A kinetic model of chylomicron core lipid metabolism in rats: the effect of a single meal

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Abstract A detailed quantitative description of the metabolism of the core lipids of chylomicrons was developed with data obtained from rats injected intravenously with radioactive chylomicrons. Groups of recipient rats were starved or else fed a single meal that was either fat-free or contained 5% fat. The kinetic model included chains of delipidation compartments and a remnant particle. Compartments for recirculation of portions of triglyceride, cholesteryl ester, and unesterified fatty acid radioactivities were all included and necessary to obtain good fits to the data. Making the simplifying assumption that remnants could be represented as particles retaining cholesteryl esters but depleted in triglycerides, a 'delipidation index' was calculated as the ratio of the cholesteryl ester residence time divided by the triglyceride residence time. Significant differences were found in the delipidation index between the starved group and both the normal-fed and fat-free-fed groups. Compared with rats fed a meal of the usual diet which contained 5% fat, starved rats showed more extensive delipidation and a trend toward a longer residence time of remnant particles. A longer residence time of triglycerides in rats fed a fat-free meal was explained by less delipidation, but remnant removal appeared to be accelerated. The kinetic model makes possible an interpretation of the patterns of metabolism in terms of the known underlying physiological mechanisms.—**Redgrave, T. G., and L. A. Zech.** A kinetic model of chylomicron core lipid metabolism in rats: the effect of a single meal. *J. Lipid Res.* 1987. **28:** 473–482.

Supplementary key words chylomicrons • lipoprotein metabolism • cholesteryl ester • triglyceride • lipoprotein lipase

The general qualitative aspects of chylomicron metabolism in rats have been described (1), but a detailed quantitative description is not yet possible for several reasons. The major difficulties arise because chylomicron metabolism is rapid, with fractional turnovers typically about 0.3/min, and complex, with multiple interactions with enzymes, apolipoproteins, plasma lipoproteins, and cell receptors. An appropriate strategy to learn more about the metabolism of chylomicrons is to develop a mathematical model of the kinetics of removal from the plasma to fit the observed data. New insights derived from the model will then allow predictions that can be tested experimentally, for reciprocal validations of the model and the kinetic processes of interest (2). In this way the sites of regulation and responses to physiological and pathological variations can be defined.

Previous mathematical descriptions of chylomicron metabolism either after injection into the bloodstream (3–6) or by hydrolysis in vitro by lipoprotein lipase (7) usually have not taken into account the receptor-mediated removal of chylomicron core remnant particles, containing residual triglycerides, which contributes to the overall kinetics of metabolism. Remnant compartments were included in the model developed by Green, Massaro, and Green (6), but cholesterol was treated as a homogeneous pool, without differentiation of cholesteryl ester from cholesterol despite their different chemical and metabolic potentialities. In the present experiments the contribution by remnant removal to the triglyceride kinetics was assessed by simultaneously tracing the plasma removal of chylomicron cholesteryl esters and triglycerides. Cholesteryl esters were separated analytically from cholesterol so that an unambiguous trace of particle kinetics could be obtained; cholesteryl ester is predominantly a core component and stays with the remnant, whereas cholesterol is predominantly a component of the particle surface much of which becomes detached from the remnant during lipolysis (8).

In the present experiments we compared the metabolism of injected chylomicrons in three groups of rats that were starved or fed meals containing either no fat or approximately 5% fat. Varying the nutritional state of the recipient rats changed the pattern of metabolism of the injected chylomicrons. The kinetic model was adequate to model the metabolism in the different nutritional states, and the parameters derived from the model predicted the underlying physiological changes.

Abbreviations: FFA, free fatty acids; VLDL, very low density lipoprotein.

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METHODS

Animals

Male rats of the Holtzman strain were obtained from Charles River Breeding Laboratories (North Wilmington, MA) and maintained on Purina Rat Chow #5012 with a fat content of 4.5% by weight. At noon on the day preceeding the turnover study, food was removed but drinking water was freely available. At 8 AM the next day groups of rats were not fed or fed either the regular chow or a specially formulated fat-free pelleted diet (Teklad #TD81116, Teklad Test Diets, Madison, WI) containing dextrose, casein, cellulose, methionine, minerals, and vitamins, but only 0.4% by weight of material extractable with chloroform–methanol 2:1.

Chylomicrons

The thoracic duct of 250-g donor rats was cannulated (9) and at the same time a cannula was secured in the stomach via a gastrostomy. Postoperatively the rats were placed in restraining cages and fed the fat-free diet described above. Tap water was freely available and 0.15 M NaCl solution was injected through the gastric cannula at 2.0 ml/hr. Lymph flow measured over consecutive 24-hr periods on the first and second postoperative days was 2.81 ± 0.81 ml/hr (mean ± SD, n = 16). On the second postoperative day a test meal of 0.5 ml of triolein (Nu-Chek-Prep, Inc., Elysian, MN) in which were dissolved 38 μCi of [1-^{14}C]palmitic acid and 75 μCi of [1,2(n)-^{3}H]cholesterol (Amersham Corp., Arlington Heights, IL) was injected into the stomach through the gastric cannula. The lymph became milky within about 0.5 hr and was collected for 6 hr at room temperature (18–25°C) into vessels that contained EDTA, gentamycin, and reduced glutathione, to attain final concentrations of about 2.7 mM, 0.1 mg/ml, and 1.6 mM, respectively. The lymph was not cooled at any time. Cells were removed by low-speed centrifugation, then solid KBr was added (0.14 g/ml) to increase the density to 1.10 g/ml. After degassing in vacuo, 14 ml of the lymph was injected under pre-formed 24-ml volume continuous linear density gradients constructed in centrifuge tubes for the Beckman SW28 rotor over the density range of 1.006–1.10 g/ml. The gradients were then centrifuged at 20°C and 27,000 rpm for 4.0 × 10^{10} rad^2/sec, plus deceleration without braking. Pure chylomicrons with S_f > 400 were recovered from the top 0.5 cm and were used for kinetic studies within 24 hr. Four separate lots were used, each prepared in duplicate.

Kinetic studies

Recipient rats weighing 219 ± 35.8 g were injected after feeding as described above. Injection of the radioactive chylomicrons was through a Teflon cannula 0.76 mm OD × 0.33 mm ID implanted through the left common carotid artery so the tip was located in the aortic arch. Surgery was under ether anesthesia between 1 and 2 hr after the meal (if any). Animals recovered in restraining cages for about 4 hr after surgery before the chylomicrons were injected. Heparin was not used and clotting was avoided by treating the cannula with Siliclad (Becton-Dickinson Co., Parsippany, NJ). The cannulas were kept filled with 0.15 M NaCl solution between samplings. The injected chylomicron bolus contained 5.1, 5.3, 8.0, or 8.3 mg of triglyceride in a volume of 0.5 ml, followed by a saline flush of 0.5 ml. After injection, blood was sampled at 3, 5, 7, 10, 15, 20, and 30 min. Volumes of 0.35 ml were taken into tubes containing 2 units of heparin after first discarding the saline solution filling the cannula. The cannula was flushed with 0.5 ml of saline after each sample. The 30-min sampling was followed by the injection of 20 mg of sodium pentobarbital, then the liver and spleen were rapidly excised and washed in 0.15 M NaCl solution. Contents of the stomach and lactescence of the intestinal lymphatics were noted at this time to confirm the ingestion and absorption of the fed meal. Plasma volume was estimated from the relationship given by Wang and Hegsted (10).

Chemical analysis

Lipids were extracted with chloroform–methanol 2:1 (v/v) from 100-μl portions of the separated blood plasma and from 1-g portions of the excised organs. The lipids were divided into classes by thin-layer chromatography on precoated layers of silica gel G 250 μm with a pre-absorbent zone (Analtech, Newark, DE) in the solvent system hexane–diethyl ether–acetic acid 80:20:1 (by volume). The triglyceride and cholesteryl ester bands were scraped into vials, then 10 ml of Liquiscint (National Diagnostics, Somerville, NJ) was added for liquid scintillation spectrometry in an LKB machine with automatic external standardization. Counts were corrected for quench and crossover between channels with curves established from counting internal standards when monitoring the automatic external standard. Chylomicrons similarly extracted and separated were analyzed for mass of free and esterified cholesterol (11), and for the fatty acid composition of the injected triglycerides by gas–liquid chromatography, after conversion to methyl esters with boron trifluoride in methanol. The methyl esters were separated on a glass column packed with 5% diethylene glycol succinate on 100/200 mesh Supelcoport (Supelco, Inc., Bellefonte, PA), at 175°C in a Hewlett-Packard Model 5710A chromatograph, equipped with a hydrogen flame ionization detector. The carrier gas was nitrogen, and flow rate was 60 ml/min. The detector and injection port temperatures were 300°C. Quantitation was by electronic integration (Hewlett-Packard Model 3385A), normalized to give values as weight percent of total fatty acids. Triglyceride mass was measured by an enzymatic procedure (Sigma Chemical Co., St. Louis, MO, Kit #335-B).

The percent lipid composition of the chylomicrons was (mean ± SD, from eight analyses of duplicate chylomicron preparations) triglycerides, 80.0 ± 3.6; phospholipids, 15.3 ± 2.6; cholesteryl esters, 2.8 ± 0.8; cholesterol, 2.0 ± 0.5. Protein was 1.7 ± 0.3% of total chylomicron mass. Triglyceride fatty acids were oleate, 86.5 ± 4.54%; palmitate, 4.4 ± 1.26%; linoleate, 3.6 ± 1.37%; stearate, 1.8 ± 0.24%; and arachidonate, 0.8 ± 0.21%. Tritium radioactivity was 56.3 ± 4.50% (range 49.4–62.8) associated with cholesteryl ester, while ^{14}C radioactivity was 94.8 ± 1.17% associated with triglyceride.

Model development

The general techniques of compartmental analysis were applied to the data, where a compartment represents a distinct homogeneous pool of material (12). In each compartment the amounts of material are represented by F(I). Material may flow to and/or from all compartments, and flows are represented diagramatically by arrows. The fractional rates of flow of material are represented by $L_{i,j}$ and have the dimensions of time^{-1}. Inputs into the model are represented by arrows flowing into a compartment but not originating in another compartment. An injection of material into the physiological system is represented by an asterisked arrow. Material leaving the model is represented by arrows that do not terminate in another compartment.

Testing of hypothetical compartmental models was with the SAAM/CONSAM simulator (13, 14) on a VAX 11/780 computer system (Digital Equipment Corp., Marlboro, MA). When satisfactory correspondence between the observed and predicted values was obtained, plasma residence times for chylomicron triglyceride and cholesteryl ester were calculated to give the average time each of these components remained in the plasma before initial removal.

Statistics

The values of the parameters given in Table 1 are unweighted arithmetic means with fractional standard deviations. In Fig. 1 the data are plotted as the geometric means, which is appropriate because of the logarithmic transformation not only in the figure but also in the rate constants used in the modeling (15). The differences in the delipidation index were tested against the hypothesis of zero difference, using the t statistic for two groups of equal sample size from populations with equal variance (16) after first testing for overall significance by analysis of variance.

Residence time calculations

The residence time of a chain of compartments that turn over at the same rate has been completely described (17). In the chylomicron model a chain of similar compartments is present except that the last compartment, RM, has an extended residence time. Because of this, equation 22 from reference 17 must contain a correction term.

$$t = t_i \left| \frac{1-\beta^6}{1-\beta} + \text{correction factor} \right| \qquad \textit{Eq. 1}$$

where: t_i = the residence time of core lipids in T_i or C_i and β = fraction going to next compartment.

The correction factor must be applied because in the last compartment, RM, both lipids have equal but increased residence time. For the residence time of chylomicron lipid, t_i, and the residence time of chylomicron remnants, t_r,

$$\text{correction factor} = \beta^5\left(\frac{t_r}{t_i} - 1\right) \qquad \textit{Eq. 2}$$

and equation 1 becomes

$$t = t_i \left| \frac{1-\beta^6}{1-\beta} + \beta^5\left(\frac{t_r}{t_i} - 1\right) \right| \qquad \textit{Eq. 3}$$

or

$$t = t_i \frac{1-\beta^5}{1-\beta} + t_r\beta^5. \qquad \textit{Eq. 3'}$$

We define through experimental design the 'index of delipidation' (ID), a ratio of chylomicron cholesteryl ester residence time to chylomicron triglyceride residence time on the first pass of radioactivity through the model. Therefore, the above equations can be combined resulting in an equation for the index of delipidation. Changing from the notation of reference 17 to a notation that corresponds to the row designations of Table 1, 'delipidation index' was calculated:

$$ID = \frac{\frac{1-(1-B)^6}{B} + (1-B)^5\,(A'\,D-1)}{\frac{1-(1-B)^6\,(1-C)^6}{B+C-BC} + (1-B)^5\,(1-C)^5\,(A'\,D-1)} \qquad \textit{Eq. 4}$$

where A' = the fractional rate of exit from compartments TX and CX; B = the fraction of triglycerides and cholesteryl ester taken up together; C = the fraction of the remaining triglycerides delipidated in the plasma; D = residence time of chylomicron remnant; and $\beta = (1-B)^*(1-C)$. However, if C′ = the fraction of total triglycerides, then $\beta = 1-(B + C')$ where $C' = (1-B)C$.

RESULTS

Kinetic curves

The plasma decay curves for the three groups of rats designated fasting, fat-free, and chow-fed are shown in **Fig. 1.** In each case the removal of triglyceride was more rapid than cholesteryl ester, consistent with many previous findings, and consistent with sequential removal by lipoprotein

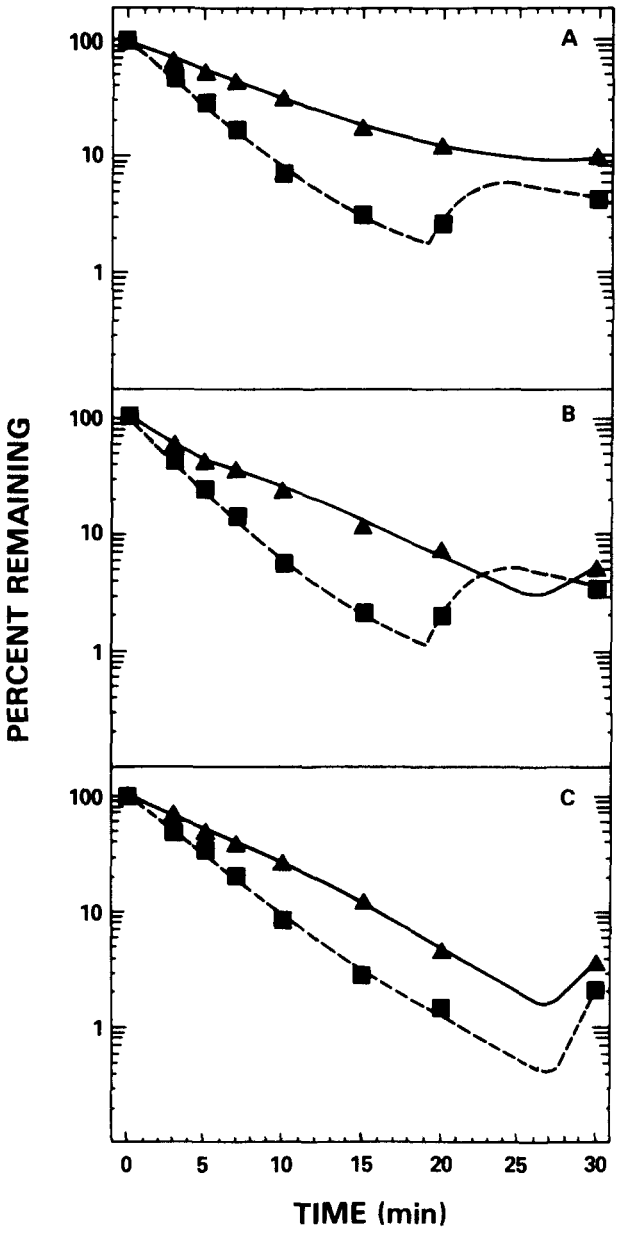

Fig. 1. Radioactivity decay curves for chylomicron triglyceride (--■--) and chylomicron cholesteryl ester (—▲—) as a function of time. A, fasting; B, chow fed; C, low fat.

lipase-mediated hydrolysis of triglycerides followed by uptake of remnant particles (1). Inspection of the initial parts of the curves, to about 15 min after injection, shows that the removal of triglyceride label was more rapid in the chow-fed group. The removal of cholesteryl ester radioactivity was slower in the fasting rats.

Mean recoveries of injected chylomicron radioactivities in the spleen 30 min after injection were 0.18, 0.06, and 0.08% for triglyceride, and 1.03, 0.54, and 0.71% for cholesteryl ester in the fasted, regular fed, and fat-free-fed groups, respectively. The higher recoveries in the fasted rats were statistically significant, with $P < 0.001$ for triglyceride, and $P < 0.025$ for cholesteryl ester. Recoveries in the liver at 30 min are plotted in **Fig. 2,** which shows that cholesteryl ester was similar in all groups at about 20% of the injected dose, but triglyceride was only about 5%, or somewhat less in the fasting rats.

Model development

As a starting point for model development, a pair of linked and constrained sequences was proposed, one for triglycerides and the other for cholesteryl esters. Each sequence contained only two compartments, **Fig. 3,** which illustrates the principles of the mathematical approach. Because the cholesteryl ester (with residual triglycerides) remained in the core remnant when the bulk of the core triglycerides was hydrolyzed by lipoprotein lipase (1), the sum of $L_{RM,T1}$ and $L_{O,T1}$ was constrained to equal $L_{RM,C1}$. Further, to represent the uptake of the remnant particles, the decay constants $L_{O,RM}$ were constrained to be the same for triglycerides and cholesteryl esters. With these constraints a minimum number of three parameter values were adjusted to fit the data: the turnover of the injected chylomicrons, $L_{RM,C1}$, the fraction of triglyceride removed before the lipoprotein particle is considered a remnant, $L_{O,T1}/L_{RM,C1}$, and the turnover of the remnant pool, $L_{O,RM}$. This relatively simple model was inadequate to fit the data for a number of reasons. First, the movement of cholesteryl ester from the CE pool to the RM pool in the cholesteryl ester portion of the model did not constitute a loss of cholesteryl ester from the plasma pool. In other words, the simple model predicted that no cholesteryl ester from the plasma pool was lost until the delipidation was complete. The data did not have such a shoulder on the cholesteryl ester curve indicating that this relatively simple model was inadequate to fit the data.

Because this relatively simple model was inadequate to fit the data and the early parts of the cholesteryl ester curves were not completely monoexponential, it was necessary to replace T1 and C1 with chains of compartments, T1-T5 and C1-C5 (**Fig. 4**) as previously described for lipolysis of triglyceride-rich particles (18, 19). The turnover times for each compartment in both chains were constrained to be equal, as were the turnover times for RM for each lipid. Pseudo-RMs were created at each step of the chain as whole particles were taken up. Mathematically this represents the concept that chylomicrons move through a series of particle sizes and compositions and therefore may be removed by the liver or other tissues at relatively different extents of delipidation. This feature of the model recognized that some chylomicrons need little triglyceride loss before removal as a particle. At this point in the model development only four parameters could be adjusted to fit the data.

Cholesteryl ester removal from the plasma represented uptake of remnant particles, whereas triglyceride removal represented the sum of that taken up as remnant particles and that hydrolyzed by lipoprotein lipase. The latter path-

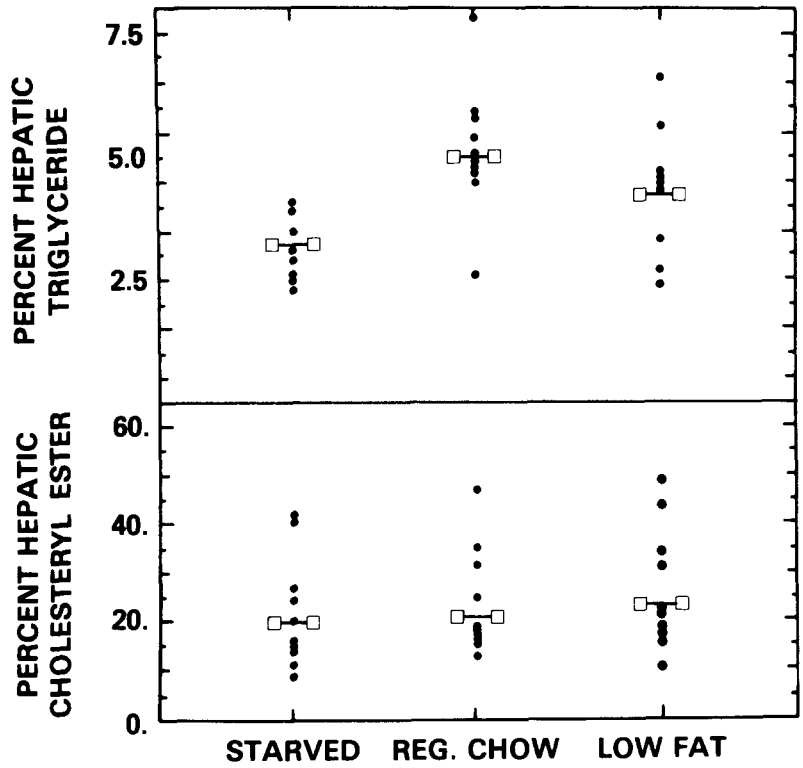

Fig. 2. Hepatic radioactivity at 30 min as percent of injected dose; geometric means (□—□). Note these recoveries at 30 min are lower than those reported 10 to 15 min after injection because of hydrolysis and resecretion of label which begins after about 20 min (20).

way has been shown in Fig. 4 forming FFA, most of which exits from the plasma compartment, but provision has been made for some flow of FFA to the liver. At this point in model development it was necessary to determine a turnover time for the plasma FFA pool and we chose a value larger than or equal to the value previously observed in rats (20) of 1 min^{-1}. It has been previously shown that as much as 40% of the plasma FFA pool could be reutilized to form hepatic triglycerides (21). Provision was also made for a fraction of remnant particles to be taken up at tissues other than the liver, shown as an exit flow from compartments UP in Fig. 4. At this point in the model development five parameters were adjusted to fit the data.

We found that both cholesteryl ester and triglyceride radioactivities in plasma reached a nadir after 15–20 min and then began to increase at 30 min (Fig. 1) which was evidence for recirculation of both lipids within the time frame of the experiment. The model therefore included pathways for recirculation shown through compartments in the liver in Fig. 4. For both lipids these pathways included delays (DL) which were constrained to be equal. This increased the number of parameters to be estimated to six. Inclusion of the recirculation pathways gave good fits to the cholesteryl ester data, but not usually to the triglyceride data. However, by adding a pathway for reutilization and recirculation of the FFA taken up by the liver, the triglyceride data were fitted. The fraction of FFA recirculated was contrained to be less than 40% (20, 21). A delay was also needed in this pathway, and it was found to be a few minutes shorter than the delay for cholesteryl ester and triglyceride taken up as remnant particles. At this point in the model construction there were eight parameters to be estimated; however, the delay for the FFA through the liver was not found to be statistically different from DL and the number of estimated parameters was reduced to seven. In addition, for any single study the fraction of FFA recycled could not be determined with confidence; however, by recycling 25% a systematic error was corrected in each study. When this parameter was fixed the number of adjustable parameters was decreased to six. Average values for four of these parameters are listed in **Table 1**, rows A, B, C, D. The other two parameters are: delay, which was between 15 and 20 20 min, and fraction of chylomicron remnants taken up by the liver. These two parameters are not included in Table 1 because they are dependent on the turnover of the secondary triglyceride-rich lipoproteins secreted by the liver. The absolute value of DL and the amount of lipid recycling through the liver are relative to the choice of model structure which puts the recirculated lipid and the corresponding lipoprotein into the top of the delipidation chain. Since the particles secreted are probably in the VLDL fraction, they surely have a longer residence time

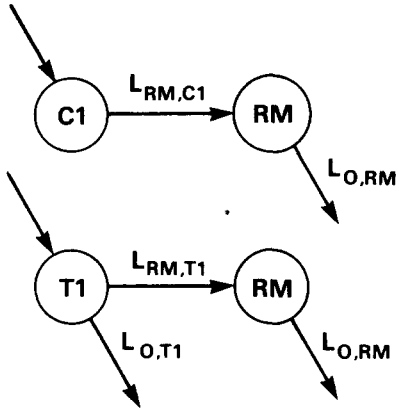

Fig. 3. Starting model. Injected radioactivity enters into C1 and T1 which represent chylomicron cholesteryl ester and triglyceride, respectively.

than chylomicrons. In addition, the fraction of lipid recirculated represents a maximum value. It is important that the first pass residence times are only dependent on the four parameters (Table 1) given in equation 4. In Fig. 4, compartment CA represents the injection catheter, in which turnover was rapid and constrained to be equal for both cholesteryl ester and triglyceride. The final model gave excellent fits of the data as shown by Fig. 1. Furthermore, it fitted the measured liver radioactivities at 30 min and predicted the time courses for appearance and decline of both lipid radioactivities in the liver. This time course is consistent with previously observed results from reference 20 and many other studies.

The average parameter values found with the model are shown in Table 1 for the three groups of rats. The values shown are the averages calculated from the individual curves fitted to data from each rat. When the averaged data (i.e., the geometric means of the plasma radioactivities plotted in Fig. 1) were fitted by the model, the values of the parameters were not statistically different. Residence times for chylomicron cholesteryl esters, triglycerides, and the delipidation index were calculated using equations 1–4.

The residence time of chylomicron cholesteryl ester ranged from 8 to 11 min; this agreed well with the predicted residence time of the remnants. The residence time of chylomicron triglyceride was considerably less, about 4 min in all groups. For each group the 'index of delipidation' of the remnant particles was then calculated as the ratio of the chylomicron cholesteryl ester and triglyceride residence times. Calculation of this index was based only on the 'first pass' of radioactivity through the plasma. Therefore the index cannot be calculated from the areas under the decay curves, which were influenced by resecretion of radioactive lipids by the liver. The variability between individual animals of this dimensionless index was less than for the residence times, shown by the smaller fractional standard deviations in Table 1, suggesting that the absolute extent of delipidation was more closely regulated than the time-dependent parameters, and also facilitating statistical comparisons between the groups. Our findings showed that the index of delipidation was significantly less in both groups of fed rats than in fasting rats.

DISCUSSION

The kinetic model fitted the observed data extremely well. We have incorporated into the model all of the metabolic principles that are known to underlie the clearance of injected chylomicrons and their metabolic products from the plasma. Our findings can be compared with the compartmental model of Green et al (6). Several differences to be noted are methodological. Because anesthesia impairs chylomicron metabolism (3) our study avoided the use of anesthesia by injecting and sampling through intravascular cannulas. In contrast, Green et al. (6) anesthetized their rats with diethyl ether before injecting the dose of chylomicrons. Also in contrast, our study avoided cooling the injected chylomicrons, which causes crystallization of the core triglycerides (22) and which was reported to change chylomicron metabolism (23). Perhaps because of these differences, Green et al. (6) found chylomicron residence times of 18–41 min, much longer than our findings of about 4 min for triglycerides and about 10 min for cholesteryl esters. Our findings were consistent with many previous measurements (1, 3–5).

Another important difference between our model and that of Green et al. (6) is that we traced radioactive label in cholesteryl esters as a component predominantly of the chylomicron particle core. In contrast Green et al. (6) did not separate cholesteryl ester label from free unesterified cholesterol. Free cholesterol is a component of the particle surface (24), and therefore participates in exchange reactions with cell membrane cholesterol and with other lipoproteins. Further, much free cholesterol label transfers to the high density lipoprotein fraction when chylomicron triglycerides are hydrolyzed (8). Because of these complications, free cholesterol label does not trace the metabolism of the chylomicron remnant as unambiguously as cholesteryl ester, which probably accounts for most of the structural differences between the model developed by Green et al. (6) and our model. In our studies, chylomicron free cholesterol was assumed not to recirculate as cholesteryl ester within the 30-min period of measurement.

Several features are included in our model and are necessary for good fits to the data. First, chains for the action of lipoprotein lipase on chylomicron triglycerides (T1-T5 and C1-C5) are similar to those included previously in models of metabolism of plasma VLDL and triglycerides (18, 19, 25, 26). Second, compartments RM representing remnants with residence times of about 10 min acknowledged the physiological participation of particles with a portion of their triglyceride removed, previ-

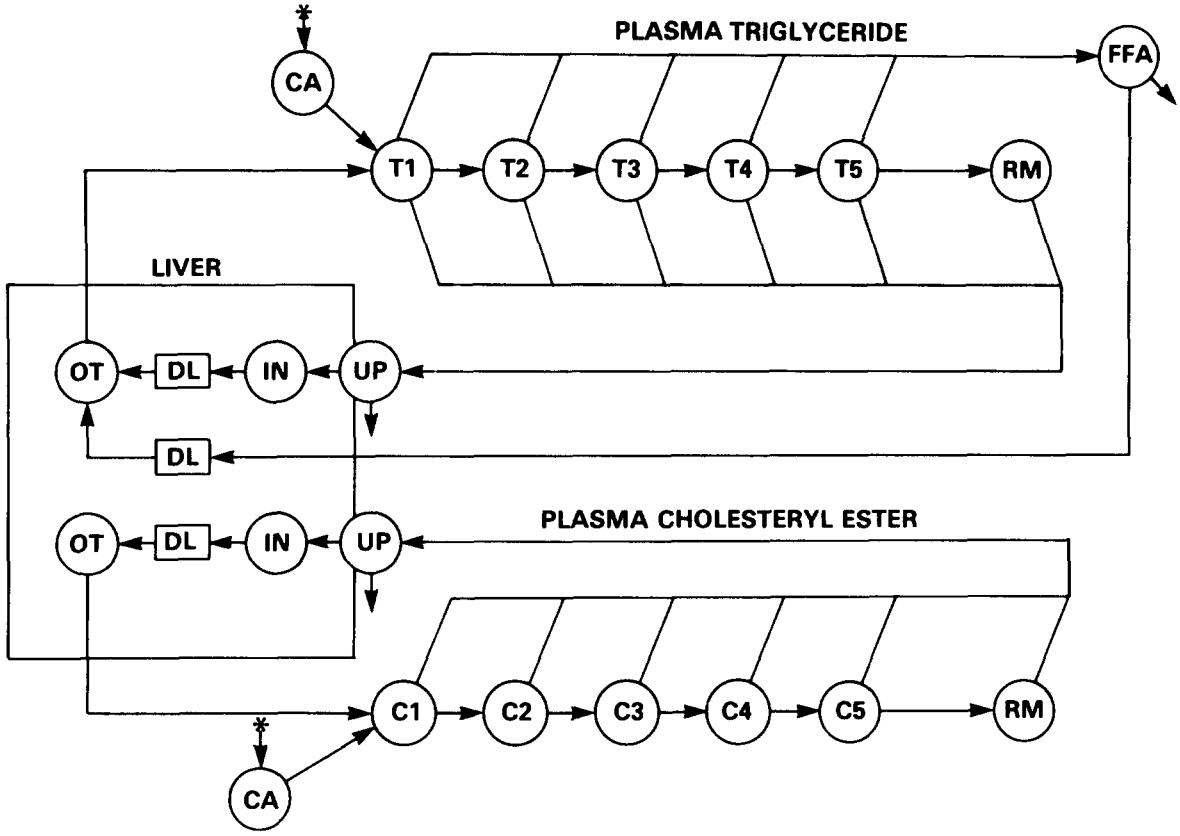

Fig. 4. Compartmental model for chylomicron core lipid metabolism. FFA, free fatty acid pool; DL, delay pools; RM, chylomicron remnant pool; CA, catheter space; UP, uptake; IN, input pools; OT, output pools.

ously recognized in simpler experiments (27). Third, provision was necessary for resecretion and recirculation of a portion of the triglycerides and cholesteryl esters taken up by the liver. Previous physiological correlates for this aspect of the model were provided from the data of Goodman (28), who showed that after an initial delay of about 15 min chylomicron cholesteryl ester taken up by the liver was hydrolyzed and subsequently resecreted. Fourth, provision was made to represent triglyceride resecretion by the liver, derived from a portion of the FFA liberated from lipoprotein lipase-mediated hydrolysis of the chylomicron triglycerides. Similar provisions were necessary in models of triglyceride metabolism in rats (20) and humans (21). A delay of about 10 min was found in the appearance of injected radioactive FFA in the triglycerides of rat plasma VLDL (20, 29), which correlated well with the delay fitting our compartmental model.

The parameters of the model enabled the extraction of underlying metabolic steps not otherwise obtainable from the decay curves, for example the 'first pass' residence times of the chylomicron core lipids. Therefore the residence time of the chylomicron triglycerides could be compared directly with the residence time of the cholesteryl esters, and the 'delipidation index' so calculated gave a simplified representation of the extent of hydrolysis of any particular chylomicron sample. Assessment of the effect of nutritional status was facilitated by the index, and its relation to remnant particle uptake by the liver could be evaluated. The index was determined only by lipoprotein lipase hydrolysis, whereas it was insensitive to uptake of particles by the liver or subsequent metabolic steps. If there was zero lipolysis, the index would be 1.0. Lipolysis of 90% of the chylomicron triglycerides would give an index of about 6, with 18.7% taken up as intact chylomicrons and with a remnant residence time of 11.2 min. The findings of our study (Table 1) show an index of 2.73 in the group of fasting rats, corresponding to 63% lipolysis. Feeding a meal with 5% fat decreased the index to 2.21, whereas a meal with 0% fat decreased the index to 1.89, corresponding with 55% or 47% lipolysis, respectively.

Contrary to what might have been expected, our findings showed that the index of delipidation (row G in Table 1) was not related to the residence time of chylomicron triglyceride (row E). Instead, an apparent positive correlation with the residence time of RM (row D) was obvious, with a correlation coefficient $r = 0.99$. Therefore, the extent of chylomicron triglyceride hydrolysis was inversely related to the rate of RM uptake by the liver. The twin

TABLE 1. Kinetic parameters

	Parameter	Units	Fasting	Chow Fed	Low Fat
A	Residence time of individual compartments TX and CX	min	2.03 ± 0.30[a]	1.86 ± 0.42	2.23 ± 0.37
A'	Fractional rate of exit from compartments TX and CX	min^{-1}	0.524 ± 0.30	0.613 ± 0.42	0.508 ± 0.37
B	Fractions of TX and CX taken up as particles		0.187 ± 0.46	0.247 ± 0.43	0.242 ± 0.34
C	Fractions of the remaining TX which are delipidated		0.409 ± 0.12	0.387 ± 0.30	0.326 ± 0.43
D	Residence time of RM	min	11.2 ± 0.43	9.27 ± 0.49	7.33 ± 0.49
E	Residence time of chylomicron triglyceride	min	4.15 ± 0.33	3.73 ± 0.29	4.68 ± 0.36
F	Residence time of chylomicron cholesteryl ester	min	10.9 ± 0.29	8.14 ± 0.30	8.38 ± 0.36
G	Mean index of delipidation		2.73 ± 0.20 (2.56)[d]	2.21 ± 0.17[b] (2.12)	1.89 ± 0.28[c] (1.89)
H	Number of animals		10	10	10

[a]Mean of individual values ± fractional standard deviation.
[b]Significantly less than fasting, $P < 0.025$.
[c]Significantly less than fasting, $P < 0.01$.
[d]Median.

questions that naturally arise are: does lipoprotein lipase activity regulate the uptake of remnants or does remnant uptake regulate the extent of lipase hydrolysis by removal of the substrate.

Our findings with respect to nutritional influences on chylomicron metabolism complement the previous findings of Harris and Felts (4), who found that the plasma removal of chylomicron triglycerides was faster in rats fed regular chow than in rats fed a 65% dextrose, fat-free diet for 14 days. Our data (Table 1) confirmed this effect, in that the residence time of chylomicron triglycerides was 20% less after a meal of regular chow than after a fat-free meal, and showed further that the effect followed just a single meal, without a preceeding period of consumption of the fat-free diet. Because our experiments included a cholesteryl ester label and with the additional insights provided by the compartmental model, our data shows clearly that the longer residence time of the chylomicron triglycerides after a fat-free meal was explained solely by the lower index of delipidation, since remnant uptake by the liver was accelerated (Table 1).

On the other hand, our findings show a converse trend for chylomicron metabolism in fasting rats compared with meal-fed rats. In the case of fasting rats, because the delipidation index was increased, a briefer triglyceride residence time might have been expected. However, because of the longer residence time of RM (containing residual triglycerides) in the fasting rats, the residence time of chylomicron triglyceride remained longer than in chow-fed rats.

The activities of lipoprotein lipase in adipose tissue and heart are reciprocally affected by fasting and carbohydrate feeding. Low fat, high carbohydrate diets decrease heart lipase and increase adipose lipase activity (30, 31), whereas fasting increases heart lipase and decreases adipose tissue activity (32, 33). A single fat meal increases heart lipoprotein lipase (34). It is therefore difficult to extrapolate to the functional activity of lipase in vivo when rats are varied in nutritional status. Our findings indicate that the extent of lipolysis of injected chylomicrons may be influenced by the rate of uptake of remnant particles by receptors in the liver, since the particles are recognized and taken up after different degrees of delipidation, depending on nutritional status.

Although the expression of apoB,E receptors mediating the hepatic uptake of low density lipoproteins in adult dogs (35) was increased by fasting, in rabbits the catabolism of low density lipoproteins was decreased by fasting (36). Mahley et al. (35) were unable to demonstrate an effect of fasting on the expression of the apoE receptors that mediate remnant uptake in canine livers.

Our finding of a longer residence time of remnants in the fasting group of rats (Table 1) may indicate decreased expression of remnant receptors, decreased affinity of the remnants for apolipoprotein E which is the essential ligand for binding to the remnant receptor (35), or other effects such as increased affinity for inhibitory ligands such as the C apolipoproteins (37). Clearly more studies are now necessary to establish the mechanism for the effect of fasting on remnant clearance.

Our compartmental model of the metabolism of the lipids of the chylomicron core makes possible a simultaneous assessment of the two important underlying metabolic events, lipolysis and remnant particle uptake. Our findings show that the two processes interacted in unexpected ways. This information would be inaccessible without development of the compartmental model. In future experiments variations can be selected that will be insensitive to changes in the rate constants (e.g., same chylomicrons injected into two strains of rats), or where the rate constants will be

deliberately varied (e.g., injecting increasing amounts of unlabeled lipoproteins with the labeled chylomicrons). At some future time it will be essential to add a surface label to the injected chylomicrons to extend the mathematical dissection of the metabolic processes. Refinement and further testing of the predictions of the model are immediately desirable by obtaining more data between 15 and 45 min after injection of the chylomicrons, where the model predicts that recirculation makes an increasing contribution to the overall kinetics.

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