Kinetics of Retinyl Esters During Postprandial Lipemia in Man: A Compartmental Model

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Orally ingested vitamin A (retinol) is incorporated into intestinal chylomicrons (CHYLO) in the form of retinyl esters (RE) along with newly absorbed dietary triglycerides (TG). As the intestinal lipoproteins undergo hydrolysis in the circulation, the majority of the RE remain with the secreted intestinal particles and have been used as a marker for intestinally derived lipoproteins during the early phase of the postprandial state. A multicompartmental model was developed for the kinetics of RE during postprandial lipemia in individuals with normal lipid levels (n = 16) and in patients with hyperlipidemia (n = 44). The assumptions used in the development of the model are presented in this report. Some of the key findings include (1) as much as 50% of the newly synthesized RE may be secreted by the intestine as very-low-density lipoprotein (VLDL)-sized particles of S_f 20 to 400 following consumption of a test meal containing a moderate amount of fat (20 to 30 g); (2) in most individuals, approximately 50% of the RE secreted in S_f greater than 400 are converted to smaller, less buoyant fractions, and 50% are irreversibly removed directly from the plasma; (3) as much as 5% to 20% of the ingested retinol may be secreted as small intestinal lipoproteins with the buoyance of low-density lipoprotein (LDL) in some individuals; and (4) less than 5% of RE flux through S_f 20 to 400 is converted to S_f less than 20, and the primary catabolic pathway for RE in this fraction is direct uptake. Comparable estimates can be obtained for the kinetic parameters when repeat studies are made in the same subjects under comparable conditions.

THE FORMATION AND SECRETION of triglyceride (TG)-rich chylomicrons (CHYLO) by the intestine has been studied by several groups.[1-4] Once in the circulation, these intestinal lipoproteins interact with lipoprotein lipase (LPL) and are rapidly depleted of their TG cargo. Grundy and Mok[5] reported that the clearance of exogenous TG following infusion of emulsified fat directly into the duodenum was extremely rapid, ranging from 4 minutes in normolipidemic to 20 minutes in hyperlipidemic men. Hydrolysis of CHYLO by LPL results in generation of lipoprotein particles that are smaller in size and enriched in cholesteryl esters, the CHYLO remnants.[3,4,6,7] The irreversible removal of these remnant particles via the liver[7,8] is suggested to involve apolipoprotein (apo)E.[9-11] The intermittent influx of these intestinal lipoproteins and their metabolic remnants during the postabsorptive state has also been suggested as a potential factor in atherogenesis.[12,13]

Data on the metabolism of intestinal lipoproteins in man are still limited. Using radiolabeled vitamin A and TG, Hazzard and Bierman[14] reported that CHYLO-TG are catabolized much faster than CHYLO-retinyl esters (RE) in normolipidemic subjects. In patients with type III dysbetalipoproteinemia, this difference in clearance rates between intestinally derived TG and RE is even more pronounced.[14] Using a fat load containing vitamin A, delayed clearance of RE has been demonstrated in patients with a variety of lipid disorders,[15-28] including individuals with normal lipids and angiographically documented coronary artery disease (CAD).[28]

The metabolism of intestinally derived RE during the postprandial state is complex and reflects the kinetics of a wide spectrum of particles differing in size and composition. Whether newly secreted intestinal particles enter the circulation as large CHYLO of S greater than 400 or as intestinal lipoproteins of S_f 20 to 400 depends on the TG content of the test meal,[14] as well as the type of fat.[29,30] From the reinfusion of autologous plasma containing retinyl palmitate–labeled lipoproteins in normolipidemic volunteers, Berr and Kern[20] suggested that several subpopulations of retinyl palmitate–containing particles might be present in plasma; one of these appeared not to be affected by heparin-stimulated plasma lipolytic activity.[21] Analyses of the kinetics of RE during postprandial lipemia have been based primarily on the areas under the RE curve associated either with TG-rich lipoproteins (very-low-density lipoprotein [VLDL] + CHYLO)[25-29] or with large CHYLO of S_f greater than 1,000 and smaller particles of S_f 20 to 1,000.[23,24,30]

We have previously described a protocol to examine the fate of RE associated with the large TG-rich, nonfasting lipoproteins of S_f greater than 400 and particles of S_f 20 to 400 that correspond to lipoproteins typically found in fasting plasma.[22] To estimate the residence times of RE in various lipoprotein fractions and the rate and extent of RE conversion from one fraction to another, a multicompartmental model (Fig 1) was developed[22] using the Simulation and Analysis Modeling (SAAM) program.[31,32] The assumptions required in the development of the model are discussed in this report.

To fully appreciate the significance of these kinetic parameters derived by the model in terms of the metabolism of intestinal lipoproteins, we need to demonstrate that when the same subject is restudied under comparable conditions the estimated model parameters will remain similar. The reproducibility of these kinetic parameters is demonstrated in this report with data from 17 hypertriglyceridemic subjects who participated in two repeated studies under comparable conditions. This represents the first instance when repeated kinetic studies were

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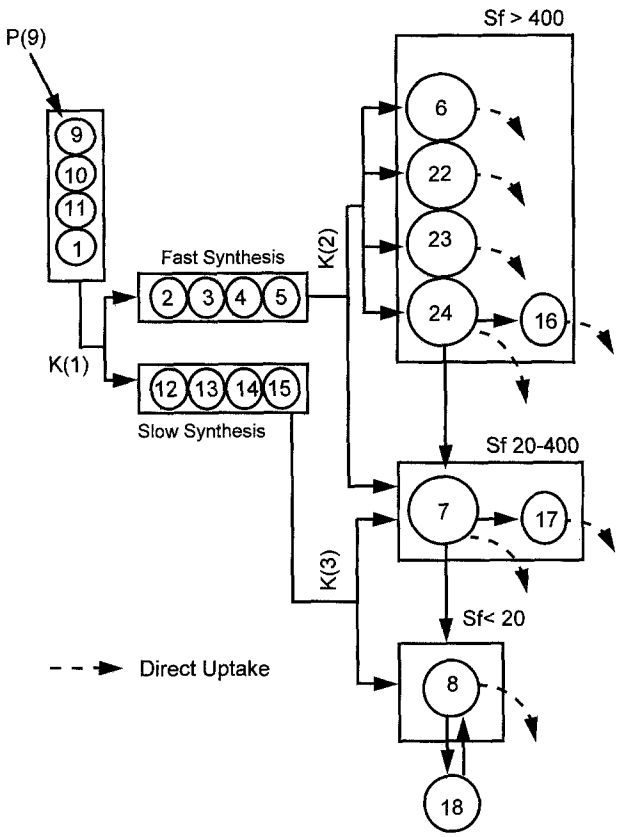


Fig 1. Multicompartmental model illustrating the incorporation of ingested retinol into intestinal lipoproteins and the subsequent removal of RE from various lipoprotein fractions in plasma.

actually available in a large number of subjects. We also need to demonstrate that these parameters are correlated with other metabolically related processes that can be assessed independently in the same subjects. In this instance, we can demonstrate that the half-life values for RE are highly correlated with the fractional synthetic rates (FSRs) of hepatic VLDL apoB in a subset of subjects with normal and elevated lipid levels.

SUBJECTS AND METHODS

Data Set for the Model Development

Details of the experimental protocol and the analytical techniques used have been described previously.[22] Briefly, 43 subjects were studied after oral intake of either 25 mL Lipomul (equivalent to 17 g corn oil; Upjohn, Kalamazoo, MI) plus 1 mL Aquasol A (equivalent to 50,000 USP vitamin A; USV Laboratories, Tuckahoe, NY) or 50 mL Lipomul plus 2 mL Aquasol A. Plasma was collected at frequent intervals over the following 10 to 12 hours for the isolation by sequential ultracentrifugation CHYLO ($S_f > 400$) and particles of S_f 20 to 400. RE in the latter fraction would be associated with both newly secreted small intestinal particles (intestinal VLDL) and CHYLO remnants, products from the hydrolysis of large CHYLO by LPL. With this isolation procedure, which is based solely on flotation characteristics of the lipoprotein classes, a distinction between apoB-48– and apoB-100–containing particles would not be possible. The concentration of RE in plasma and in each lipoprotein fraction was measured by high-performance liquid chromatography (HPLC) as previously described.[22] These studies were conducted with the approval of the Committee for the Protection of Human Subjects at The Children's Hospital of Philadelphia. Compartmental analysis was performed using CONSAM[32] implemented on the VAX 11/750 system (DEC, Marlboro, MA) in the CLINFO facilities of the University of Pennsylvania School of Medicine.

Of the subjects studied, 16 had normal plasma lipid levels, 22 had fasting hypertriglyceridemia, and the remaining five had the apoE 2/2 phenotype with β-migrating VLDL. Of these five subjects, four had hyperlipidemia and one had normal lipid levels. Mean lipid levels for the three groups of subjects are presented in Table 1; individual clinical data in a subset of these subjects have been previously reported elsewhere.[22]

Data Set for the Reproducibility Study

Data were obtained from 17 patients with type IV or type V hyperlipidemia who participated in a multicenter study. This was a randomized trial that included four 6-week phases designed to examine two formulations of gemfibrozil on postprandial lipid levels. A total of 21 subjects were randomized, with 17 individuals completing at least the first three phases of the protocol to be included in this analysis. Phases I and III corresponded to the two placebo periods, and phases II and IV to active treatment with two different formulations of gemfibrozil. Subjects were maintained on the American Heart Association (AHA) step I diet throughout the entire 24-week period and were admitted to the respective clinical research center for 3 days at the end of each 6-week phase for the postprandial study. Only postprandial data from the two placebo periods (12 weeks apart) are presented here to demonstrate reproducibility of the kinetics of RE. Informed consent was obtained at each of five research institutions. Table 2 presents mean fasting lipid levels of the subjects determined on the morning before consumption of the fat load. Fruit shakes[33] containing standardized amounts of fat in the form of Lipomul were prepared by the staff of the Nutrition Center of the Medlantic Research Foundation (Washington, DC) and shipped frozen to the various clinical centers. Plasma samples were collected at frequent intervals over a 24-hour period after consumption of the fat load and shipped to Penn Med Labs (Medlantic Research Foundation) for lipoprotein fractionation and determination of RE concentrations using a modified isocratic HPLC method.[33]

Association Between the Kinetics of RE and the Production Rate of Hepatic VLDL ApoB

To examine the relationship between the kinetics of RE during postprandial lipemia and the kinetics of hepatic apoB, 24 subjects (14 with normal lipid profiles and 10 with moderate hypertriglyceridemia) participated in both a postprandial study with vitamin A and a study of apoB kinetics with a primed constant infusion of ^{15}N-glycine.[34] These subjects represent a subset of participants in the postprandial study conducted at The Children's Hospital of Philadelphia described earlier. The two protocols were performed within a 7-day period while the participants were maintained on the AHA step I dietary regimen.

MODEL DEVELOPMENT AND RESULTS

The fate of oral vitamin A from the time the meal is ingested to the time esterified retinol is irreversibly removed from the

Table 1. Plasma Lipid Levels for Subjects in the Model Development Phase

Parameter	Normal Plasma Lipids	Endogenous Hypertriglyceridemia	ApoE 2/2
No. of subjects	16	22	5
TG*	96 ± 34	324 ± 155	640 ± 520
Cholesterol*	179 ± 63	230 ± 64	407 ± 205
HDL cholesterol*	44 ± 11	26 ± 7	31 ± 9

*mg/dL, mean ± SD.

Table 2. Plasma Lipid Levels for 17 Subjects Included in the Reproducibility Study

Lipid	Study A	Study B
TG	590 ± 179	631 ± 219
Cholesterol	247 ± 38	247 ± 35
HDL cholesterol	29 ± 2	29 ± 3
LDL cholesterol*	94 ± 17	88 ± 23

NOTE. The 2 studies were separated by 12 weeks. Results are the mean ± SD in mg/dL.

*Direct LDL cholesterol as determined by ultracentrifugation.

circulation can be described in five stages. They include (1) a presynthesis stage that accounts for the movement of the dose of vitamin A and other nutrients through the digestive tract and their subsequent absorption; (2) fast and slow synthesis steps associated with the esterification, incorporation, and secretion of absorbed retinol in newly synthesized intestinal lipoproteins; (3) a metabolic scheme for the processing of RE associated with lipoproteins of S_f greater than 400 in plasma; (4) a metabolic scheme for the processing of RE associated with lipoproteins of S_f 20 to 400 in plasma; and (5) a slowly metabolized pool of RE-labeled lipoproteins corresponding to plasma particles of density greater than 1.006 g/mL. The proposed compartmental model (Fig 1) allows estimation of 13 model parameters from the simultaneous analysis of four RE concentration curves corresponding to whole plasma, S_f greater than 400, S_f 20 to 400, and S_f less than 20. Table 3 defines the metabolic parameters estimated by the multicompartmental model. Figure 2 presents the experimental data points and computer-fitted curves for the plasma and all three lipoprotein fractions for one hyperlipidemic subject.

Presynthesis Subsystem

The plasma RE curve typically remains unchanged or may exhibit an initial decay component from the baseline level during the first 15 to 45 minutes (Fig 2A). This is followed by an upswing as newly formed RE enter the circulation. The onset of this upswing was observed between 30 and 60 minutes after the meal, depending on the transit time of retinol through the gastrointestinal tract.[35] This variability is accounted for in the model by differences in the fractional rate constant among subjects: L(10,9) = L(11,10) = L(1,11).

Of the ingested dose, a fraction P(9) will appear in the circulation as RE, and this is reflected in the area under the plasma RE curve. Plasma volume in each subject was estimated from total body weight adjusted for excess body weight.[36] From the present data set, approximately 85% to 99% of administered retinol can be recovered as plasma RE (Table 4). Parameter K(1) denotes the fractional transfer rate from this presynthesis cascade, representing the absorptive process, to the intestinal cells where retinol will be esterified and packaged into lipoproteins.

Synthetic Subsystem

Previous studies have suggested that intestinal particles can be continuously secreted into the bloodstream for several hours after consumption of a single fat-containing meal.[2,3] This continuous influx of newly formed CHYLO is represented in the model by a series of pools (pools 2, 3, 4, and 5) that receive input from the presynthesis subsystem and allow for unidirectional flow down the cascade until the assembled particle is secreted into the bloodstream.

This synthetic cascade is characterized kinetically by several parameters, including P(21), L(3,2), L(13,12), K(2), and K(3) (Table 3). Specific parameters may be assigned to each of these processes, but it is possible that the measured RE concentrations are insensitive to variations in some of these turnover rates. For instance, Fig 3A illustrates that changes in either the input rate, $K(1) \cdot P(21)$, or the output rate, K(2), have similar effects on the shape of the RE curves. Since, with the exception of patients with apoE 2/2, the estimated values for $K(1) \cdot P(21)$ were similar among subjects (Table 4), we have chosen to assign a mean value of 1.25/h to the input rate and allow the changes in K(2) to explain the variabilities in RE kinetic curves among different subjects.

Table 3. Definition of the Metabolic Parameters for RE Kinetics

Parameter	Definition
1. Presynthesis subsystem	
P(9)	Fraction of administered dose of retinol appearing in plasma as RE
L(10,9) = L(11,10) = L(1,11) (h^{-1})	Fractional turnover rate of the pools in the presynthesis cascade (pool 9, 10, and 11)
K(1) (h^{-1})	Fractional transfer rate from the presynthesis cascade to the synthesis pathways
2. Synthesis subsystem	
Fast	
P(21)	Fraction of RE flux from the presynthesis cascade channeled to the fast synthesis pathway
L(3,2) (h^{-1})	Fractional turnover rate of the pools in the fast synthesis cascade (pool 2, 3, and 4)
K(2) (h^{-1})	Fractional transfer rate from the fast synthesis cascade to plasma lipoproteins
Slow	
1-P(21)	Fraction of RE flux from the presynthesis cascade channeled to the slow synthesis pathway
L(13,12) (h^{-1})	Fractional turnover rate of the pools in the slow synthesis cascade (pool 12, 13, and 14)
K(3) (h^{-1})	Fractional transfer rate from the slow synthesis cascade to plasma lipoproteins
3. Sf >400 subsystem	
P(65)	Fraction of RE flux secreted from the fast synthesis cascade as Sf >400
K(6) (h^{-1})	Fractional turnover rate of the pools in the delipidation cascade for Sf >400
P(60)	Fraction of RE flux converted down the cascade within Sf >400
4. Sf 20-400 subsystem	
1-P(65)	Fraction of RE flux secreted from the fast synthesis cascade as Sf <400
K(7) (h^{-1})	Fractional turnover rate of RE from Sf 20-400 VLDL-sized lipoproteins
P(70)	Fraction of RE flux converted to Sf <20

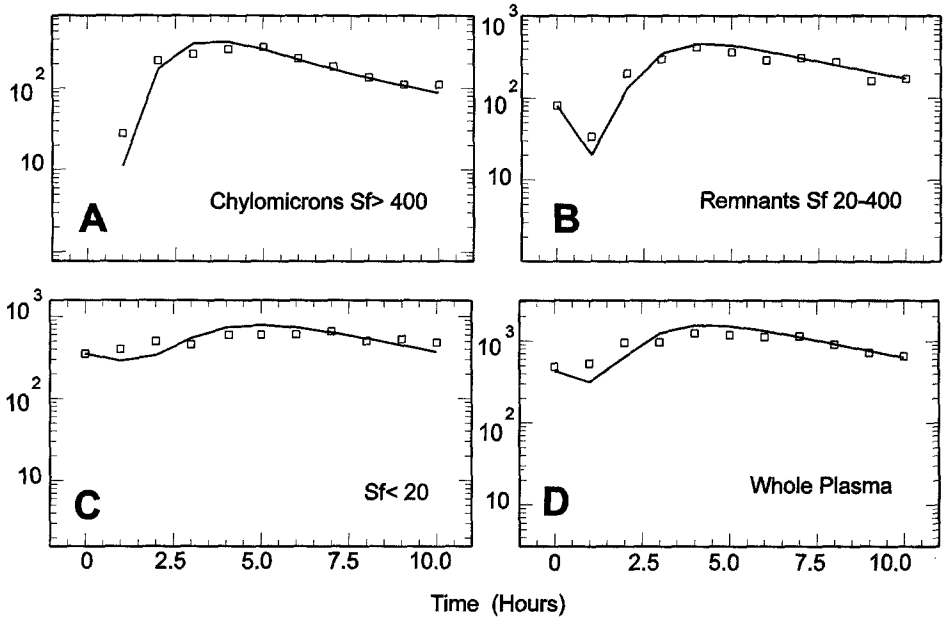


Fig 2. Kinetic curves representing concentrations of RE in (A) CHYLO of $S_f > 400$, (B) VLDL of S_f 20 to 400, (C) density fraction d > 1.006 g/mL corresponding to lipoproteins of $S_f < 20$, and (D) whole plasma. Solid lines represent the best-fit curves for the observed experimental data for 1 subject with mild hypertriglyceridemia.

Other kinetic parameters, on the other hand, may have a critical role in determining unique features of the RE concentration curves. For instance, changes in the value of the fractional turnover rate L(3,2) within the fast synthesis pathway can be shown to affect several components of the plasma RE curve (Fig 3B). They include (1) the time required to reach peak concentration, (2) the broadness of the peak, and (3) the initial slope of the decay after the peak. In this scheme, individuals with a slow rate of synthesis, ie, a delayed rate of appearance of the newly absorbed RE in plasma, would have a low value for L(3,2). The mean length of time spent by retinol in the respective synthetic cascade before secretion in the plasma as RE can be calculated from this fractional turnover rate and the transfer rate K(2) into the vascular space: $[\text{synthesis time}]_{Fast} = 3 \times [1/L(3,2)] + [1/K(2)]$. By analogy, for the slow synthesis pathway, $[\text{synthesis time}]_{Slow} = 3 \times [1/L(13,12)] + [1/K(3)]$.

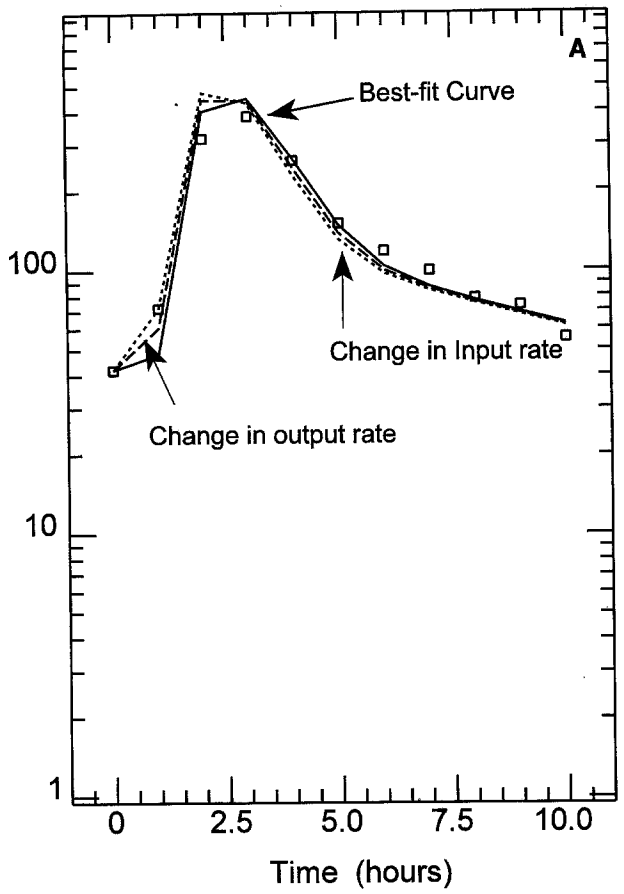
Table 4. Kinetic Parameters for RE Metabolism During Postprandial Lipemia

Parameter	Normal Plasma Lipids	Endogenous Hypertriglyceridemia	ApoE 2/2
1. Presynthesis subsystem			
P(9)	0.92 ± 0.12	0.97 ± 0.07	0.93 ± 0.07
L(9,10) (h^{-1})	3.63 ± 2.62	4.87 ± 3.95	8.78 ± 5.86
K(1) (h^{-1})	1.18 ± 0.84	1.33 ± 0.75	0.61 ± 0.25
2. Synthesis subsystem			
Fast			
P(21)	0.62 ± 0.14	0.58 ± 0.18	0.53 ± 0.20
L(3,2) (h^{-1})	3.07 ± 1.38	2.47 ± 1.79	2.10 ± 1.70
K(2) (h^{-1})	1.50 ± 0.91	2.24 ± 1.36	1.60 ± 1.27
Slow			
L(13,12) (h^{-1})	1.57 1.25	0.95 0.75	1.05 1.10
K(3) (h^{-1})	1.25 0.95	1.75 0.95	1.45 0.95
3. Sf >400 subsystem			
P(65)	0.54 ± 0.16	0.65 ± 0.14	0.48 ± 0.08
K(6) (h^{-1})	8.30 ± 2.97	3.70 ± 2.29	2.52 ± 2.41
P(60)	0.56 ± 0.16	0.57 ± 0.14	0.41 ± 0.23
4. Sf 20-400 subsystem			
1-P(65)	0.46 ± 0.08	0.35 ± 0.14	0.52 ± 0.08
K(7) (h^{-1})	4.25 ± 2.67	1.46 ± 1.52	0.32 ± 0.23
K(70)	0.16 ± 0.02	0.24 ± 0.03	0.26 ± 0.03

Subsystem for S_f Greater Than 400

The metabolic fate of RE in plasma lipoproteins depends on the TG content of the particles being secreted, which in turn is affected by the intestinal pool of dietary fatty acids available for TG synthesis. Consumption of larger amounts of fat will result in the synthesis and secretion of larger, more buoyant CHYLO. In our model, the parameter P(65) characterizes the fraction of newly formed lipoproteins that appear in the bloodstream as large CHYLO of S_f greater than 400. The remaining fraction, 1-P(65), enters the circulation as smaller intestinal lipoproteins in the S_f range of 20 to 400. When subjects are given a meal containing a modest amount of fat (17 to 34 g fat), only 40% to 54% of the newly formed RE are associated with large CHYLO of S_f greater than 400 (Table 4). In one normolipidemic volunteer who was studied on three separate occasions, the estimated value for P(65) changed from 0.40 to 0.50 and to 0.75 when the fat content in the test meal was increased from 17 to 34 to 68 g fat, respectively. This feature of the model is consistent with data reported by Hazzard and Bierman,[14] which demonstrated that in the absence of a fat load, the majority of labeled retinyl palmitate was associated with lipoprotein particles of S_f 20 to 400.

It is accepted that a wide spectrum of intestinal particles differing in size and composition may be found in the circulation,[3-5] but little information is available on the characteristics of the different subpopulations. In our model, CHYLO of S_f greater than 400 are represented by a series of pools corresponding to four subpopulations with different kinetic fates. They are kinetically distinct, since CHYLO in pool 24 of S_f greater than 400 can be converted to S_f 20 to 400 in a single step, whereas CHYLO in pool 6, being more TG-rich, must be sequentially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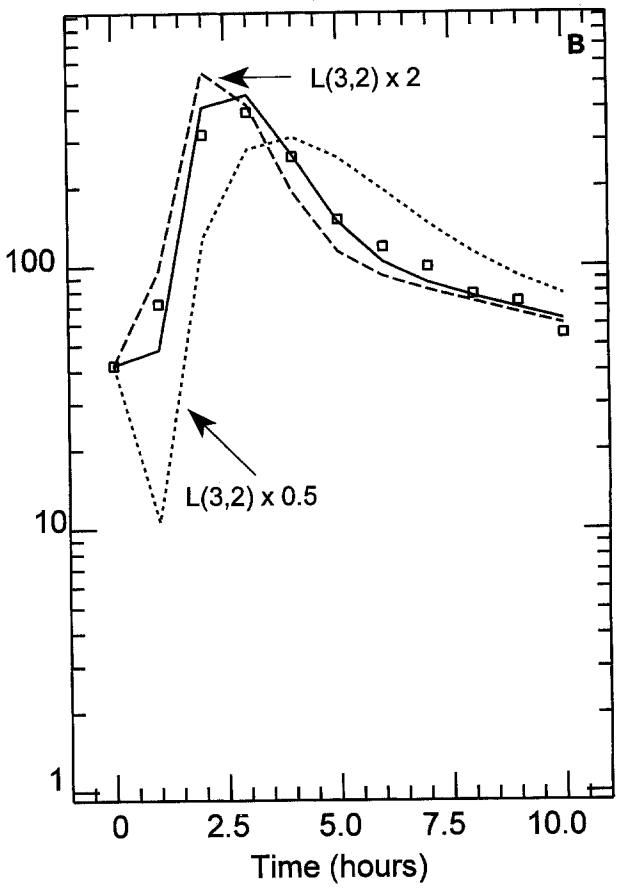
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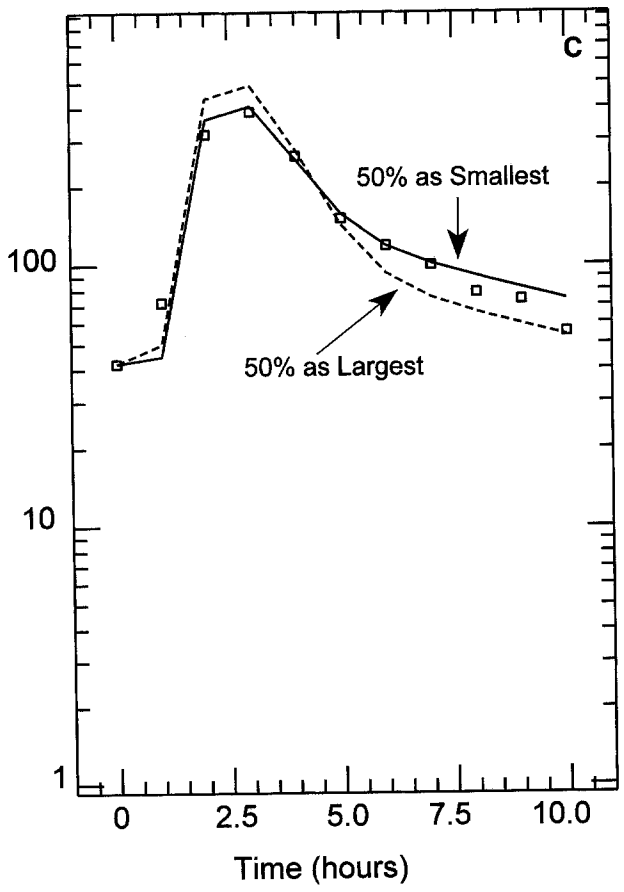
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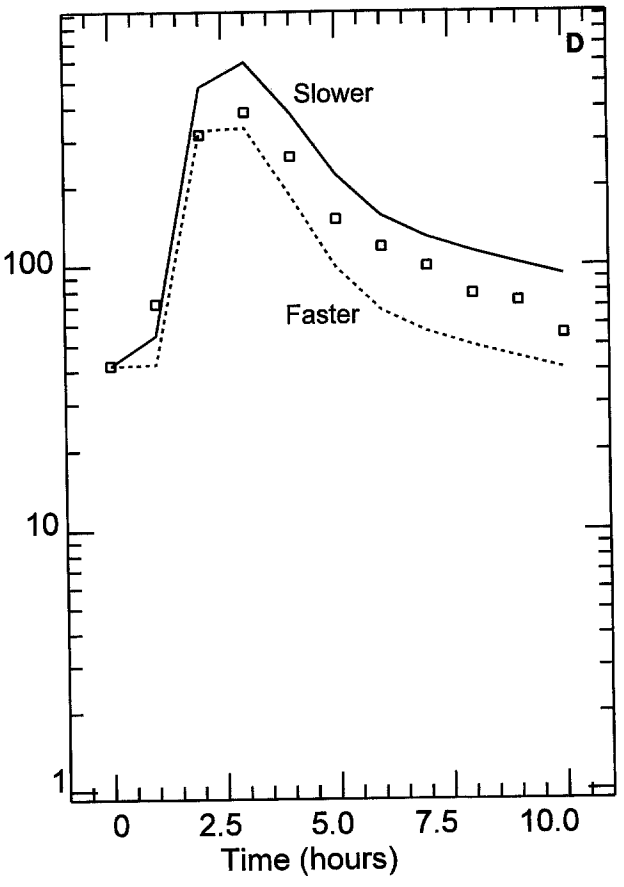
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converted to pools 22, 23, and 24 before reaching S_f 20 to 400. However, newly secreted CHYLO are not limited to pool 6. Compositional data on CHYLO isolated from lymph before exposure to any lipolytic activity would suggest that the intestine can package and secrete particles with a wide range of sizes.[35] To reflect this empirical observation, the compartmental model also allows for direct secretion of RE-containing particles into all four pools comprising the CHYLO cascade (Figs 1 and 4).

How can we determine the distribution of newly secreted RE among these four subpopulations of CHYLO? While keeping all of the fractional turnover rates constant, we can simulate the effect of changing the distribution of RE among these subpopulations on the shape of RE curves in plasma and other lipoprotein fractions. Figure 3C illustrates that only minimal differences in the shape of the CHYLO-RE curve can be seen when 50% of the RE flux is allowed to be secreted directly into the largest subpopulation (pool 6), as compared with the case in which 50% is secreted into the smallest subpopulation (pool 24). In both instances, 16% of total RE flux is assumed to be secreted into each of the remaining three subpopulations. Thus, knowledge of the exact distribution of RE among CHYLO subpopulations may not be critical to describe the CHYLO-RE curve. In the present analysis, we have assumed the simplest scheme in which newly formed RE are equally distributed among all four subpopulations of CHYLO.

Once these lipoprotein particles of S_f greater than 400 enter the vascular space, they may be metabolized via one of three possible pathways. As TG in CHYLO are hydrolyzed by LPL, some of the particles will become less buoyant and be gradually converted to lipoproteins of S_f less than 400. Partial hydrolysis of other CHYLO particles, on the other hand, may cause these particles to be directly removed from the circulation in toto without conversion to other lipoprotein classes. This will depend on the accessibility of ligands on CHYLO and the availability of receptors on cell surfaces. Finally, some of the partially hydrolyzed particles may become resistant to further hydrolysis[21] while still retaining a sufficient amount of TG to remain buoyant in the fraction of S_f greater than 400 (pool 16). Three parameters are required to characterize these metabolic pathways of CHYLO-RE, including K(6), P(60), and L(16,24).

In the model, K(6) represents the LPL-associated fractional turnover rate of RE within the CHYLO delipidation cascade. In view of in vitro data that suggest LPL may have a higher affinity for TG-rich particles,[37,38] one might expect that the fractional turnover rate for TG would be different for large and small CHYLO, with a faster turnover rate associated with the larger particles. However, the fractional turnover rate for RE in the core of these CHYLO, may or may not change as TG are removed. By allowing K(6) to either increase or decrease progressively with each pool in the cascade, we can show that although the area under the CHYLO-RE curve is affected by

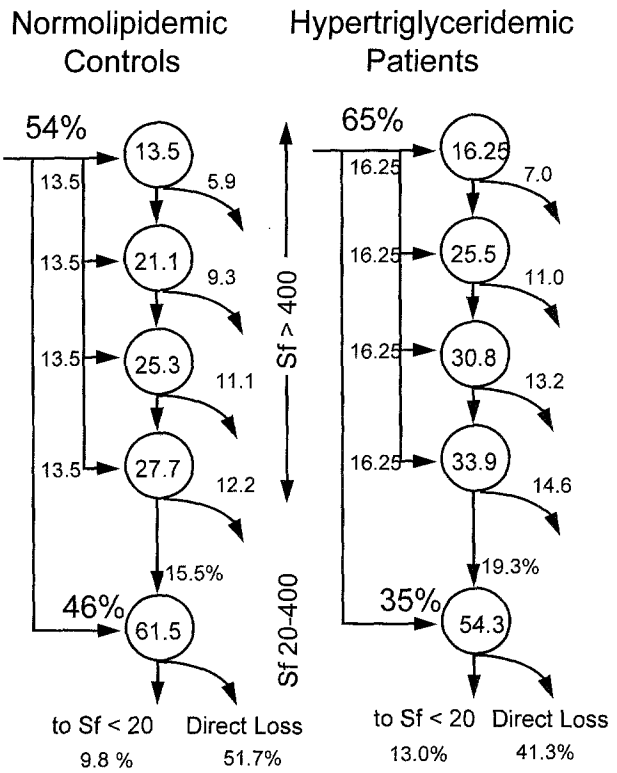

Fig 4. Representative flow of RE through the 4 subpopulations of CHYLO in normolipidemic and hyperlipidemic subjects. The first 4 pools correspond to pools 21 to 24, with pool 24 feeding into pool 7 associated with the S_f 20 to 400 fraction. In normolipidemic controls, approximately 54% of the absorbed dose is secreted as $S_f > 400$, with one quarter, or 13.5%, going directly into each of the 4 subpopulations. In hypertriglyceridemic subjects, 65% of the absorbed dose enters the circulation as $S_f > 400$. In both groups of subjects, on average, 56% is converted down the cascade and 44% is directly removed from the circulation. The net result is that 9.8% of the absorbed RE reaches $S_f < 20$ in normals, as compared with 13.0% in hyperlipidemic subjects. In normals, 13.5% of the total RE flux enters the plasma as the largest CHYLO, 7.6% is converted to the next subpopulation, and 4.23%, 2.37%, and 1.32% are converted to the subsequent 3 subpopulations, with 0.74% ultimately being converted to the $S_f < 20$ fraction. This accounts for 7.6% (0.74/9.8) of the total RE flux converted to $S_f < 20$. In hypertriglyceridemic subjects, 16.25% enters the plasma as the largest CHYLO, but only 0.89% reaches $S_f < 20$.

Fig 3. Effect of changing specific fractional turnover rates on the appropriate RE concentration curves. (A) Solid line depicts the curve with default values for K(1) and K(2). Changes either in the input rate K(1) · P(21) by a factor of 2 (- - - -) or in the output rate K(2) by a factor of 1.5 (– – –) can be shown to have a similar effect on the plasma RE curve. Since the input rate K(1) · P(21) can be shown to exhibit only minimal variability among individuals, we chose to fix the input rate to allow for a better estimate of K(2). (B) Changes in L(3,2) by either a factor of 2 (– – –) or a factor of 0.5 (- - -) can be shown to have a profound effect on the shape of the curve (——), specifically the time to peak and the broadness of the peak. (C) Changes in the distribution of newly secreted RE among the 4 pools of plasma CHYLO do not significantly alter the shape of the RE curve: (□) 25% of newly secreted RE entering the CHYLO cascade as pools 6, 22, 23, and 24; (— — —) 50% entering the plasma as large CHYLO pool 6 and 16.67% as each of the other subpopulations, pools 22, 23, and 24; (——) 50% as small CHYLO (pool 24) and 16.67% as each of the other subpopulations, pools 6, 22, and 23. (D) Changes in the fractional turnover rate of each pool in the delipidation cascade do have a significant impact on the plasma RE curve: (——) fractional turnover rates progressively increase with smaller subpopulations; (— — —) fractional turnover rates progressively decrease with smaller subpopulations. (□) Represents the situation when all 4 subpopulations have the same turnover rates.

progressive changes in the turnover rates, the overall shape is not changed (Fig 3D). For our analysis, K(6) was assumed to be the same for all four subpopulations of CHYLO and estimated as a single unknown parameter.

With TG hydrolysis, the core RE in CHYLO can either remain with the particles as they are converted to less buoyant lipoproteins or be removed irreversibly from the sampling space as the particles are taken up in toto. P(60) defines this fraction of CHYLO-RE flux that is converted to the next subpopulation of particles of slightly higher density. The fraction of CHYLO-RE flux that is directly removed from the circulation without conversion to other lipoproteins is 1-P(60). On average, 56% of CHYLO-RE flux is converted to small CHYLO and 44% is directly removed from each subpopulation (Table 4). This fraction, P(6), tends to be smaller in hypertriglyceridemic subjects, suggesting a less efficient conversion to smaller lipoproteins. According to our model, CHYLO-RE secreted as the largest CHYLO (pool 6) will be preferentially removed from the circulation by direct uptake, with only 9.8% ($13.5 \times [0.56 \times 0.56 \times 0.56 \times 0.56]/13.5$) of its RE flux being converted to S_f less than 400 (Fig 4). In contrast, 56% of the RE flux entering the plasma as small CHYLO (pool 24) is converted to S_f 20 to 400. This concept of preferential uptake of larger TG-rich particles without conversion to a less buoyant fraction has previously been described by Packard et al[39] for different subpopulations of hepatic VLDL.

Figure 4 illustrates another feature of the proposed metabolic scheme. In the present model, metabolic pools containing small particles will include newly secreted RE, as well as RE associated with remnant lipoproteins. In normolipidemic controls, our kinetic analysis would indicate that small CHYLO in pool 24 account for 31.6% (27.7/[13.5 + 21.1 + 25.3 + 27.7]) of the total RE in S_f greater than 400, as compared with only 15.4% (13.5/[13.5 + 21.1 + 25.3 + 27.7]) in pool 6 corresponding to the largest CHYLO. Thus, this mathematical scheme is consistent with a gradual enrichment of esterified retinol (and of esterified cholesterol) as CHYLO are hydrolyzed to smaller particles.[6,7]

Another aspect of the metabolism of intestinal lipoproteins of S_f greater than 400 involves the partially hydrolyzed CHYLO that have become resistant to lipolysis despite their relatively high TG content.[21] This pathway, characterized by L(16,24), has to be included to account for the slowly metabolized tail component of the decay curve. The half-life of this component ranges from 6.5 to 15 hours, as compared with a half-life of 10 to 45 minutes for particles in the normal delipidation cascade. The kinetics of the slowly metabolized component that accounts for 1% to 3% of total RE flux is not taken into consideration in our definition of the half-life for CHYLO-RE, which specifically describes the kinetics of the delipidation cascade.

Subsystem for S_f 20 to 400

RE recovered within S_f 20 to 400 may be associated either with newly secreted VLDL-sized intestinal lipoproteins or with partially hydrolyzed CHYLO remnants. The contribution of these two sources can only be assessed from simultaneous analysis of the kinetic curves of RE concentrations in S_f greater than 400 and S_f 20 to 400. Studies that only examine RE concentrations in whole plasma and in TG-rich lipoproteins would not produce the kinetic data necessary to evaluate this process.

Three additional model parameters can be estimated from the RE curve corresponding to lipoproteins in S_f 20 to 400, including K(7), P(70), and L(17,7). By analogy to the kinetics of RE in S_f greater than 400, the fractional turnover rate K(7) reflects three distinct metabolic pathways: (1) conversion to less buoyant lipoproteins by further hydrolysis, characterized by the fraction P(70); (2) removal via direct receptor-mediated uptake, characterized by 1-P(70); and (3) conversion to the LPL-resistant subpopulation of VLDL-sized particles, L(17,7), which are catabolized at a significantly slower rate.

To be consistent with our concept of the stepwise hydrolysis of TG-rich lipoproteins, the subsystem for S_f 20 to 400 should also be described by a delipidation cascade similar to that used for describing the kinetics of RE in S_f greater than 400. However, available kinetic data do not warrant this increased complexity in the model. One possible explanation is that, in contrast to the fate of RE associated with large CHYLO, the majority of RE associated with particles of S_f 20 to 400 are directly removed without conversion to more dense lipoproteins, S_f less than 20. Thus, based on the kinetics of RE, a stepwise process is not required for delipidation of small intestinal lipoproteins of S_f 20 to 400, with less than 15% of the RE flux through this fraction being converted to smaller, less buoyant particles. In contrast, 56% of the RE flux through the larger S_f greater than 400 must be converted to one or more subpopulations of smaller particles before leaving the density fraction.

As with CHYLO, a fraction of the RE may remain within S_f 20 to 400 but is resistant to further lipolysis. The fractional turnover rate of pool 17 is assumed to be similar to that of pool 16, its counterpart in S_f greater than 400. The half-life for RE in lipoproteins of S_f 20 to 400 used in Fig 6 did not include the contribution of this slowly catabolized subpopulation.

Subsystem for S_f 0 to 20

From the simultaneous analysis of the curves describing the concentrations of retinyl palmitate in plasma, CHYLO, and VLDL available with our protocol, a conversion pathway from S_f 20 to 400 to a lipoprotein fraction of density greater than 1.006 g/mL must be postulated. The majority of RE can be recovered in the density fraction 1.006 to 1.063 g/mL corresponding to S_f 0 to 20. On average, 10% to 15% of the RE flux through S_f 20 to 400 is converted to S_f 0 to 20. In most individuals, this is equivalent to less than 3% of the RE flux entering the plasma as CHYLO of S_f greater than 400. In more recent studies when direct determinations of RE in the density fraction greater than 1.006 g/mL were available, it can be shown that the proposed model can accurately describe the experimental RE curve (Fig 2C). From the present analysis, the half-life of RE in pool 8 would be in the magnitude of days (comparable to the half-life reported for plasma LDL), in contrast to minutes for S_f greater than 400 and hours for S_f 20 to 400. This difference in turnover rates can account for the association of 30% to 40% of RE with particles in the density fraction greater than 1.006 g/mL fasting plasma, despite the fact that less than 3% of total RE flux is actually converted to LDL-sized particles.

Reproducibility of the Postprandial Response

Figure 5 illustrates the reproducibility of key metabolic parameters estimated by the compartmental model using data from repeated studies in a group of 17 hypertriglyceridemic subjects. The set of four RE curves, corresponding to plasma, S_f greater than 400, S_f 20 to 400, and S_f less than 20, from each study were independently fitted. Final values for these metabolic parameters reflect both the day-to-day variability in the postprandial response for each participant between the two study periods and the mathematical error in parameter estimation using the compartmental model. Correlation coefficients for simple regression between the two independent estimates of the model parameters are .86 or better. The mean difference (approximated by the slope of the regression line passing through the origin) in the estimates for these parameters ranges from 4% for the fractional turnover rate K(6) of RE in S_f greater than 400 (Fig 5A) to 8% for the fractional turnover rate K(7) of RE in S_f greater than 400 (Fig 5B), with estimates for most of the remaining parameters in the 5% to 10% range. For instance, the mean difference between the two estimates was 5% for both the fractional turnover rate L(3,2) for the fast synthetic pathway and for P(60), the fraction of RE converted down the cascade.

Association Between the Kinetics of Intestinally Derived RE and the Kinetics of Hepatic ApoB

Figure 6 illustrates the correlation between the half-life of RE in S_f 20 to 400 and the production rate for VLDL apoB. The production rate for VLDL apoB was calculated from the fractional synthetic rate (FSR) of VLDL apoB and an independent estimate of VLDL apoB pool size. The FSR of VLDL apoB was estimated from the tracer to tracee ratio of apoB following the prime constant infusion of ^{15}N-glycine. The correlation between VLDL apoB production rate and half-life of RE in S_f greater than 400 lipoproteins, although statistically significant, was weaker (r = .45, data not shown).

DISCUSSION

The metabolism of postprandial lipoproteins in man is still poorly understood. Wilson et al[18,19] have shown that the elevated concentrations of RE in plasma samples taken 24 hours after consumption of a fat meal containing vitamin A can be related to delayed clearance of intestinal lipoproteins in several groups of patients with lipid disorders. However, clearance of RE from plasma lipoproteins was shown by Berr et al[20,21] to be quite complex. Most of the reports to date used areas under the

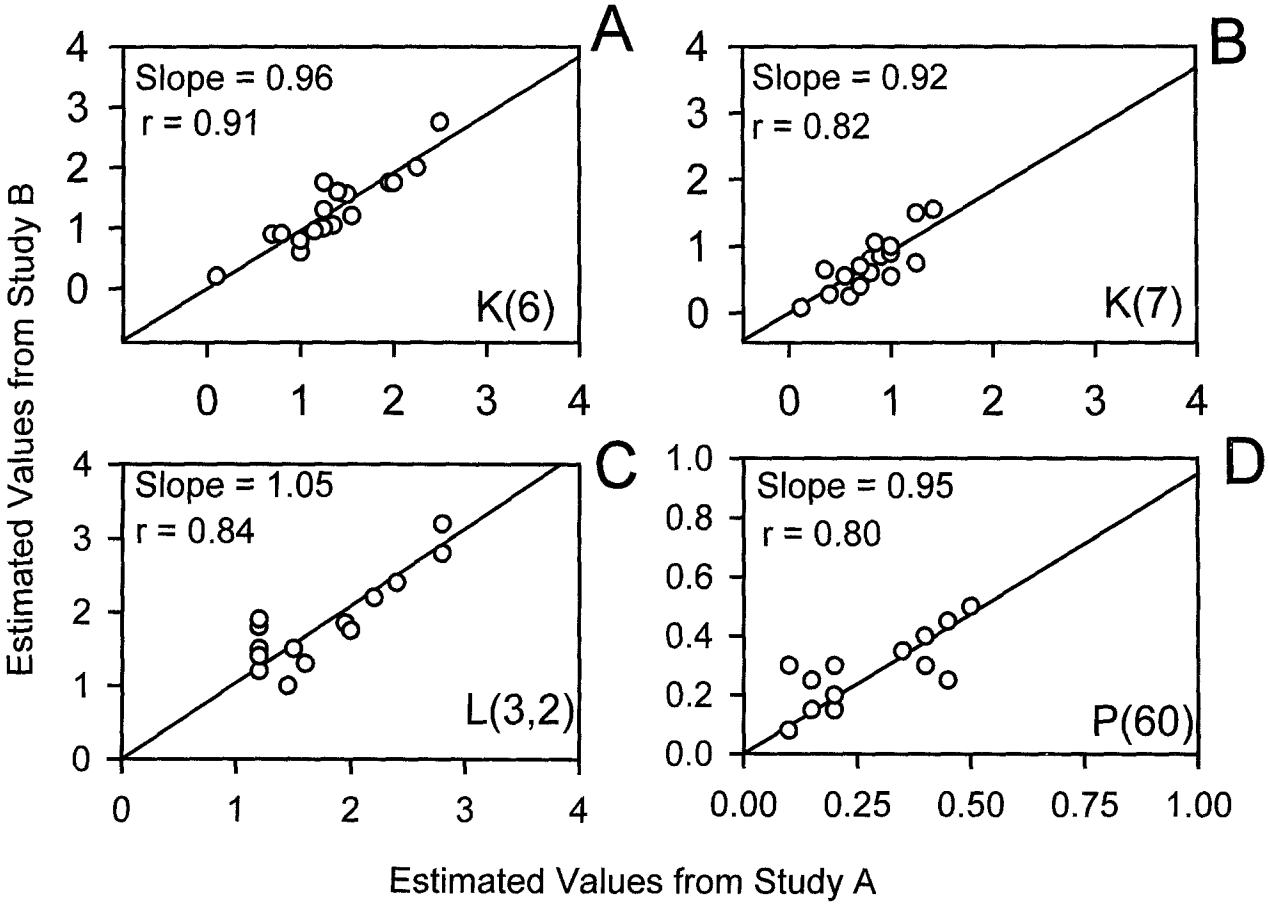

Fig 5. Reproducibility of the estimates for the primary metabolic parameters based on repeat postprandial studies in the same individuals. The slope of the regression line that included the origin was used as an estimate of the mean difference between the 2 independent estimates of the model parameters. (A) Fractional turnover rate K(6) of RE in S_f > 400 CHYLO; (B) fractional turnover rate K(7) of RE in S_f 20 to 400 lipoproteins; (C) fractional turnover rate L(3,2) of the cascade in the fast synthesis pathway; (D) Fraction P(60) of the RE flux that is converted to the next subpopulation in the cascade instead of being directly removed without conversion to smaller, less buoyant lipoproteins.

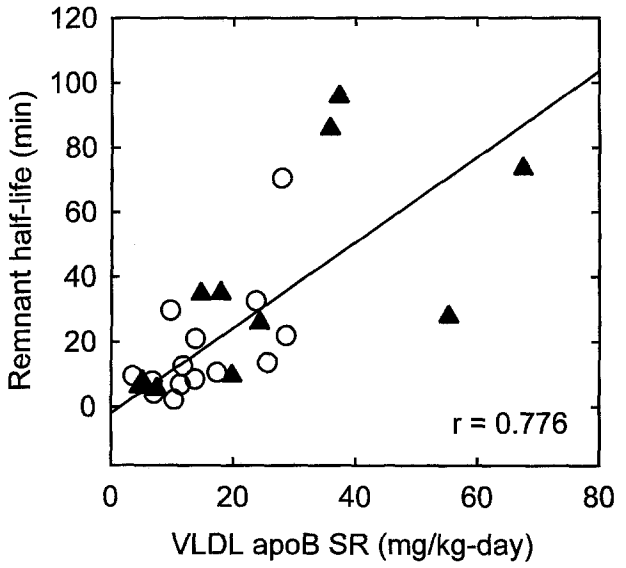

Fig 6. Overproduction of VLDL apoB as assessed by a primed constant infusion of ^{15}N-glycine is correlated with delayed clearance of CHYLO remnants as assessed by the half-life of RE in S_f 20 to 400 during postprandial lipemia. This relationship can be demonstrated in normolipidemic controls (○) and in patients with various forms of hyperlipidemia (▲).

RE curves to assess differences in the kinetics of intestinally derived RE during postprandial lipemia. This approach does not distinguish the rate of RE secretion from the actual residence time of RE in plasma. Furthermore, this approach does not allow evaluation of certain metabolic processes such as the fraction of RE entering the circulation as S_f greater than 400 as compared with S_f 20 to 400, the conversion of RE from large to small particles, and the synthesis times. Our data would indicate that these metabolic parameters may be affected by specific interventions such as therapy with bile acid–binding resins,[40] gemfibrozil,[40] hepatic methylglutaryl coenzyme A reductase inhibitors,[41] and weight loss.[42]

Using the proposed compartmental model, some insights into the metabolism of intestinal lipoproteins can be established. Our data indicate that with 17 to 30 g fat, as much as 40% to 50% of newly formed RE enter the circulation as small intestinal VLDL. With larger fat loads, more RE will enter the circulation as S_f greater than 400.[40] This is consistent with previous observations by Hazzard and Bierman.[14] Compartmental analysis also suggests that the majority of RE flux entering the circulation as large CHYLO would be directly removed before conversion to VLDL- or LDL-sized lipoproteins. As illustrated in Fig 4, of 9.8 U RE converted to S_f less than 20, 7.4, or 75.5%, entered the plasma initially as small VLDL (pool 7, Fig 1) and only 0.2 U or 2%, entered the plasma initially as the largest CHYLO (pool 6, Fig 1). Thus, by varying the fat content of the test meal, we may affect the type of particles being secreted and the contribution of different metabolic pathways may be reflected in the kinetics of RE.

The relative contribution of the stepwise delipidation cascade and that of the direct removal pathway on the kinetics of RE in S_f greater than 400 could be distinguished in our studies because experimental data on the kinetics of RE in VLDL-sized particles (S_f 20 to 400) were available. As a result of interactions with lipolytic enzymes, newly secreted CHYLO become progressively smaller and are converted down the delipidation cascade. This path is characterized by a fractional turnover rate K(6) that is significantly faster in normolipidemic as compared with hypertriglyceridemic subjects (Table 4). Alternatively, the partially hydrolyzed CHYLO can be removed via interaction with specific receptors and be irreversibly removed without conversion to smaller particles. Approximately 50% of the RE can be directly removed at each step (Table 4), independent of fasting lipid profiles. Whether interventions that are known to have a direct effect on the activity of the remnant receptor (either the LDL receptor-like protein or the VLDL receptor) could alter this parameter are yet to be examined.

An important outcome of this kinetic scheme is the preferential loss of RE associated with larger CHYLO as compared with RE in smaller particles. It should be emphasized that the model assumes that at each stage of the delipidation cascade, a particle has the same chance of being converted to a smaller particle or directly removed via receptor-mediated uptake, and it does not postulate a different metabolic process for larger CHYLO. By having to undergo more steps within the delipidation cascade, larger particles are more likely to be removed in toto and fewer of them will survive to become VLDL-sized particles. This same scheme could potentially explain the reduced conversion of radioiodinated apoB from S_f 200 to 400 VLDL to S_f 0 to 12 LDL described by Packard et al.[39]

Using immunoaffinity chromatography, Cohn et al[43,44] have delineated another level of complexity in the use of oral retinol as a marker of intestinal lipoproteins. As much as 25% of the RE in postprandial plasma may be associated with hepatic apoB-100 in normolipidemic controls receiving 1 g fat/kg body weight, or approximately 75 g fat without cholesterol.[44] There was a time-dependent increase in the percent of RE in apoB-100–containing lipoproteins, from a few percent at 2 and 4 hours to 20% to 30% at 9 hours postprandially. It is not clear whether this presence of RE in hepatic lipoproteins could represent a redistribution of core lipids between intestinal and hepatic lipoproteins as mediated by the lipid transfer proteins. Alternatively, this could represent the recycling of newly internalized core lipids from TG-rich intestinal lipoproteins in newly formed hepatic lipoproteins of RE. Additional studies to address factors that may affect the rate of appearance and the contribution of intestinally derived RE in hepatic lipoproteins are needed.

Thus, although the oral fat challenge test has become an important tool in understanding the metabolism of intestinal lipids and lipoproteins in the postprandial state, the complexity of the system must not be overlooked. Generalization of results should not be made unless comparable test meals standardized for fat content and composition are used. Inconsistencies in findings reported by different investigators may be explained by differences in the composition of the fat-containing meal. The use of large fat loads (≥75 g fat) may accentuate existing abnormalities in the lipolytic system in some individuals, and a smaller fat load may allow the kinetics of small intestinal lipoproteins to be examined. Using a moderate fat load, we have been able to demonstrate that 50% of the RE are directly

secreted as small VLDL-sized intestinal particles, and the half-life of RE in this density fraction was highly correlated with the production rate of VLDL apoB by the liver (Fig 6). In other words, delayed clearance of CHYLO remnants may be an indirect marker for overproduction of apoB by the liver. This correlation would be consistent with the delayed clearance of CHYLO remnants observed by Groot et al[28] in men with documented CAD compared with male controls with matching fasting lipid profiles, since these patients are more likely to have overproduction of hepatic apoB, as suggested by Vega et al.[45] Preliminary data from ongoing studies on the acute effect of different cholesterol contents in the test meal would suggest that higher cholesterol content in the test meal is associated with greater conversion to S_f 20 to 400 particles. This may explain the apparent contradiction between the results of Groot et al[28] and Sharrett et al.[46] Using a comparable fat load but only 300 mg cholesterol, Sharrett et al failed to demonstrate an association between carotid intima-media thickness and postprandial RE in whole plasma. An association was found only with postprandial TG. By challenging their subjects with a higher load of cholesterol (500 mg/m^2 body surface area), differences in both postprandial TG and remnant RE were observed in CAD patients.[28]

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A TRIBUTE TO DR LOREN ZECH

This report is dedicated to Dr Loren Zech, a caring teacher and dear friend. Dr Zech's guidance in the early stages of learning and understanding SAAM will always be missed. Many of the approaches used in the development of this model were based from Dr Zech's work on the multicompartmental model for endogenous triglycerides. Dr Zech passed away on January 29, 1996.

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