Kinetic evidence for both a fast and a slow secretory pathway for apolipoprotein A-I in humans

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Abstract The kinetics of apolipoproteins A-I and A-II were examined in human subjects using leucine tracers administered intravenously. High density lipoproteins were separated and apoA-I and A-II were isolated. The specific activity or enrichment data for these apolipoprotein were analyzed by mathematical compartmental modeling. In 11 of 14 subjects studied with a bolus-injected [^{3}H]leucine tracer, in 3 subjects studied similarly with [^{3}H]leucine, and in one subject studied by primed dose, constant infusion of [^{3}H]leucine, a rapidly turning-over apoA-I fraction was resolved. A similar component was observed in 7 of 10 studies of apoA-II. The apoA-I data were analyzed using a compartmental model (Zech, L. A. et al. 1983. *J. Lipid Res.* **24:** 60–71) modified to incorporate plasma leucine as a precursor for apoprotein synthesis. The data permitted resolution of two apoA-I pools, one, C(2), turned-over with a residence time of less than 1 day, the other, C(1), a slowly turning-over pool, appeared in plasma after a delay of less than half a day. C(1) comprised the predominant mass of apoA-I and was also the primary determinant of the residence time of apoA-I. Although the mass of the fast pool, C(2), was considerably less than that of C(1), because of its rapid turnover, the quantities of apoA-I transported through this fast pathway were 2- to 4-fold greater. These kinetic studies indicate that apoA-I is secreted into both fast and slowly turning-over plasma pools. The latter is predominantly measured with radioiodinated apoA-I tracers. The data can be analyzed by postulating either separate input pathways to each of the pools or by assuming the fast pool is the precursor to the slow pool. Thus, apoA-I could be initially secreted as a family of particles that are rapidly cleared from plasma, and a portion of this apoprotein then reappears in a slowly turning-over pool that constitutes the major mass of apoA-I. The physiologic identity of these kinetically distinct apoA-I species is unknown; however, the fast pool of apoA-I demonstrated in these studies is strikingly similar to that seen in subjects with Tangier disease who lack the slow pool.—**Fisher, W. R., V. Venkatakrishnan, L. A. Zech, C. M. Hall, L. L. Kilgore, P. W. Stacpoole, M. R. Diffenderfer, K. E. Friday, A. E. Sumner, and J. B. Marsh.** Kinetic evidence for both a fast and a slow secretory pathway for apolipoprotein A-I in humans. *J. Lipid Res.* 1995. **36:** 1618–1628.

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Apolipoproteins (apo) A-I and A-II are the main proteins associated with high density lipoprotein (HDL), a plasma lipoprotein of major significance because of its role in plasma cholesterol transport. Although apoA-I and apoA-II exist in a variety of particles differing in apolipoprotein and lipid composition, they are predominantly found in two families of particles: those containing apoA-I only, and those containing both apoA-I and apoA-II (1, 2). The metabolism of these apolipoproteins has been studied in vivo by a number of investigators using tracer kinetic methodologies, either with a radioiodinated apoprotein administered as a bolus (3–9) or a heavy isotopically labeled amino acid, such as leucine or phenylalanine, administered during an 8–12 h infusion (10–12). Our laboratories have been interested in the use of leucine as an endogenous tracer, administered either as a bolus or as a primed dose, constant infusion but with data collected after the termination of the infusion. Using these methods we have been able to demonstrate the occurrence of a rapidly turning-over apoA-I pool, which contributes little to the total mass or residence time of apoA-I but through which flows the major portion of apoA-I entering plasma. ApoA-II appears to have similar kinetics.

Abbreviations: apo, apolipoprotein; HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; IV, intravenous; FCR, fractional catabolic rate; FSR, fractional synthetic rate.

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STUDY DESIGN

Protocol for subjects studied by bolus injection of [^{3}H]leucine tracer

Eleven kinetic studies were performed in 6 subjects heterozygous for familial hypercholesterolemia and one study in a normal subject (subjects N-3 and FH-2,3,5,6,7 (ref. 13), also subject FH8, a 59-yr-old white female, with a total cholesterol of 464, HDL-cholesterol 53, low density lipoprotein (LDL)-cholesterol 303, and triglyceride of 132 mg/dl). The clinical protocol has been described in detail (13). In brief, subjects who were housed in a metabolic ward on a constant diet received an IV bolus injection of 0.005 mCi/kg of [^{3}H]leucine at 11 AM, after which 20 blood samples were drawn over 2 weeks at the times shown in the figures that follow. Beginning early on the morning of tracer injection, and continuing for 48 h, the subjects received their nourishment as four equal feedings at 8 AM, noon, 6 and 11 PM.

Protocol for subjects studied by bolus injection of [^{2}H]leucine

Three normal subjects were studied while consuming their usual diets (no intake of alcohol occurred for 4 days prior to each study). The subjects fasted for 12 h prior to the injection at 8 AM of an IV bolus of 60 μmol/kg of [^{2}H]leucine (5,5,5-D_3, 99%, Cambridge Isotopes Labs, Inc., Andover, MA), and blood samples were removed via an indwelling catheter during the first 12 h. The subjects returned home for an evening meal and reported the following morning, fasting, for blood sampling and again on subsequent days at the times indicated in the figures that follow.

Protocol for the normal subject (IF-1) studied by a primed dose, 12-h constant infusion of [^{2}H]leucine

The protocol was identical except that the priming dose of [^{2}H]leucine at the start of the study was 10 μmol/kg, followed by an IV infusion of 10 μmol/kg per h for 12 h.

METHODS

These studies were conducted in two different laboratories and the methodology used for recovering and analyzing the HDL reflected the different tracers used in the studies.

Methodology for [^{3}H]leucine samples

Apolipoprotein B-containing lipoproteins were initially removed from plasma samples by vertical rotor ultracentrifugation, as described (14). The infranatant remaining after removal of LDL was recovered, adjusted to density 1.20 g/ml with KBr, and recentrifuged at 40,000 rpm in a Beckman 50.3 rotor for 44 h. To remove residual LDL, the recovered HDL was overlayered with 1.06 g/ml KBr solution and recentrifuged for 22 h. The HDL recovered from the tube bottom was dialyzed against 10 mM Tris-HCl, pH 8.0, and then subjected to preparative PAGE electrophoresis using a modification of a method described for albumin and apoB (15). To separate apoA-I and A-II, a Laemmli system was used with a lower, separating gel of 12.5% acrylamide in a buffer containing 0.05% SDS and 0.56 M Tris-HCl, pH 8.8, and a stacker gel of 4% acrylamide with 0.05% SDS and 0.125 M Tris-HCl, pH 6.8. The run buffer was Tris-glycine, pH 8.3, with 0.05% SDS. With this system the C apolipoproteins migrated ahead of apoA-I, which resolved well from apoA-II. The apoA-I- and A-II-containing bands were excised, and the apolipoproteins were electroeluted in a manner similar to that previously described except that the recovery of these small proteins was much improved by using a double membrane technique, with the gel slice sandwiched between layers of dialysis membrane (15). Protein specific activity was measured, as described, by using a fluorescamine protein assay with scintillation counting (15). Plasma leucine concentration and specific activity were measured on blood samples as described (16).

Methodology for the [^{2}H]leucine samples

Three ml samples of plasma (EDTA) were subjected to sequential density ultracentrifugation, and the $1.063 < d < 1.21$ g/ml high density fraction was isolated after centrifugation at 48,000 rpm for 24 h in a Beckman Ti50 rotor at 5°C. After dialysis against 0.15 M NaCl, 0.01 M EDTA, aliquots were subjected to SDS-PAGE (17), and the stained band corresponding to apoA-I was removed, lyophilized, and hydrolyzed under N_2 for 24 h at 110°C in 12 N HCl. The amino acids were obtained by ion exchange isolation, and the *t*-butyldimethyl silyl (TBDMS) derivatives were prepared as described (18). GC–MS analysis was carried out after separation on a 25-m capillary column (HP-ultra-2) in a Hewlett-Packard model 5988A mass spectrometer operated in the EI selected ion mode. The leucine peak was monitored at *m/z* 200 and 203 (for d3-leucine). Isotope enrichment was measured using standard mixtures of leucine and 99% d3-leucine.

Mathematical compartmental modeling of the kinetic data was performed using the SAAM:CONSAM computer program (19).[2]

[2]This software is available at no cost from L. Zech, NCI, 10/6B13, Bethesda, MD 20892 USA; (301) 496-8915; Internet: GREIF@SAAM.NCI.NIH.GOV or via Modem: (301) 480-3295 login as zmodem.

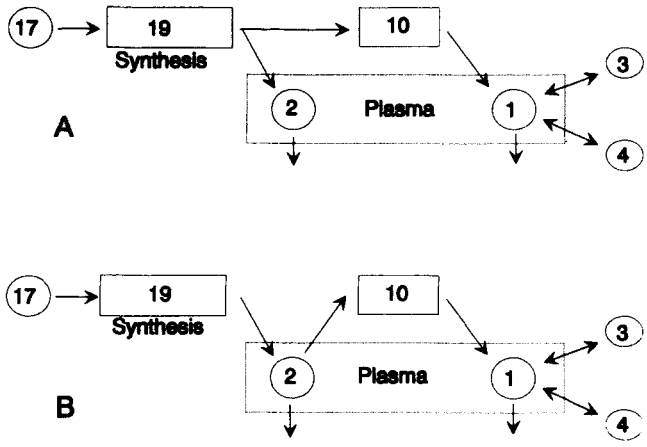

Fig. 1. Models of apoA-I metabolism used in fitting the leucine tracer. C(17) is plasma leucine which is utilized as a forcing function to drive the biosynthesis of apoA-I, C(19). ApoA-I initially appears in plasma as a rapidly turning-over pool, C(2), or after a delay, C(10), as a species that has a much longer residence time, C(1). This C(1) pool also exchanges with a nonplasma space, although the kinetics of this exchange cannot be resolved with the leucine tracer. A: This model assumes independent secretion of apoA-I into the two plasma pools. B: In this model the rapidly turning-over pool, C(2), is viewed as a precursor of C(1).

RESULTS

Studies with a bolus injected leucine tracer

ApoA-I is a protein that turns over slowly in plasma, and because of the long kinetic tail of plasma leucine it was problematic whether this tracer, when administered as a bolus, would permit the correct measurement of the fractional catabolic rate (FCR). A set of kinetic data from a normal subject, N-3, was analyzed to investigate this question, and the data were fit to a modified version of the apoA-I model of Zech et al. (3) which had been developed using a radioiodinated apoA-I tracer (**Fig. 1A**). As modified to incorporate a biosynthetic input for the endogenous tracer, the model now has a plasma leucine pool which serves as a forcing function followed by a delay reflecting the synthesis time for the protein. ApoA-I continues to be modeled as two plasma compartments, one of which, C(2), turns over rapidly; the other, a slowly turning-over compartment, C(1), is in equilibrium with two extravascular exchange compartments. It was necessary to introduce an additional delay in the input pathway to C(1) in order to fit a notch in the early portion of the data, the significance of which will be discussed subsequently. As measured with the radioiodinated tracer, the residence time of apoA-I is reported to be about 120 h (3). **Figure 2A–C** shows three different model-generated solutions (see figure legend for details) each of which yielded reasonable fits to the data; however, the system residence time, i.e., the residence time for the tracer within the kinetically defined system including the plasma and exchange compartments, for the data was arbitrarily adjusted from 60 to 120 h. The difference in these solutions rests with the slowly turning-over compartment, C(1). Throughout, the rate of the catabolic pathway, L(0,1), was held constant, but the output rates, i.e., FCRs, for the exchange compartments were permitted to adjust as necessary to achieve the desired residence times while maintaining a fit to the data. Alternatively, as shown in Fig. 2D, when the exchange compartments, C(3) and C(4), were excluded by setting their rate constants to zero, the data were fit by adjusting L(0,1), but only after the kinetic parameters for the exchange compartments were fixed is the solution determined by L(0,1). Under these circumstances the residence time of apoA-I was then calculated as 28 h. This experiment emphasizes the importance of being able to determine the kinetics of the exchange compartments. The residence time of apoA-I, therefore, is nondeterminable with the [^{3}H]leucine tracer as it depends upon the values at which the parameters for the exchange compartments are set, and these cannot be identified with this tracer methodology. Accordingly, in subsequent analyses, the residence time of apoA-I was initially set at 120 h and then was adjusted by as much as one-third as required to fit the data. The failure of the leucine tracer, when administered as a bolus, to permit determination of the residence time of apoA-I, or of apoA-II, is a result of both the complexity of cellular leucine metabolism in the liver and recycling of leucine that obscure the kinetic features of the slowly turning-over compartment, C(1).

ApoA-I kinetics were examined in 11 studies following an IV bolus injection of [^{3}H]leucine. Four subjects with familial hypercholesterolemia were studied on two occasions each, and of these 8 studies a notch in the data was clearly appreciated, as the radioactivity initially reached its plateau, in 7 studies; one was equivocal and was scored as negative. Single studies were also performed in two additional hypercholesterolemic subjects and one normal subject and of these the normal also demonstrated the notch in the data. Thus in 8 of 11 studies this discontinuity was evident, as shown in **Fig. 3.** When [^{2}H]leucine was administered as an IV bolus in three normal subjects they also showed discontinuities in the leading shoulder (**Fig. 4**). ApoA-II kinetics were likewise examined with an IV bolus of [^{3}H]leucine, and in 7 of 10 studies the findings were similar to those with apoA-I. Figure 3 shows apoA-I and A-II kinetic data for the same two subjects.

The apoA-I kinetic data from two subjects, N-3 and FH-5, studied with the IV [^{3}H]leucine tracer (Fig. 3) were analyzed using the apoA-I model (Fig. 1A). The model-

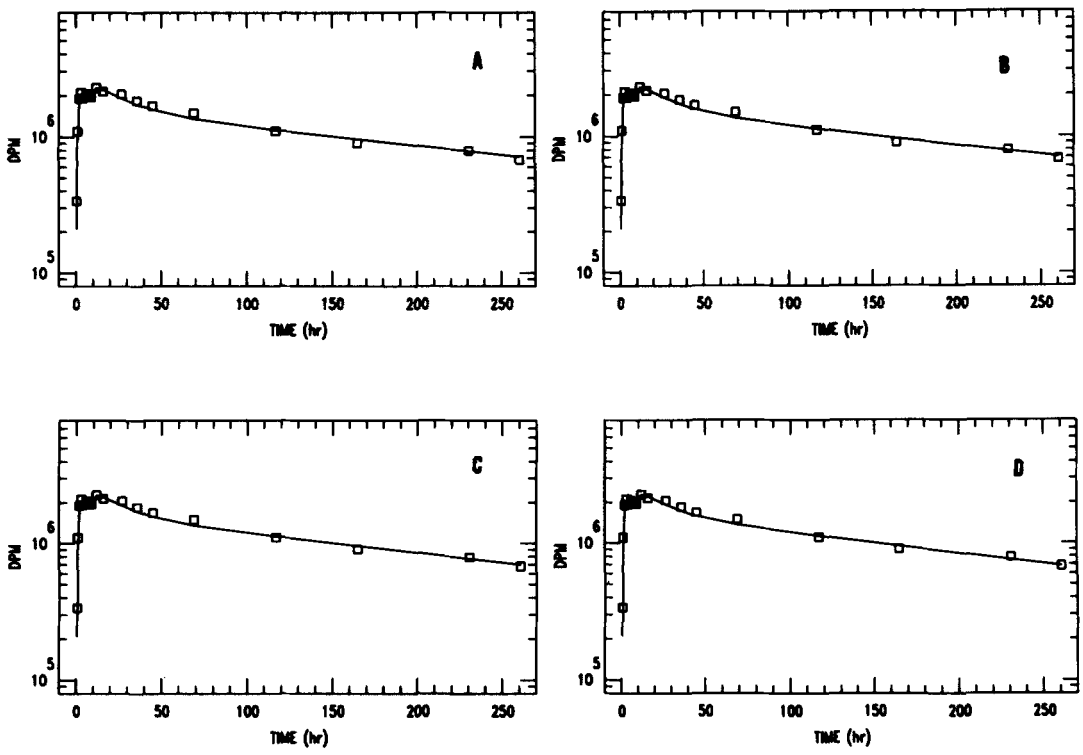

Fig. 2. A demonstration of the importance of the exchange compartments, C(3) and C(4), in establishing the residence time of apoA-I. In A, B, and C the catabolic pathways L(0,1) and L(0,2) are maintained constant, but the system residence time of apoA-I is arbitrarily changed from 60 to 96 to 120 h, respectively. Yet the fit to the data is maintained by adjusting the values of the rate constants, L(1,3) and L(1,4), for the exchange compartments, see below. D: Here the exchange compartments are excluded by setting their rate constants to zero, and the fit is achieved by adjusting L(0,1). With the exchange compartments excluded the residence time (T) is determined by L(0,1) and is 28 h.

	A	B	C	D
T(h)	120	96	60	28
L(0,1)	8.5 E-03	8.5 E-03	8.6 E-03	1.05 E-02
L(0,2)	0.1	0.1	0.1	0.1
L(1,3)	1.55	2.0	10.0	0
L(3,1)	2.24 E-02	2.24 E-02	2.24 E-02	0
L(1,4)	5.70 E-04	7.88 E-04	1.75 E-03	0
L(4,1)	1.96 E-03	1.96 E-03	1.96 E-03	0

generated solutions are seen in **Fig. 5** and the calculated kinetic parameters are presented in **Table 1,** data set 1A. The labeled component, C(2), has a fast turnover with a residence time that varied somewhat between the subjects but was considerably less than 1 day. An additional delay, represented by C(10), is required for tracer entering C(1), and C(1) is the primary determinant of apoA-I residence in plasma. The simulated solutions for C(1) and C(2), shown in the inserts of Fig. 5, demonstrate the kinetics of these two pools. Table 1 gives values calculated for the masses and transports of apoA-I in C(2) and C(1) as well as the ratio of the transports through these compartments, R(2)/R(1). It must be appreciated that these values are approximations because of the incomplete resolution of these two pools. Still, C(1) constitutes the major mass of apoA-I in plasma, but C(2) is not negligible. However, because of its rapid turnover the transport of apoA-I through C(2) is substantially greater than for C(1).

Because of the rapid turnover of C(2) and the additional delay in the input pathway for C(1), we posed the question of whether C(2) could be a precursor of C(1). Although the limited data preclude firm conclusions, they can be satisfactorily fit to a model in which C(2) is the precursor of C(1) (Fig. 1B). The calculated solutions for these same two subjects are not shown as they produced plots that were not distinguishable from those shown in Fig. 5, while the calculated parameters are shown in Table 1, data set 1B. This model presupposes that the major plasma apoA-I component, C(1), is totally derived from a rapidly turning-over plasma precursor, C(2); however, only a portion, perhaps one-half, of this precursor pool is utilized to generate the apoA-I in C(1); the rest disappears from plasma.

Studies with a primed, constant infusion of tracer

One subject, IF-1, was studied by a primed dose, constant infusion of [^{2}H]leucine over a 12-h period, and

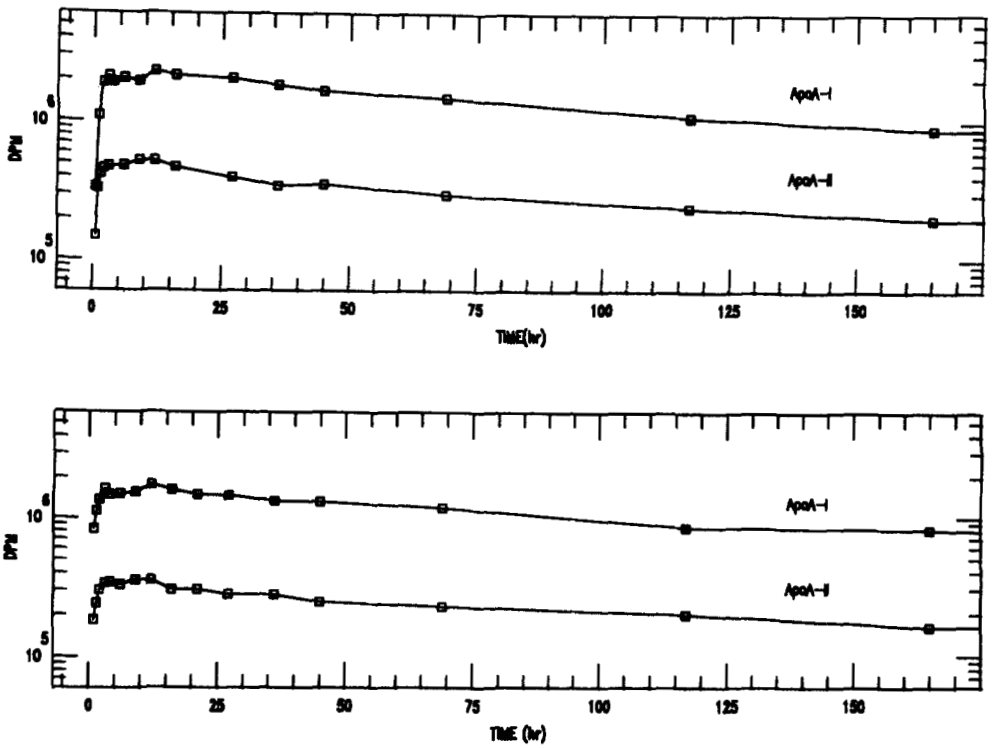

Fig. 3. ApoA-I and A-II specific radioactivity data from two subjects using a [^{3}H]leucine tracer administered as a bolus. The experimental data are joined by connecting lines. In each panel the upper data are apoA-I, the lower are apoA-II. Top: Subject N-3 (normal male); Bottom: subject FH-5 (heterogenous hypercholesterolemic female).

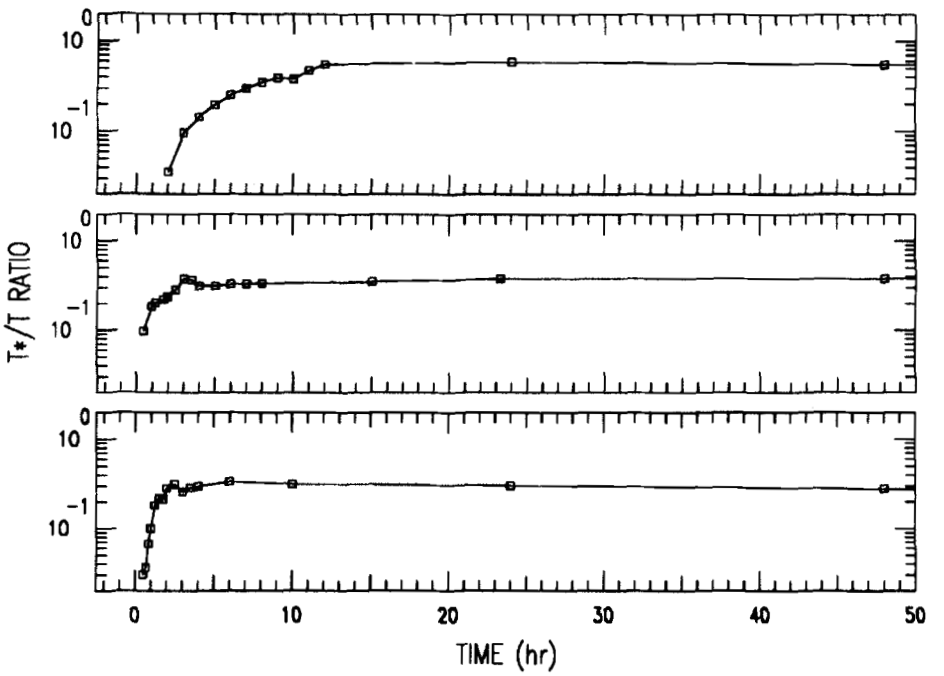

Fig. 4. ApoA-I isotopic enrichment data from three normal subjects using a [^{2}H]leucine tracer administered as a bolus. The experimental data are joined by connecting lines.

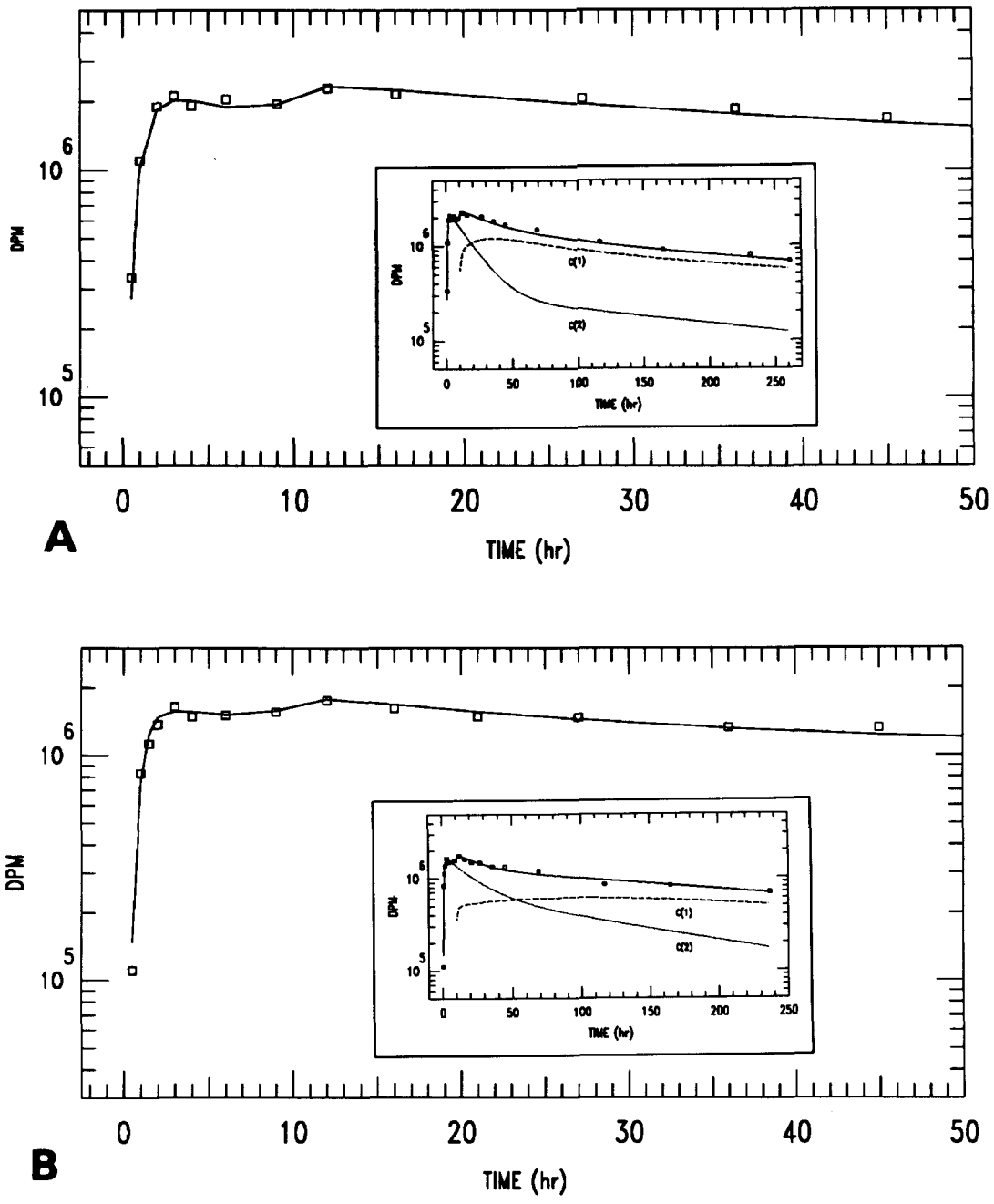

Fig. 5. ApoA-I kinetic data from subjects N-3, (A) and FH-5 (B) that have been fit to Model A, Fig. 1. The inset shows the complete data through 250 h and the computer-simulated solutions for C(1) and C(2).

sampling continued for 192 h. The tracer:tracee (T*/T) ratio was determined for plasma leucine, very low density lipoprotein (VLDL)-apoB, LDL-apoB, and apoA-I, and these data are plotted in **Fig. 6.** The data show an abrupt fall in T*/T for both plasma leucine and VLDL-apoB after the termination of the tracer infusion at 12 h. By contrast, LDL-apoB and apoA-I tracer enrichment continued to rise through 24 h. Thereafter, LDL-apoB enrichment began a steady decline, but apoA-I remained at a plateau and only began to decrease after 96 h. VLDL-apoB is known to be synthesized and directly secreted into plasma, and LDL is largely a product formed from VLDL, thus explaining the difference in their kinetics (20). The kinetic profile of apoA-I more

TABLE 1. Kinetic data

	Subject:	N-3 Normal Male		FH-5 FH Female		IF-1 Normal Male	
	Protocol:	IV Leucine Bolus		IV Leucine Bolus		IV Leucine Infusion	
	Model: Fig. 1	1A	1B	1A	1B	1A	1B
Masses (mg)							
M(1)		2432	2431	2652	3212	4594	4447
M(2)		598	529	988	428	806	952
M(3) + M(4)		6434	5786	3243	5116	7617	8736
Transports (mg/h)							
R(2,19)		61	74	47	64	74	68
R(10,19)		20	0	13	0	13	0
R(10,2)		0	39	0	48	0	20
Ratio R(2)/R(1)		3.0	1.9	3.6	1.3	5.7	3.4
C(2) Residence time (h)		8.7	7.1	21	6.7	11	11
C(10) Delay (h)		8.1	4.1	8.0	2.7	21	1

closely resembles that of LDL than VLDL, and the kinetic data obtained with the tracer infusion appear compatible with the apoA-I models shown in Fig. 1.

The apoA-I kinetic data obtained during the infusion were analyzed to determine a fractional synthesis rate (FSR) by two methods. The slope of the initial rise in enrichment was calculated by a least-square regression and when divided by the enrichment of VLDL at its plateau, which yielded an FSR of 0.0056 h (T = 178 h). Alternatively, when the T*/T values during the 12-h infusion were fit to a single exponential, a similar value of the FSR was determined (0.0055 h).

These apoA-I data from subject IF-1 were also analyzed using the model in Fig. 1A. **Figure 7** shows a model-generated fit in which an additional delay of 21 h was required in the secretory pathway for C(1), and the inset shows the effect of altering the duration of this delay and thus provides the means to determine its duration. The calculated kinetic parameters are also reported in Table 1 under Leucine Infusion, data set 1A, and are very similar to those determined for the two subjects who were studied using an IV bolus of tracer, except that the delay is somewhat longer.

In the modeling of these infusion data, the delay into C(1) was envisioned as occurring in its secretory pathway (Fig. 1A); however, as in the studies in which the tracer was injected as a bolus, we tested the hypothesis that C(2) could serve as a precursor for C(1) (Fig. 1B). The solution for a model in which the input of nascent apoA-I into the delay pathway is set to zero, L(10,19) = 0, and a portion of the output from C(2) is diverted into the delay pathway and thus becomes a precursor for

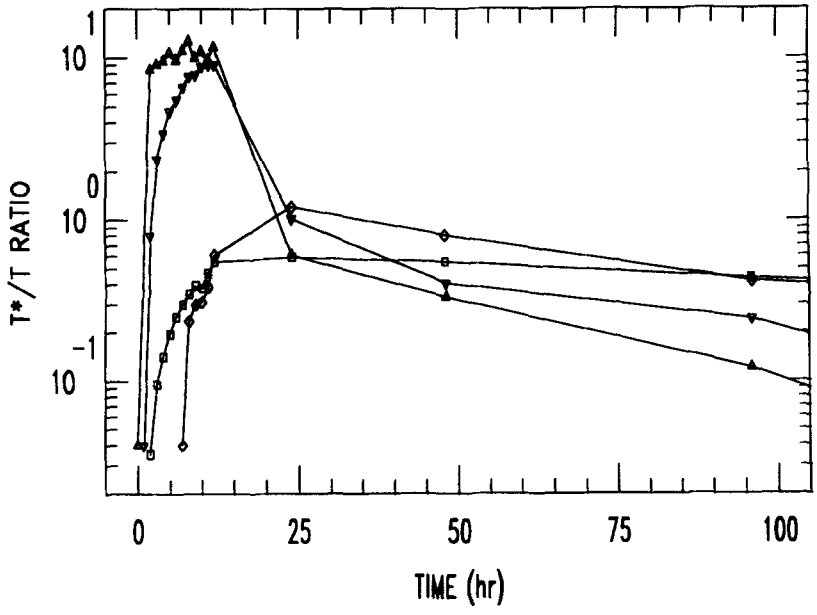

Fig. 6. Isotopic enrichment data for subject IF-1. A primed dose, constant infusion of [^{3}H]leucine was administered for 12 h and the data collection was continued thereafter. This figure shows data from the initial 96 h of the study; (△) plasma leucine; (▽) plasma VLDL-apoB; (◇) plasma LDL-apoB; (□) plasma apoA-I. The experimental data are joined by connecting lines.

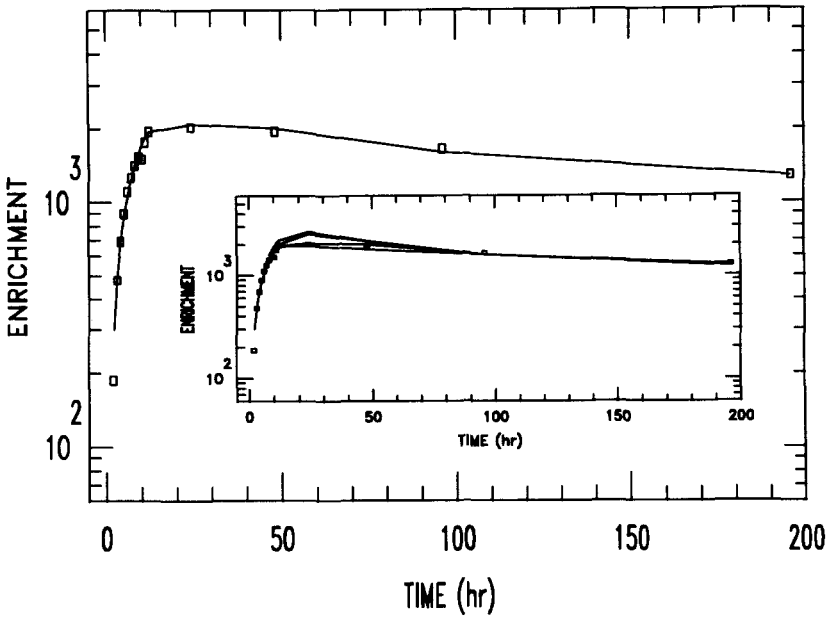

Fig. 7. ApoA-I kinetic data obtained using the primed dose, constant infusion protocol from subject IF-1. The data are fit to Model A, Fig. 1, with a delay, C(10), of 21 h. The inset shows the sensitivity of the fit to the duration of the delay. The computer solutions to the model shown for the four plots, from top to bottom, are for delays of 5, 10, 21, and 30 h.

C(1) yielded a plot indistinguishable from that in Fig. 7, and the recalculated parameters are presented in Table 1 under Leucine Infusion, data set 1B. In the example shown, 29% of the mass of apoA-I exiting C(2), L(0,2), is recycled to provide the entire input to C(1).

Thus, the kinetic data from the two subjects whose apoA-I was traced using a bolus injection of [^{3}H]leucine and from the subject whose apoA-I was labeled by a primed-dose infusion of [^{2}H]leucine, could by analyzed by similar models (Fig. 1, Model A). In these totally different experiments the analysis demonstrated the initial input of tracer into a fast turning-over compartment, and after a delay of 8–21 h the tracer appeared independently in a second apoA-I pool that had a much reduced total transport. The kinetic apoA-I data from the three other subjects who received a bolus injection of [^{2}H]leucine reproduce these findings in yet a third, different experimental protocol. Furthermore, the kinetics of apoA-II resemble those of apoA-I.

Alternatively, the kinetic data from both the bolus and infused tracer protocols can also be analyzed by a compartmental model in which C(1), the major pool of apoA-I, is depicted as a metabolic product of an initially secreted and rapidly turning-over apoA-I pool (Model B, Fig. 1). If so, then only a lesser portion of the apoA-I initially secreted into the fast pool reappears in plasma. In either case it is this slowly turning-over pool which is the primary determinant of the plasma residence time of apoA-I.

DISCUSSION

In 1983 kinetic studies on apoA-I were reported using a radioiodinated apoA-I tracer, and the data were analyzed by a model containing two plasma pools (3, 4). Subsequently, a number of studies using similar tracers have been reported examining the relationship of the plasma concentration of HDL to its FCR (5–9). The current studies using endogenous, leucine tracers have permitted examination of this proposed model in greater detail. They have also permitted the determination that considerable caution is warranted when using leucine as a tracer to measure apoA-I residence time. It has, however, been reported that the FSR of HDL measured with a leucine tracer infusion and the FCR reported using radioiodinated HDL give similar values (12).

Nevertheless, the leucine tracer is particularly useful in studying the early events in the metabolism of apoA-I. In the subjects studied, apoA-I was initially secreted and resided in plasma in a pool with a residence time of under a day. A separate apoA-I fraction appeared in plasma after a delay of less than a day, and it had a prolonged residence time that could not be measured with the [^{3}H]leucine tracer but was the predominant apoA-I pool measured with radioiodinated apoA-I tracers. Presumably these two fractions constitute different sets of apoA-I particles. The analysis of the kinetic data does not suppose apoprotein exchange between these pools but neither can it be excluded. The mass of C(1)

appears to be 4- to 5-fold greater than that of C(2); however, the transport or production rate of apoA-I through C(2) is approximately 3-fold greater than for C(1), reflecting the much shorter residence time of the protein in C(2). One may model the data so that apoA-I in C(2) is either totally catabolized or a portion of it may recycle and become a precursor for C(1), in either case appearing in plasma after a delay constituted by C(10). The physiologic implications of these models are very different. To our knowledge there is no known hepatic pool of newly synthesized apoA-I that is sequestered and then secreted from the liver after a delay of about one-half day.

Several physiological interpretations of these models of apoA-I kinetics should be considered. One hypothesis is that the rapidly turning-over pool, C(2), constitutes proapoA-I, which is known to be secreted and then proteolytically cleaved to form mature apoA-I (21). The small differences in the molecular weights of these two proteins would not result in resolution on the SDS-gels used in these experiments. However, the kinetic data on apoA-II, which were generated with the [^{3}H]leucine tracer, are relevant to this hypothesis. Ten studies were performed, of which seven gave evidence of a rapidly labeled component appearing at a time similar to that seen with apoA-I. Figure 3 shows the apoA-I and A-II data for two subjects. A comparison of these data strongly suggests that apoA-I and A-II have similar kinetics. If this is true, then the hypothesis that the delay preceding the appearance of C(1) reflects the time for the conversion of proapoA-I to the mature protein appears untenable, as apoA-II is not known to be proteolytically modified after its secretion into plasma.

A second hypothesis would equate these two plasma pools of apoA-I with apoA-I only and apoA-I:A-II families of particles (1, 2). There is much current interest in the structure, metabolism, and function of these apparently separate families of HDL. A kinetic study of apoA-I on such particles has been reported which demonstrated different kinetics for apoA-I only and apoA-I:A-II particles, with the former having a shorter residence time in plasma. However, the residence time of these particles, 4.39 and 5.17 days, is much greater than that observed for the rapidly turning-over pool of apoA-I observed with the endogenous leucine tracer (8). Furthermore, were this hypothesis valid, one would expect the apoA-II kinetics to differ from those of apoA-I by not exhibiting both the rapid and the delayed input pathways (Fig. 3). In this scenario one would predict that apoA-II might be secreted with either the early or the late appearing apoA-I components, and the double peak would not occur.

A variant of this hypothesis is suggested by a recent study performed in rabbits with radioiodine-labeled human HDL_2 and HDL_3 subfractions (22). ApoA-I in the smaller HDL_3 particles had a considerably faster FCR than apoA-I in larger HDL_2; however, the former was not shown to be a precursor to the latter. The prolonged FCR of apoA-I in HDL_2 was the primary determinant of plasma HDL levels. In our studies, HDL_2 and HDL_3 were not fractionated; however, in humans these subfractions have been reported to have similar residence times (23).

A third hypothesis proposes that there is a time delay in the secretion of two different apoA-I (and apoA-II)-containing lipoprotein particles, perhaps one pool derived from the intestine and the other from the liver. The delay in reaching the plasma from the intestine and the necessity for lipolysis prior to release of intestinal apoA-I could account for a delay in the input pathway to C(1); however, it is unlikely that all of the slow pool is derived from the intestine as most apoA-I in humans appears to be of hepatic origin and C(1) constitutes the major apoA-I mass in plasma (12). This possibility could be directly addressed by using an oral leucine tracer and thus measuring the kinetics of apoA-I for the gut-derived apoprotein. In preliminary experiments we have found similar kinetic properties of apoA-I irrespective of whether the tracer was administered by the oral or intravenous route (A. E. Sumner and J. B. Marsh, unpublished results).

A fourth hypothesis proposes that a portion of newly synthesized apoA-I in HDL is removed from the circulation and remodeled prior to its reappearance in plasma. Consistent with this hypothesis is the suggestion that apoA-I that resides outside the vascular space, C(10), may be undergoing a sequential processing and that a portion could recycle back to C(2) during this processing. This idea has been simulated and is not inconsistent with the data; it has the effect of slightly prolonging the delay represented by C(10). A number of observations support this hypothesis. Nascent HDL of hepatic origin has been shown to be quite different from mature HDL. In perfused rat livers the newly secreted apoA-I, in the form of nascent HDL, is present in a larger particle containing apoE, and most of the lipid is triglyceride and free cholesterol in contrast to mature HDL which has little triglyceride and is rich in cholesteryl esters (24). Remodeling of nascent HDL through the action of lipoprotein lipase has been reported (25). After lipolysis of the triglyceride and before LCAT converts free to esterified cholesterol, HDL can assume a discoidal shape (26). Yet little if any nascent HDL can be found in plasma, although discoidal HDL has been found in intestinal lymph (27), and this could constitute a precursor to the apoA-I found in C(1). Much evidence supports the concept that apoA-I and HDL can bind to cell surface receptors (28, 29), and retroendocy-

tosis of HDL particles has also been reported (30). HDL can thus be removed from plasma and released back again into the circulation.

Finally, a particularly interesting disorder of HDL metabolism, Tangier disease, may be cited in support of the existence of a rapidly turning-over pool of apoA-I and apoA-II (31). In these patients a structurally normal apoA-I protein is rapidly and completely metabolized (32, 33). Using radioiodinated HDL, its residence time in two subjects has been estimated at 0.13 and 0.3 days (34), values that are similar to those observed for C(2) in the present study. Though the kinetics of apoA-II in Tangier disease have not been reported, its very low plasma concentration suggests that it is metabolized in a manner similar to that of apoA-I.

In Tangier disease we suggest that, in contrast to the normal situation, nascent HDL which binds to the cell is not released back into plasma. Tangier HDL are abnormal in lipid and apolipoprotein composition, especially in the apoA-I:A-II particles and have increased apoA-IV (35). These structural differences may reflect the structure of HDL in the rapidly turning-over pool, C(2), in contrast to C(1) apoA-I particles which predominate in normal plasma. Kinetic studies of labeled HDL isolated from Tangier patients have not been carried out in normal subjects. We predict that different kinetics would be observed if Tangier disease HDL were injected into normal subjects, in contrast to the normal kinetics observed when HDL from healthy subjects is infused into Tangier patients.

In conclusion, in studies of apoA-I metabolism in human subjects performed by three totally different protocols which had in common the use of an endogenous leucine tracer, a model of apoA-I metabolism having two plasma pools has been demonstrated. Such a model has been previously proposed using an exogenous, radioiodinated apoA-I tracer (3), but our current studies more firmly establish this finding. ApoA-I appears to enter plasma by two pathways. The major portion of apoA-I enters plasma by the fast pathway, it has a rapid turnover with a residence time of less than 1 day, and most of this apoprotein is catabolized. ApoA-I also enters plasma after an additional delay by a second pathway, and this pool presumably constitutes the major apoA-I fraction in plasma. It is predominantly the kinetics of this pool which are measured with radioiodinated apoA-I tracers. Our kinetic data are compatible with, but do not prove, a precursor–product relationship between these two plasma apoA-I pools, and at this time it is only possible to speculate about the physiologic significance of these findings.

Note added in proof: Inadvertently, we omitted a reference to a report of impaired retroendocytosis in the monocytes of Tangier patients. Normal monocytes were reported to internalize HDL particles into the cytoplasm and subsequently resecrete them, whereas Tangier monocytes failed to resecrete the HDL (36). These observations are clearly relevant to the interpretation of our kinetic data.

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