Proposal of a Multicompartmental Model for Use in the Study of Apolipoprotein E Metabolism

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Apolipoprotein (apo) E is a 299–amino acid glycoprotein that serves a number of functions in lipoprotein metabolism. Apo E binds to the triglyceride-rich lipoproteins (TRL), very–low-density lipoprotein (VLDL), and chylomicrons, as they are lipolyzed, mediating their removal from plasma via lipoprotein receptors. Apo E is also found associated with high-density lipoprotein (HDL) and has been suggested to play a role in reverse cholesterol transport. Studies on the kinetic behavior of apo E from the TRL and HDL fractions provide insights into the metabolic relationships between TRL and HDL in vivo. We sought to develop a compartmental model that can be used for analysis of kinetic data in studies on the metabolism of TRL and HDL apo E. Using radioactive tracers, it has been previously observed that, in some instances, a portion of VLDL apo E that is removed from plasma subsequently reappears in VLDL. Four multicompartmental models were considered that could account for this type of behavior: model A, in which there is transfer of apo E from HDL to VLDL; model B, in which there is a bidirectional extravascular exchange; model C, in which there is removal and subsequent reintroduction of TRL apo E into plasma; and model D, in which there is secretion of TRL apo E into plasma directly and via an extravascular pathway. Models C and D provided the best fit to the experimental data. While no physiologically plausible analog to model C could be found, an extravascular delay, analogous to newly secreted apo E that enters the lymphatic system before appearing in plasma, was postulated for model D. It was this model that was used to analyze kinetic data from metabolic studies of apo E. The model was able to provide a satisfactory fit to kinetic data in studies in which subjects were given a primed-constant infusion of $^2H_3$-leucine. It was determined that TRL apo E from the six subjects studied had a mean residence time of 0.11 ± 0.05 days and a mean production rate of 10.6 ± 7.2 mg/kg/d, while HDL apo E had a mean residence time of 2.96 ± 0.99 days and a mean production rate of 0.07 ± 0.07 mg/kg/d. We conclude that this model describes a potential pathway for the metabolism of a portion of apo E in plasma and can be used to calculate the residence time and production rate of TRL and HDL apo E under a variety of conditions.

APOLIPOPROTEIN (apo) E is a 299-amino acid glycoprotein that is synthesized by most tissues throughout the body.[1] Apo E is thought to be involved in the uptake of lipid into different tissues.[2] Plasma apo E is derived primarily from the liver[3] and is found associated with triglyceride-rich lipoproteins (TRL) of both liver (very–low-density lipoproteins [VLDLs]) and intestine (chylomicrons), and high-density lipoproteins (HDLs).[4] Apo E associated with HDL may have a role in reverse cholesterol transport,[2] while apo E associated with TRL plays a role in the removal of both chylomicrons and VLDL from plasma via receptor-mediated processes.[5-8]

Determination of the metabolic pathway of apo E associated with different lipoprotein fractions in plasma is complicated by observations that there can be a transfer of apo E between VLDL (or TRL) and HDL.[8-11] The transfer is not well characterized and Blum[8] has pointed out that "mass transfer of apo E from HDL to TRL may occur, while under other circumstances mass transfer in the opposite direction may occur." An apparent transfer of apo E from VLDL to HDL occurs during heparin-induced lipolysis[9,10] and after hepatectomy,[12] both of which are non–steady-state conditions that result in an increase in HDL cholesterol. Transfer of apo E from VLDL to HDL has been reported to occur during alimentary lipemia in both humans[8] and rats,[12] and likely occurs during normal eating patterns as observed in kinetic studies by Gregg et al.[13] In the latter studies, there was slow clearance of labeled apo E from HDL, while there was both rapid and slow loss of apo E from VLDL, with the slow tail of the VLDL curve being parallel to the relatively slow HDL curve, suggesting that the VLDL tail is derived from transfer of apo E from HDL to VLDL. Back transfer of apo E from VLDL to HDL must be negligible under the conditions of this study, since the specific activity of apo E label in the two fractions did not approach equilibrium.

Previous kinetic studies that used radiolabeled apo E associated with VLDL have shown the existence of a unique kinetic feature of apo E: loss and reintroduction of label from VLDL.[14-16] Although the most likely explanation for this behavior would be exchange of apo E with HDL as has been described for apo C,[17] exchange with HDL is unlikely, since the label leaves the plasma space. Other possible explanations would include sequestration and release of apo E from the space of Disse, retroendocytosis of apo E, or movement of apo E into the lymphatics and its subsequent return to plasma.

It is the purpose of this report to propose and evaluate compartmental models for the TRL and HDL portion of the plasma apo E metabolic pathway.

MATERIALS AND METHODS

Subjects

Six male volunteers underwent a complete medical history and physical examination. The subjects were in good health and had normal hepatic, renal, and thyroid function. They did not smoke and were not taking medications known to affect plasma lipid levels. The experimen-

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tal protocol was approved by the Human Investigation Review Committee of the New England Medical Center and Tufts University. Subjects consumed a diet containing 49% carbohydrate, 15% protein, 36% fat (15% saturated, 15% monounsaturated, and 6% polyunsaturated fatty acids), and 180 mg/1,000 kcal/d cholesterol (target values) for 6 weeks before the kinetic studies.[18] All food and drink were provided to the subjects and caloric intake was adjusted to maintain body weight.

Protocol for Tracer Studies

Subjects underwent a primed-constant infusion with $^2H_3$-leucine as described previously.[18] Briefly, after a 12-hour overnight fast, subjects consumed hourly meals over a 20-hour period, with the first representing 1/20th of the daily caloric intake,[18] and subsequent meals were equally divided among the remaining daily caloric intake. Five hours after the first meal, at 0 hour of the infusion protocol, subjects were given a bolus injection (10 μmol/kg) immediately followed by a constant infusion of $^2H_3$-leucine (10 μmol/kg/h). The duration of the infusion period was 15 hours. Blood samples were drawn periodically for the determination of apo E enrichment with the infused isotope.

Plasma Lipids and Lipoprotein Fraction Preparation

Blood was collected in tubes containing EDTA. Fasting blood samples were obtained after a 12- to 14-hour overnight fast. Plasma was separated from whole blood after centrifugation at 3,000 rpm for 20 minutes at 4°C. The TRL fraction ($d < 1.006$ g/mL), which contains chylomicrons and VLDL in the postprandial state, and HDL (1.063 to 1.21 g/mL) were isolated by sequential ultracentrifugation.[19] Plasma was assayed for total cholesterol and triglyceride using enzymatic reagents as previously described.[20] HDL cholesterol was measured after precipitation of apo B–containing lipoproteins with dextran sulfate–magnesium chloride from plasma as previously described.[21]

Quantitation, Isolation, and Phenotyping of apo E

Apo E was assayed in plasma and TRL and HDL with a commercially available noncompetitive, enzyme-linked immunosorbent assay (ELISA) that was standardized against purified apo E.[22] Coefficients of variation within and between runs for the assay were ≤5%. This assay correlates well with a reference apo E assay, but gives results that are higher than reference values.[22,23] For the sake of comparison, we have divided the ELISA results by 2.4 to bring the average plasma values within the range reported in the literature.

Apo E phenotyping was performed by isoelectric focusing of whole plasma followed by immunoblotting using purified antihuman apo E antiserum.[24]

Apo E was isolated from the TRL and HDL fractions by preparative sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (4% to 22.5%) using a TRIS-glycine buffer system as previously described.[25] As this method allows for the isolation of total apo E, the kinetic results in subjects with a heterozygous apo E phenotype will represent the weighted average of the kinetic behaviors of the individual isoforms in the TRL fraction.

Determination of Isotopic Enrichment

Polyacrylamide gel bands containing apo E were hydrolyzed in 12N hydrochloric acid at 100°C for 24 hours. The hydrolyzates were then dried under nitrogen. The dried amino acids were then propylated, converted to the *N*-heptafluorobutyramide derivatives, and extracted into ethyl acetate before analysis on a Hewlett-Packard (Palo Alto, CA) 5890/5988A gas chromatograph/mass spectrometer as previously described.[26] The coefficient of variation for samples with tracer/tracee ratios less than 1.0% is 13.7% and decreases to 7.3% for samples with tracer/tracee ratios greater than 1.0%.

Model Descriptions

The models proposed for the analysis of kinetic data are based on the observation that when radiolabeled apo E associated with the VLDL fraction is injected into humans or animals, there can be a loss followed by a reintroduction of label into VLDL,[14-16] similar to what has been observed for the apo C.[17] Four models were formulated that could account for this type of behavior (Fig 1). All of the models have the same basic structure in which the precursor pool, analogous to the hepatic leucine pool, is followed by a delay, which represents the time required for the appearance of apo E in plasma. From this delay, apo E enters plasma associated with TRL and HDL. While transfer of apo E from TRL to HDL has been shown to occur under certain conditions,[9,11] we found no evidence of a second input into the HDL tracer data and make the assumption that there is a negligible transfer of apo E from TRL to HDL. Also, we are unable to distinguish between clearance of HDL apo E from plasma and transfer of HDL apo E to TRL. Therefore, the assumption is made that HDL apo E is removed from plasma through transfer to TRL. TRL can have one of four different fates: apo E is cleared irreversibly from this fraction (model A); there is a bi-directional exchange with a non-TRL, non-HDL compartment along with clearance from TRL (model B); there is removal and subsequent reintroduction of TRL apo E into a second TRL apo E compartment from which there is irreversible clearance (model C); and there is secretion of TRL apo E at two separate times into a single plasma

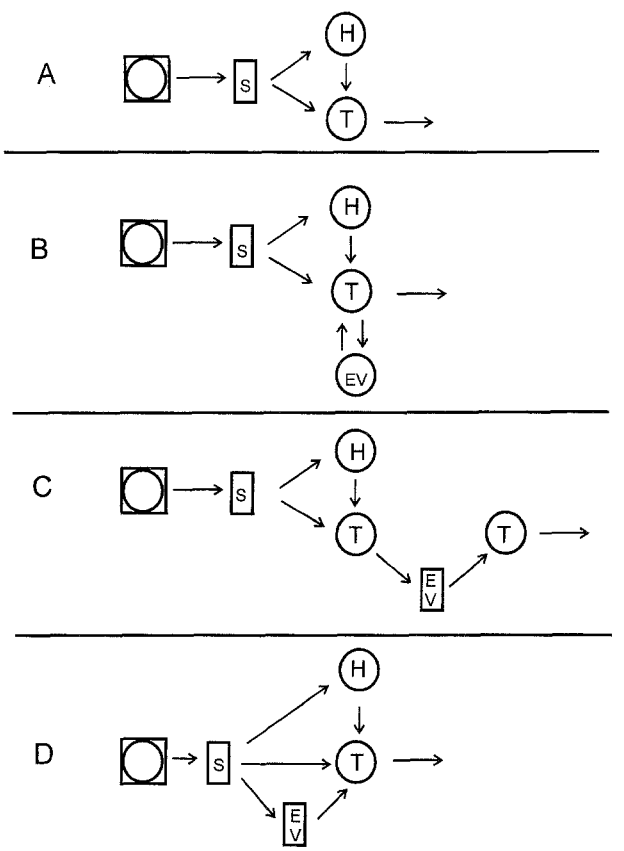

Fig 1. Four compartmental models that were fitted to the kinetic data. Models B through D would be able to account for the removal and reintroduction of apo E into TRL observed previously. Boxed circle represents the forcing function precursor that is analogous to the liver. Rectangles are secretory (S) and extravascular (EV) delay elements. Circles are the TRL compartment (T) or HDL compartment (H) or an extravascular exchange compartment (EV). Arrows represent the flux of apo E between compartments.

Table 1. Subject Characteristics

Subject No.	Age (yr)	Total Cholesterol*	Triglyceride*	LDL-C*	HDL-C*	apo E Phenotype
1	69	233	81	172	42	3/3
2	41	156	76	95	43	4/3
3	73	204	125	133	49	3/3
4	53	155	152	95	27	3/2
5	62	141	117	106	29	3/3
6	47	181	98	117	45	4/3

Abbreviations: LDL-C, LDL cholesterol; HDL-C, HDL cholesterol.
*Fasting levels.

compartment from which there is irreversible clearance (model D). While we have selected four models to test that have physiological analogs that are consisted with the metabolism of apo E as we currently know it, there are other empirical models that could result in similar types of simulations and these may have physiological analogs that have not been considered.

Kinetic Analysis

Kinetic analysis was performed on data expressed as percent tracer/tracee using the CONSAM 30[27] modeling program. A forcing function, which allows analysis of the model without describing the plasma and liver leucine subsystems, was used to describe the precursor pool of apo E leucine. An assumption was made that the tracer/tracee of the precursor pool remained constant throughout the experiment and a constant was used as a forcing function to define the precursor pool percent tracer/tracee ratio. The residence time was calculated by taking the reciprocal of the fractional synthetic rate (FSR). Plasma volume, in liters, was assumed to be 4.5% of body weight. The TRL and HDL apo E pool sizes were calculated by multiplying the plasma volume by the TRL and HDL apo E concentrations, respectively. Production rates per kilogram body weight were calculated by multiplying the FSR by the pool size and dividing by body weight.

Statistical Analysis

The Wilcoxon signed rank test was used to determine any significant differences between the residual sum of squares obtained for each model tested. A P value less than .05 was considered significant.

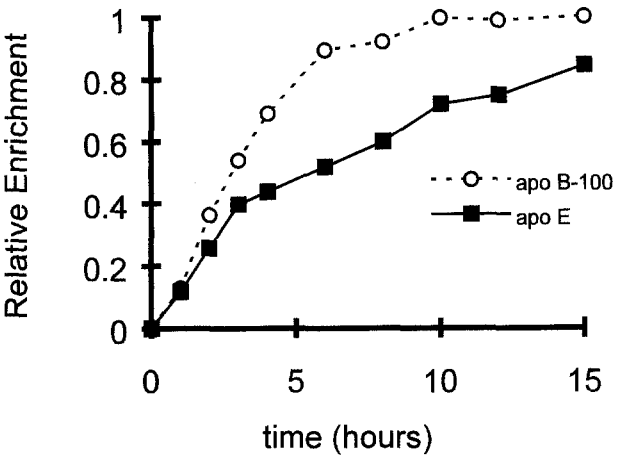

Fig 2. Graph of average relative isotope enrichments for TRL apo B-100 and apo E showing the differences in the increase in enrichment for the 2 proteins.

RESULTS

Total cholesterol levels of the study subjects ranged from 141 to 233 mg/dL, plasma triglyceride levels from 76 to 152 mg/dL, and HDL cholesterol levels from 27 to 49 mg/dL (Table 1). One subject had an apo E 3/2 phenotype, three had a 3/3 phenotype, and two had a 4/3 phenotype. There was a wide range of TRL apo E values among the subjects, ranging from 0.9 to 5.0 mg/dL, with a mean value of 2.3 mg/dL (Table 2). HDL apo E levels ranged from 0.1 to 0.7 mg/dL, with a mean value of 0.4 mg/dl (Table 2).

Figure 2 shows the relative differences between the TRL apo B-100 and TRL apo E enrichment data during the course of the primed-constant infusion. While apo B-100 has a monoexponential appearance, apo E appears to have two separate inputs of tracer, one appearing at approximately 30 minutes and the other after approximately 6 hours. Selection of the model that best fit the experimental data was performed by comparing the values

Table 2. Concentration, Residence Time, Production Rate of apo E in TRL and HDL, and Model Parameters for the Subjects Studied

Subject No.	Plasma apo E (mg/dL)	TRL apo E (mg/dL)	HDL apo E (mg/dL)	TRL apo E Residence Time (d)	HDL apo E Residence Time (d)	TRL apo E Production (mg/kg/d)	HDL apo E Production (mg/kg/d)	Secretory Delay (h)	TRL Transfer From HDL (pools/h)	Clearance From TRL (pools/h)	Fraction of Total Secreted Into HDL	Fraction of Total Secreted Into TRL	Fraction of Total Secreted Into Extravascular Delay*	Extra-vascular Delay (h)
1	3.1	0.9	0.4	0.06	2.62	7.1	0.07	0.77	0.02	0.64	0.11	0.53	0.36	6.9
								(.13)	(0.06)	(0.12)	(0.12)	(0.05)		(0.04)
2	4.9	1.2	0.7	0.10	1.50	5.4	0.21	0.28	0.03	0.40	0.11	0.53	0.36	7.5
								(0.83)	(0.07)	(0.19)	(0.20)	(0.10)		(0.06)
3	8.6	3.2	0.5	0.06	3.61	24.1	0.06	0.43	0.01	0.67	0.003	0.76	0.24	6.9
								(0.22)	(0.08)	(0.15)	(0.16)	(0.04)		(0.07)
4	7.8	5.0	0.3	0.17	2.46	13.3	0.06	0.62	0.02	0.24	0.07	0.87	0.06	6.8
								(0.27)	(0.08)	(0.24)	(0.24)	(0.14)		(0.27)
5	4.6	1.9	0.4	0.15	3.21	5.7	0.05	0.44	0.01	0.28	0.04	0.52	0.44	1.6
								(0.91)	(0.10)	(0.08)	(0.10)	(0.45)		(0.27)
6	3.2	1.6	0.1	0.09	4.34	7.8	0.01	0.66	0.01	0.46	0.002	0.63	0.37	7.9
								(0.19)	(0.09)	(0.18)	(0.18)	(0.06)		(0.05)
Mean	5.4	2.3	0.4	0.11	2.96	10.6	0.07	0.53	0.02	0.45	0.06	0.61	0.31	6.3
SD	2.3	1.6	0.2	0.05	0.99	7.2	0.07	0.18	0.01	0.18	0.05	0.14	0.14	2.3

NOTE. The fractional standard deviation is shown in parentheses below the parameter measurements for each subject.
*Calculated by difference.

Table 3. Residual Sum of Squares for TRL and HDL Kinetic Data Fitted to Four Different Models

Subject No.	Model A	Model B	Model C	Model D
1	1.866	1.157	1.897	**0.731**
2	1.300	1.083	0.853	**0.801**
3	4.551	4.559	3.097	**2.747**
4	0.364	0.369	**0.281**	0.285
5	0.321	0.307	0.288	**0.220**
6	1.130	1.199	**0.504**	0.854

NOTE. The best fit for each subject is in bold print.

for the residual sum of squares (Table 3). Using this criterion, the fit of the data model D was significantly better than those of models A and B ($P < .05$). There were no significant differences between the fits of the data to models C and model D. Model C is an empirical model, the physiological analog of which would be loss from and reintroduction into TRL of all of newly secreted TRL apo E. While this cannot be ruled out, it seems unlikely and the more physiologically plausible analog of model D, where a portion of newly secreted TRL apo E enters an extravascular compartment such as the lymphatics, is favored.

Representative fits of the predicted solution of the final model to the observed data are shown in Fig 3 and the individual kinetic data are presented Table 2. The results of the kinetic analysis show that apo E has a residence time in TRL of 0.11 ± 0.05 days and in HDL of 2.96 ± 0.99 days (Table 2). The production rate of TRL apo E was 10.6 ± 7.2 mg/kg/d, while

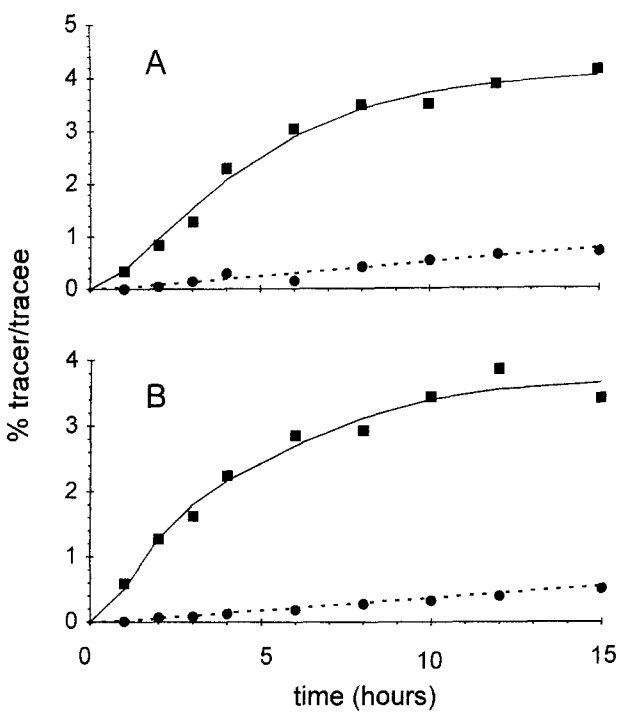

Fig 3. Plots of representative calculated and observed data for subjects no. 5 (A) and no. 6 (B). The solutions were obtained using model D. Squares and solid line are the TRL apo E data and model solution respectively; circles and dashed line are HDL apo E data and model solution respectively.

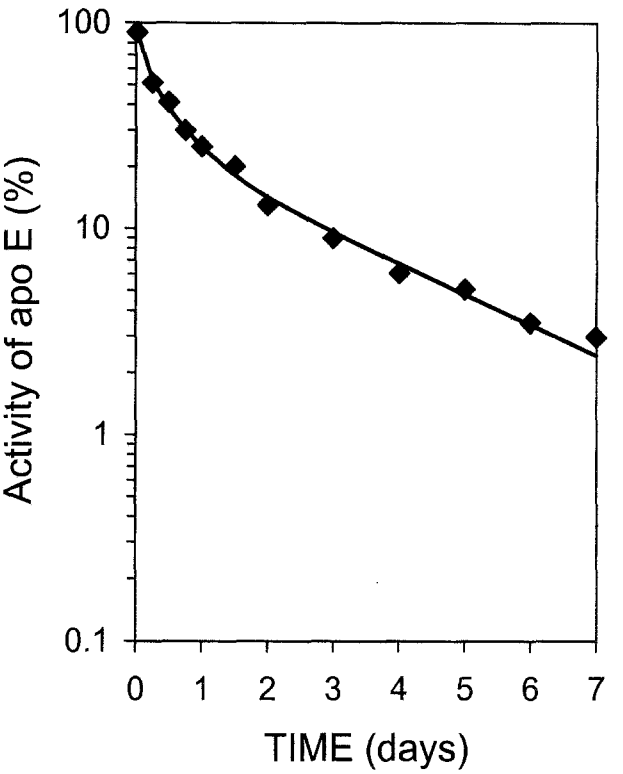

Fig 4. Triexponential fit to plasma apo E data adapted from Fig 3 of Gregg et al.[13] The fast component, representing 30% of the total apo E mass was fixed to the average calculated value for TRL apo E FCR of 9.09 pools/d. The slow component, representing 26% of the total apo E mass, was fixed to the average calculated value for the HDL apo E FCR of 0.34 pools/d. A third component with an intermediate FCR of 1.92 pools/d accounted for 42% of the total apo E mass. A triexponential equation provided a significantly better fit to that data than a biexponential equation as determined by an *F* test ($P = .0003$). Adapted with permission of Lipid Research, Inc.[13]

the production of HDL apo E was much lower at 0.07 ± 0.07 mg/kg/d. The time required for synthesis and secretion of apo E into plasma was 0.53 ± 0.18 hours. The extravascular TRL delay time, which represents the average time that apo E takes to appear in TRL by this pathway, ranged from 1.6 to 7.9 hours, with a mean time of 6.3 hours.

The average residence times for TRL and HDL apo E were used in fitting a triexponential curve to data taken from Gregg et al[13] (Fig 4). These data show the clearance of radiolabeled apo E from plasma. The triexponential curve was able to fit these data with the fast component fixed to the FCR of TRL apo E and the slow component fixed to the FCR of HDL apo E. The intermediate component was adjustable and the FCR determined to be 1.92 pools/d, equivalent to a residence time of 0.52 days. The amount of apo E with the rapid FCR was 30%, with an intermediate FCR of 42% and with a slow FCR of 26%. These results show that the residence times calculated for TRL and HDL apo E in the present study are consistent with data obtained from previous studies of plasma apo E metabolism.

DISCUSSION

Despite there being a number of previous kinetic studies on apo E, no compartmental model exists to characterize the

movement of apo E between TRL (or VLDL) and HDL. The kinetic behavior of apo E in these fractions is complicated by the fact that transfer of apo E between VLDL and HDL has been demonstrated. Van't Hooft and Havel[11] and Weisgraber et al[9] have shown that apo E from VLDL exchange labeled with apo E transfers to HDL and that apo E from HDL exchange labeled with apo E transfers to VLDL. However, this may be an artifact of the labeling procedure, since transfer from VLDL to HDL did not occur if the exchange labeling was done during an overnight incubation or at 37°C.[11] Other investigators have observed that during heparin-induced lipolysis, there in an apparent transfer of apo E from VLDL to HDL.[8,10] Another possible explanation for this, apart from there being transfer of apo E from VLDL to HDL, is that during heparin-induced lipolysis, VLDL-associated apo E is cleared rapidly as part of a VLDL particle, which accounts for the decrease in VLDL-associated apo E. Apo E, which similar to lipoprotein lipase binds to endothelial cell-surface heparin sulfate proteoglycans, is released free into plasma and rapidly associates with newly formed HDL_2, which accounts for the increase in HDL-associated apo E. The latter explanation is purely hypothetical and would need to be tested experimentally.

We made the assumption that there is no movement of apo E from TRL to HDL. The reason for this is that there is no evidence of anything other than a single input of label into HDL apo E. Any considerable input of tracer from TRL apo E into HDL would impart the shape of the TRL curve on the HDL data, since TRL apo E would be a precursor of HDL apo E, in addition to the apo E formed in the liver. Since the HDL apo E enrichment data appear linear in all cases (presumably due to the constant enrichment of the hepatic precursor), any input from TRL would have to be minor or nonexistent. We therefore make the assumption that there is no transfer in this direction. This assumption is reasonable based on data from Gregg et al.[13] If there were any considerable bidirectional exchange of apo E from VLDL to HDL, there should be an equilibrium that develops between the amount of label on VLDL and HDL apo E. However, there is no evidence of the label approaching equilibrium between these lipoprotein fractions. There is evidence from these data of HDL to VLDL transfer, since the slower component of the VLDL is parallel to the HDL apo E curve. This would suggest that HDL apo E is the precursor of this portion of the VLDL curve and that transfer only occurs in this direction.

Apo E also, in some instances, exhibits a distinctive metabolic behavior similar to the apo C,[17] whereby the protein leaves and reenters the TRL compartment at a later time.[14-16] However, apo E may be different in the respect that the labeled protein appears to leave the plasma space, rather than exchanging solely with another lipoprotein species such as HDL. Quarfordt[14] injected humans with radiolabeled VLDL and found that the radioactivity associated with VLDL apo E began to increase at approximately 1 hour after an initial decrease in radioactivity, which he hypothesized was due to sequestration and return of the labeled protein to VLDL. Wong and Rubinstein[15] injected rats with radiolabeled apo E associated with the $d < 1.21$ g/mL fraction and noted a biphasic rate of clearance of the protein from plasma. They also noted there was removal and reintroduction of the labeled protein from plasma of some, but not all, of the animals studied. They hypothesised that this could be due to extravascular sequestration of the protein and subsequent reintroduction into plasma. Kushwaha et al[16] injected radiolabeled apo E associated with VLDL or HDL into baboons. They observed a rapid removal of label from the plasma followed by a slower removal, in some, but not all, animals. Those animals whose decay curves indicated a rapid initial removal of label from plasma also demonstrated reintroduction of label into the plasma.

The current model for apo E was developed by formulating solutions that could be responsible for the kinetic behavior observed in a number of subjects in which there appeared to be a second input of tracer into the TRL fraction after approximately 6 hours. This resulted in a TRL apo E enrichment curve that had a shape that was distinctly different from that of TRL apo B-100 (Fig 2). There are several potential mechanisms that could be responsible for this behavior. One mechanism, depicted by model A, would be that a portion of newly synthesized apo E was secreted or associated first with HDL and then transferred from HDL to the TRL fraction. However, the amount of label secreted into HDL was not sufficient to account for a second input of tracer into the TRL fraction after approximately 6 hours. This model would also be unable to account for the previous observations, where labeled apo E leaves and reenters the plasma space. A second possible mechanism, depicted by model B, is that there is a exchange of apo E between TRL and an extravascular compartment, similar to what has been hypothesized to occur with LDL.[17] This possibility was excluded, as it provided a poor fit to the data. A third mechanism would be that there was removal and reintroduction of apo E from the TRL fraction, such as might occur if there were retroendocytosis of TRL apo E. The fourth mechanism was that there are two distinct pathways for secretion of apo E into TRL, one that appeared in plasma approximately 30 minutes after the start of the infusion and the other that appeared approximately 6 hours after the start of the infusion that could be explained by uptake of apo E into the lymphatics with subsequent entry into plasma. Based on the residual sum of squares data for each model, models C and D provided the best fit to the data. Model C, in which there is removal of TRL apo E from the plasma space and reintroduction back into TRL, was ruled out, as there is no physiological precedent. Although retroendocytosis has been described for LDL[28] and HDL,[29] there are no reports of this occurring with apo E, nor is it likely that there would be complete removal and reintroduction of all of newly secreted TRL apo E. Model D, in which a portion of newly secreted apo E enters a nonplasma compartment such as lymph, has a physiologic analog and was therefore selected as the most appropriate model to use to describe the data.

The presence of an extravascular delay has been described by Reeve and Bailey[30] for albumin metabolism. Their model combines a number of pathways, which are analogous to interstitial fluid pathways and which leave and reenter the plasma space. Miniati et al[31] have demonstrated removal and reintroduction of albumin from plasma in dogs. They demon-

strated that radiolabeled albumin injected into pleural space enters plasma similarly to what is predicted using a model of albumin metabolism that includes an extravascular delay. They also demonstrated that ligation of lymph ducts significantly decreased the rate of entry of labeled albumin from the pleural space to the plasma, suggesting that lymph was the source of label reentering the plasma space.

Since the behavior of albumin is similar to what has been observed for apo E, we hypothesize that the extravascular compartment found necessary for removal and reentry of label is analogous to a fraction of plasma apo E entering hepatic lymph. Although not synthesized by the small intestine, apo E is found in lymph.[32] A likely source of this material is plasma apo E that has been filtered into lymph either as the free protein or associated with lipoproteins. While apo E is found in plasma only associated with lipoproteins,[33] free apo E has been localized in the space of Disse.[34] It is likely that a portion of this is filtered into hepatic lymph and either associates with lipoproteins in lymph or associates with lipoproteins after reentering plasma. It is also likely that in experiments in which apo E is radiolabeled in vitro and injected intravenously, a portion of the injected apo E enters lymph either associated with lipoproteins or after dissociating from the lipoproteins on which they were injected, and reappears in plasma several hours later.

Model D, as presented, has a number of constraints that were necessary to obtain unique solutions when fitting the data to the model. The HDL data, which appear as a straight line that can be fit to a single exponential, do not allow the distinction to be made between transfer or exchange processes and catabolism. The loss of label from apo E in the HDL compartment is likely the summation of catabolic and transfer processes. Likewise, it is likely that apo E in the TRL fraction may be lost to catabolism of the TRL particle, through transfer to intermediate-density lipoprotein (IDL), or through transfer to cell-surface proteoglycans. As there is not enough kinetic information to allow these processes to be distinguished, they are combined into a single loss function. Also, the relatively higher error associated with the time points with lower enrichment decreases the confidence associated with measuring the secretory delay for both TRL and HDL apo E as reflected by the relatively increased fractional standard deviations for these estimates. The assumption is made that apo E of hepatic origin is secreted into TRL, HDL, and extravascular compartments after the same delay, so that both TRL and HDL apo E data can be used to determine a single secretory delay time.

The mean residence time of TRL apo E in the present study was 0.11 days, while that for HDL apo E was 2.96 days. These results support observations made by van't Hooft and Havel[11] that apo E associated with VLDL can be removed from plasma rapidly, while that associated with HDL has a clearance rate from plasma similar to that of HDL apo A-I. Schaefer et al[35] reported an average residence time of VLDL apo E of 0.11 days in a patient with familial defective apolipoprotein B-100 and 0.19 ± 0.06 days in three control subjects. Gregg et al[13] reported that clearance of apo E from plasma was greatest in VLDL and slowest in HDL. The kinetic values obtained in the current study are consistent with those reported for previous studies and are likely the fastest (TRL) and slowest (HDL) kinetic components involved in the clearance of apo E from plasma.[13]

The average production rate of TRL and HDL apo E in the present study was 10.6 ± 7.2 and 0.07 ± 0.07 mg/kg/d, respectively. The large standard deviations associated with the production rates suggest that, like apo B-100, apo E production rates can be very different, even among individuals with similar plasma cholesterol levels, and are likely influenced by a number of factors, including diet, age, and sex. These values are approximately twice the VLDL apo E production rate calculated by Schaefer et al of 5.9 ± 1.2 mg/kg/d for three male control subjects and 7.3 mg/kg/d for a male subject with familial defective apolipoprotein B-100.[35] The total production (TRL plus HDL) is also higher than that reported by Gregg et al for total plasma apo E3 (4.20 ± 1.73 mg/kg/d) in male subjects.[13] This may be due to the different conditions under which the subjects were studied: continuously fed in the current study, as opposed to fasted in the study by Schaefer et al[35] and both fasted and postprandially in the study by Gregg et al.[13] There is redistribution of apo E from HDL, a fraction with a relatively slow turnover of apo E, to TRL, a fraction with a relatively rapid turnover of apo E, in response to feeding, with no change in the plasma concentration of apo E.[8] There would have to be either an increase in the production rate or a decrease in the clearance rate of apo E from TRL to account for the plasma concentration of apo E remaining unchanged between the fasted and postprandial or constantly fed states. Further work would need to be performed to determine if either of these changes is occurring.

The results obtained in this study apply to apo E associated with lipoproteins isolated by ultracentrifugation. Apo E is known to dissociate from lipoproteins during ultracentrifugation and is also found associated with the IDL and LDL fractions. Therefore, there is a portion of apo E that was not measured in the current study. When lipoprotein fractions are separated according to size using size exclusion chromatography, apo E is found in two major fractions: one comigrating mainly with apo B–containing lipoproteins, and the other comigrating mainly with apo A-I–containing lipoproteins. Mackie et al[33] and Gibson et al[36] have shown that apo E is preferentially dissociated from the VLDL-associated fraction during ultracentrifugation. Indeed, Gregg et al measured the specific-activity decrease of apo E in dissociated apo E and found it to be cleared rapidly, like VLDL-associated apo E.[13] Gregg et al observed that apo E displays three major kinetic behaviors: a rapidly cleared portion that is associated with VLDL-containing lipoproteins, a portion cleared at an intermediate rate associated with IDL and LDL, and a slowly cleared portion that is associated with HDL.[13] This is consistent with the results obtained by fitting the data taken from Fig 3 of Gregg et al,[13] where a triexponential curve with fast, medium, and slow components fit the data.

A compartmental model that is able to describe the metabolism of TRL and HDL apo E has been proposed. This model can be used to assess the metabolic behavior of apo E under a

variety of conditions, allowing for the determination of both the residence time and production rate of apo E in TRL and HDL.

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