabolism of apoA-I from the HDL compartment only. We assumed that all intestinal TRL apoA-I destined to become HDL apoA-I was transferred from chylomicrons to HDL.

The apoB-48 model contains three compartments (**Fig. 2**). Compartment 1 represented the apoB-48 plateau enrichment and was used as an estimate of the precursor pool enrichment. Compartment 2 was the intracellular delay compartment. Compartment 3 represented the apoB-48 TRL T/T ratio with direct catabolism of apoB-48 from this compartment.

Kinetic analysis

In order to analyze the kinetic data, we assumed that a primed constant infusion provides a constant enrichment of plasma leucine and a constant enrichment of the precursor pool. Previous studies from our laboratory have reported that under similar study conditions, plasma leucine reaches plateau after 1 h and remains constant throughout the infusion. We also assumed that each subject was studied under steady state conditions with regard to the lipid and apolipoprotein concentrations. The caloric content and composition of each hourly meal remained constant and was designed to achieve a steady state (19, 22).

Under steady state conditions, the FSR equals fractional catabolic rate (FCR). The secretion rates (SR) of TRL and HDL apoA-I were calculated as: fractional secretion rate (pools/day) × apolipoprotein pool size (mg)/body weight (kg) and are expressed as mg/kg per day. Pool sizes were defined as: apolipoprotein plasma concentration (mg/dl) × plasma volume (0.045 l/kg body weight). The residence time (RT) represents the inverse of the FCR.

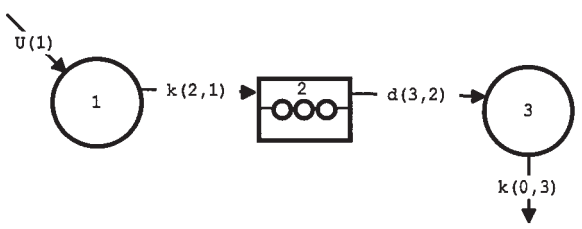

Fig. 2. ApoB-48 compartmental model, where compartment 1 is the precursor pool and compartment 3 is TRL.

Statistical analyses

The SYSTAT 7.1 software program (SPSS, Inc., Chicago, IL) was used for all statistical analyses. Wilcoxon's nonparametric paired *t*-test was used to test for the statistical significance ($P < 0.05$) of differences between TRL and HDL apoA-I FCRs.

RESULTS

Study subjects

Study subjects had a mean age of 57 ± 15 y (5 males, mean age = 49 ± 12 y and 3 females, mean age = 71 ± 4 y) and a mean body mass index (BMI) of 25 ± 3 kg/m^2 (males = 24 ± 1.5 and females = 26 ± 4.3). Plasma lipid and lipoprotein levels during the constant fed state were within the normal range (**Table 1**). Compared to the fasting values (data not shown), there was a significant decrease in HDL-C and apoA-I levels associated with an increase in triglyceride levels during the constant feeding period.

TRL and HDL apoA-I T/T ratios and pool sizes

Figure 3 shows the mean percent tracer/tracee ratios for apoA-I and apoB-48 within TRL and apoA-I within HDL over the 15-h infusion period. The slopes of the TRL and HDL apoA-I curves were 0.044 and 0.059 with confidence intervals between 0.040–0.048 and 0.054–0.064, respectively. The slope of the apoB-48 curve was 0.303 with a confidence interval between 0.206 and 0.400. The mean apoA-I concentration in total plasma was 134 ± 15 versus 0.204 ± 0.057 mg/dl within the TRL fraction (**Table 2**). Mean apoA-I concentration and pool size within the TRL fraction represented 0.15% of the total mean apoA-I concentration in plasma. Although apoA-I levels in TRL are part of the whole plasma apoA-I concentration, subtracting this amount has a negligible effect on total plasma apoA-I levels. The estimated mean apoB-48 concentration was 0.41 ± 0.24 mg/dl and the pool size was 14 ± 8.7 mg. The mean tracer/tracee ratio plateau enrichment of TRL apoB-48 was significantly lower than that of TRL apoB-100 (5.28 ± 1.68 vs. 6.88 ± 1.24, $P < 0.015$).

Kinetic parameters of apoA-I and apoB-48 within TRL and HDL apoA-I

A representative graph of the fitting of the data to the model is shown in **Fig. 4**. Mean TRL and HDL apoA-I FCRs were 0.250 ± 0.069 and 0.239 ± 0.054 pools/day, respectively ($P = 0.76$) (**Table 3**). The estimated mean TRL and HDL apoA-I RTs were 4.27 ± 1.15 and 4.37 ± 0.93 days, respectively. The mean HDL apoA-I SR was about 650-fold higher than the TRL apoA-I SR (14.6 ± 4.4 vs. 0.0223 ± 0.007 mg/kg per day). The greater HDL apoA-I SR resulted from the larger pool size. The mean TRL apoB-48 FCR was 5.2 ± 2.0 pools/day and the mean RT 0.214 ± 0.076 days (**Table 4**). The mean TRL apoB-48 RT was 5.1 h compared to 4.3 days for TRL apoA-I.

TABLE 1. General characteristics and plasma lipid, lipoprotein, and apoA-I values during the constant feeding condition

Subject	Gender	Age	TC	TG	VLDL-C	LDL-C	HDL-C	ApoA-I
1	M	45	170 ± 4	206 ± 23	18 ± 1	112 ± 3	40 ± 2	127 ± 5
2	M	41	178 ± 9	111 ± 32	10 ± 3	125 ± 8	42 ± 4	136 ± 11
3	F	67	199 ± 5	130 ± 14	14 ± 2	138 ± 3	47 ± 2	129 ± 9
4	F	74	238 ± 7	212 ± 25	24 ± 6	163 ± 8	52 ± 3	164 ± 2
5	M	41	217 ± 5	137 ± 14	11 ± 2	170 ± 5	37 ± 2	134 ± 9
6	F	71	168 ± 5	80 ± 8	6 ± 1	115 ± 4	47 ± 2	126 ± 6
7	M	47	170 ± 4	138 ± 11	14 ± 2	123 ± 3	34 ± 1	114 ± 2
8	M	69	250 ± 13	169 ± 19	19 ± 2	182 ± 10	50 ± 4	145 ± 8
Mean ± SD		57 ± 15	199 ± 33	148 ± 45	15 ± 6	141 ± 27	44 ± 6	134 ± 15

Lipid and lipoprotein values represent the individual mean (mg/dl) SD of 10 timepoints whereas apoA-I values are the mean of 4 timepoints during the 15-h infusion.

DISCUSSION

We have examined the kinetic behavior of apoA-I within TRL and HDL fractions in plasma. Using a multicompartment model we have determined the turnover rates of apoA-I within each lipoprotein fraction. The postprandial reponses in our study were similar to those observed during a fat feeding with regard to the changes in HDL-C and apoA-I levels (23). However, the magnitude of the postprandial increase in plasma triglyceride was smaller during the constant feeding of small meals compared to a single large fat-rich meal (data not shown).

The apoA-I FCR (0.250 pools/day) was 21 times slower than the TRL apoB-48 FCR (5.2 pools/day), but comparable to the apoA-I FCR in HDL (0.239 pools/day). ApoB-48 is a protein found exclusively in chylomicron and chylomicron remnants. Thus it serves as a marker for chylomicron turnover. The mean apoB-48 RT from this study is consistent with our previous studies (22). Other investigators have examined the kinetics of chylomicron remnants using retinyl palmitate (24). However, in contrast to apoB-48, we have noted that retinyl esters can exchange between lipoprotein particles containing apoB-48 and apoB-100 (13). Therefore apoB-48 appears to be a suitable marker of chylomicron remnant metabolism.

As most of the apoA-I in TRL is transferred to plasma HDL once chylomicrons enter the circulation (7, 10), the estimated TRL apoA-I SR is probably an underestimation and does not represent the amount originally synthesized and secreted from the intestine. In addition, some of the TRL apoA-I may not have been synthesized directly from the intestine but may be present due to an exchange of lymph HDL apoA-I with newly formed chylomicrons (8).

Our data indicate that the leucine enrichment of apoA-I in the TRL fraction was lower than that in the HDL fraction. Although there is always the possibility of contamination during sample processing, we follow measures to minimize its occurrence. Our samples were not

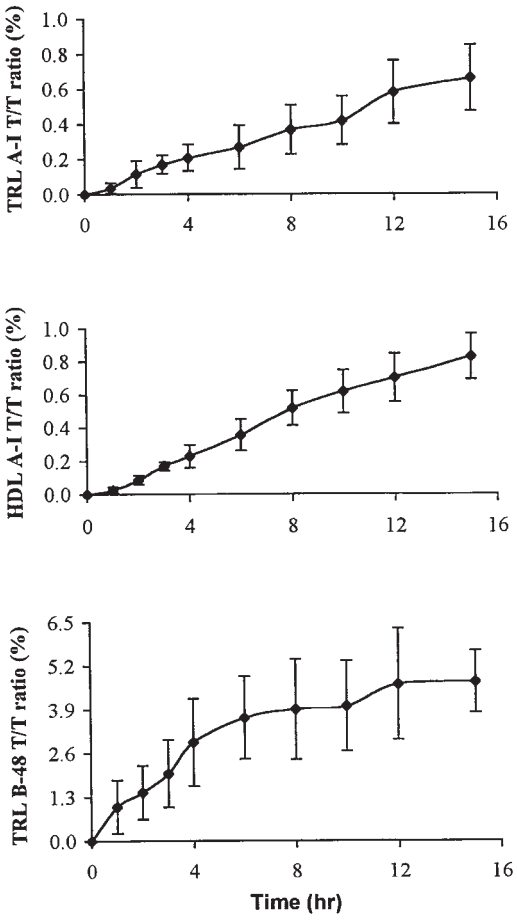

Fig. 3. Mean plasma apoB-48 and apoA-I TRL, and apoA-I HDL tracer/tracee (T/T) ratios during the constant leucine infusion.

TABLE 2. ApoA-I concentration and pool size

Subject	TRL A-I	TRL A-I Pool Size	Plasma A-I	Plasma A-I Pool Size
	mg/dl	*mg*	*mg/dl*	*mg*
1	0.212	6.77	127	4058
2	0.287	10.57	136	5015
3	0.272	8.69	129	4115
4	0.159	6.28	164	6483
5	0.237	7.45	134	3802
6	0.150	4.05	126	3399
7	0.168	5.97	114	4056
8	0.145	4.10	145	4555
Mean ± SD	0.204 ± 0.057	6.74 ± 2.20	134 ± 15	4435 ± 957

Values for each subject represent the mean of 4 measurements made during the 15-h infusion period.

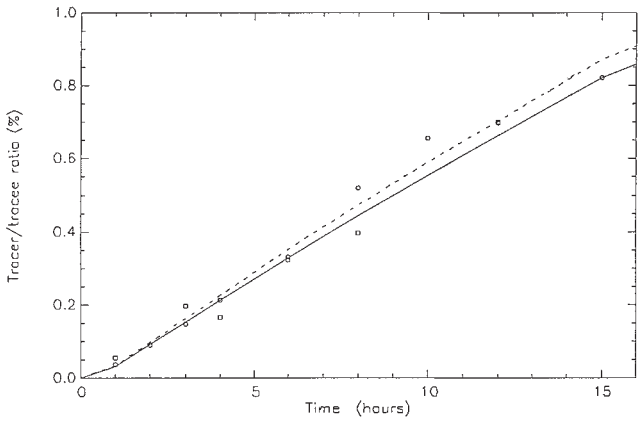

Fig. 4. ApoA-I tracer/tracee ratios within the TRL (squares; ——) and HDL (circles; -------) fractions after a constant leucine infusion in a representative subject. The lines represent the fits obtained using the model shown in Fig. 1.

TABLE 4. TRL apoB-48 kinetic parameters

Subject	B-48	Pool Size	FCR	RT	SR
	mg/dl	*mg*	*day*$^{-1}$	*1/FCR*	*mg/kg/day*
1	0.32	10.2	4.3	0.233	0.62
2	0.33	12.2	7.1	0.141	1.06
4	0.68	26.8	3.0	0.333	0.91
5	0.13	3.7	5.3	0.189	0.28
6	0.32	8.7	8.6	0.116	1.25
7	0.31	11.2	3.6	0.278	0.51
8	0.81	25.5	4.8	0.208	1.94
Mean	0.41	14.0	5.2	0.214	0.94
± SD	0.24	8.7	2.0	0.076	0.55

dialyzed before electrophoresis nor were isolated bands subjected to cation exchange columns in order to minimize such contamination (25). Both of these measures minimize the exposure of samples to unlabeled leucine. In addition, we measured the leucine mass peak on gel blanks and negligible amounts of unlabeled leucine were detected.

We modeled the data assuming an exchange of apoA-I between the plasma TRL and the plasma HDL compartments. With this model the amount of apoA-I transferred from the plasma HDL compartment into the plasma TRL compartment was undetectable. In our study, the TRL apoB-48 FCR at 5.2 pools/day was much greater than that of TRL apoA-I and HDL apoA-I which were 0.250 and 0.239 pools/day, respectively. One possible explanation for our results is that TRL and HDL apoA-I have a similar kinetic behavior in plasma through an exchange of apoA-I between the two lipoproteins. However, this exchange occurs too fast in vivo for the model to be able to measured it.

The fact that the mean slope of the TRL apoA-I was lower than the mean slope of the HDL apoA-I curve could be explained by the differences in the enrichment of the precursor pool. As described in our model, we assumed that TRL apoA-I was produced in the intestine whereas HDL apoA-I was made in the liver. Although one of the assumptions with regard to the use of a primed-constant infusion is that it provides a constant level of the tracer in the precursor pool, our study was carried out under constant feeding conditions. We observed that this exogenous source of unlabeled leucine has the effect of diluting the intestinal pool more so than the hepatic pool. We observed that the mean TRL apoB-48 plateau enrichment was 22% lower than the mean apoB-100 plateau enrichment. This difference agrees with the 25% lower mean TRL apoA-I curve slope compared to the mean HDL apoA-I slope. Therefore, our data suggest that the dilution of the intestinal pool with dietary leucine results in a lower enrichment of intestinally derived proteins such as TRL apoA-I.

In summary, we have shown that plasma TRL apoA-I and HDL apoA-I have similar fractional catabolic rates. In contrast, TRL apoB-48 is catabolized about 20 times faster than TRL apoA-I, suggesting the presence of a rapid exchange of apoA-I between TRL and HDL in plasma.

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TABLE 3. ApoA-I TRL and HDL kinetic parameters

	TRL A-I					HDL A-I				
Subject	A-I	Pool Size	FCR	RT	SR	A-I	Pool Size	FCR	RT	SR
	mg/dl	*mg*	*day*$^{-1}$	*1/FCR*	*mg/kg/day*	*mg/dl*	*mg*	*day*$^{-1}$	*1/FCR*	*mg/kg/day*
1	0.212	6.77	0.211	4.74	0.0202	127	4058	0.178	5.62	10.15
2	0.287	10.57	0.214	4.67	0.0276	136	5015	0.342	2.92	20.89
3	0.272	8.69	0.158	6.33	0.0193	129	4115	0.241	4.15	13.94
4	0.159	6.28	0.229	4.37	0.0163	164	6483	0.260	3.85	19.17
5	0.237	7.45	0.323	3.10	0.0344	134	3802	0.250	4.00	13.56
6	0.150	4.05	0.363	2.75	0.0245	126	3399	0.185	5.41	10.46
7	0.168	5.97	0.294	3.40	0.0222	114	4056	0.195	5.13	10.02
8	0.145	4.10	0.210	4.76	0.0137	145	4555	0.259	3.86	18.71
Mean	0.204	6.74	0.250	4.27	0.0223	134	4435	0.239	4.37	14.61
± SD	0.057	2.20	0.069	1.15	0.0066	14.9	957	0.054	0.929	4.42

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